Scientia

RECENT ADVANCES IN CONFRONTING THE CHALLENGE OF CANCER

HIGHLIGHTS:

- Community Science: Studying Cancer in Pets and People
- Are Poly-aneuploid Cancer Cells the Keystone Cure for Cancer?
- Understanding Sex Differences in Cancer Promises Better Treatment and Survival

EXCLUSIVES:

- Worldwide Cancer Research
- Against Breast Cancer

Issue: #136

edanz
learning lab

learning.edanz.com/sd

Free Premium Membership for Scientia readers

Build skills and solve your your toughest research & publication challenges

25
Quick Courses

100+
Guides & Infographics

1000+
Searchable Topics

Excelling in research together

Edanz Learning Lab is part of the Edanz Smart Tools platform - a platform for self-learning, productivity and collaborative writing with insight and support from our team of experts.

edanz
SMART TOOLS
EXCELLING IN RESEARCH TOGETHER

WELCOME...

This special issue of Scientia is dedicated to the scientists working to confront cancer, one of the biggest challenges facing medical science in the 21st century. According to the World Health Organization, cancer is the second leading cause of death around the world, accounting for nearly 10 million deaths in 2020. Although research into cancer has taken place in some form for over 200 years, still to this day we do not have a cure.

The first section in this special issue is dedicated to research on the causes and risk factors that may lead to cancer, with a focus on the need for public health interventions to reduce preventable deaths. Our exclusive interview with Worldwide Cancer Research's Chief Executive, Dr Helen Rippon, sets the scene for meeting scientists working on research as diverse as identifying the similarities between cancers in humans and dogs to identifying the environmental causes of cancer through exposure to toxins.

Our second section focuses on the work of researchers striving to improve the timing and accuracy of diagnosing cancer. This is a critical area of research because early diagnosis is associated with much better rates of survival than later diagnosis. Here, we read of the development of low-cost, non-invasive procedures that have exciting applications for the detection of novel biomarkers in cancer, to algorithmic approaches to decision-making in breast cancer that are data-driven and equitable with the intention of reducing stress on patients. We conclude the section with an exclusive interview with Richard Bahu, Chair of Trustees at the UK research charity Against Breast Cancer.

Our final section is dedicated to the development of treatments for cancer. In acknowledgement of the need to develop a broad range of therapies to cover the whole spectrum of cancer, we meet the researchers dedicated to the development of novel and innovative therapeutics, ranging from combined therapy to improve the therapeutic response of tumours in late-stage pancreatic ductal adenocarcinoma to the potential benefits of an alkaline diet on the tumour microenvironment and the enhancement of anti-cancer treatments. We close this section and special issue of Scientia with an exclusive interview with Worldwide Cancer Research's Director of Research, Dr Lynn Turner, where we read how the COVID-19 pandemic has impacted the battle against cancer, and what challenges must now be faced as a result.

Science Diffusion

CONTACT

Published in the UK, by Science Diffusion ltd

ISSN 2059-8971 (print)
ISSN 2059-898X (online)

E: info@sciencediffusion.com
W: www.sciencediffusion.com
W: www.scientia.global

@scientia_social
www.facebook.com/socialscientia
www.linkedin.com/company-beta/11065635

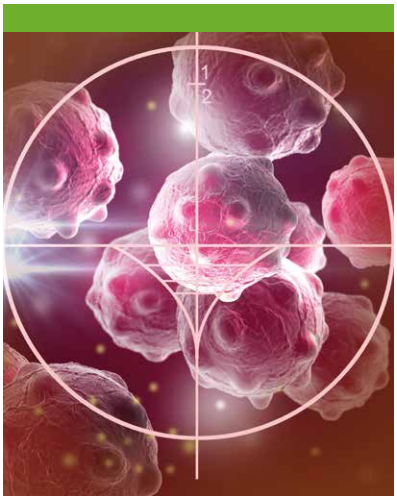
Meet The Team...

CONTENTS

ISSUE : #136

CAUSES AND RISK FACTORS OF CANCER

DIAGNOSING CANCER



TREATING CANCER

CAUSES AND RISK FACTORS OF CANCER

SHEDDING LIGHT ON CANCER: CAUSES, RISK FACTORS AND THE NEED FOR PUBLIC ADVOCACY

Cancer is a complex group of diseases and as such, there are many possible causes and risk factors. Critically, elucidating the causes and risk factors for different types of cancer provides a potential for preventing its occurrence in the first place. With this in mind, we open this section with an exclusive interview with Worldwide Cancer Research's Chief Executive, Dr Helen Rippon, and read of their vital work funding the most innovative and promising cancer research across the globe.

Our first researcher to be featured in this section uses pets as a model to study the development of cancer. Dr Elinor Karlsson, based at the University of Massachusetts Medical School and the Broad Institute of MIT and Harvard, observed that the presentation and characteristics of cancer in dogs often resemble those seen in humans. With her multidisciplinary team of collaborators, Dr Karlsson is working to identify the similarities between cancers in humans and dogs, and inform the development of better therapeutic approaches for the benefit of both species.

We then turn to the work of Dr Chen Zhao (University of Iowa) and Dr Qianze Dong (China Medical University). We read of their early-stage laboratory work exploring the cellular mechanisms of haematopoietic stem cells – the processes through which cellular blood components are produced. When this process is disrupted, one of the devastating processes that may result is that of blood cancer, such as acute myeloid leukaemia. Their research is making significant strides forward in explaining how some cancers may develop in the body, and also holds promise for the development of new anti-cancer treatments.

Explaining the potential role for an immune system protein, GANP, and its coding gene, ganp, in the development of cancer is the focus of Dr Yasuhiro Sakai and Dr Kazuhiko Kuwahara (both at Fujita Health University School of Medicine). Their multidisciplinary, cutting-edge research has shown that differential levels of GANP appear to correlate with breast cancers and with lymphocytic cancers.

We then shift our focus to the potential environmental causes of cancer through exposure to toxins. Dr Jennifer Elizabeth Kay and Professor Bevin Page Engelward are leaders of the Massachusetts Institute of Technology Superfund Research Program and are using their expertise to understand how chemical contaminants are linked to the development of cancer and other long-term health problems. We read how their work is revealing the genetic factors that influence susceptibility to cancer in particular following exposure to environmental chemicals, and the importance of taking a public health approach to ensure disease prevention and disease mitigation.

Professor Janet Gray at Vassar College and Ms Nancy Buermeyer at Breast Cancer Prevention Partners in the USA also adopt a public health approach in their work. Breast Cancer Prevention Partners are the leading policy and advocacy organisation focusing on preventing breast cancer before it even starts. We read how the use of everyday substances such as beauty, personal care and cleaning products can increase the risk of breast cancer through exposure to toxins, and the importance of population-level changes to reduce the unnecessary deaths and illness caused by breast cancer.

Finally, we turn to the work of Dr Bhuvaneswari Ramaswamy and Dr Sarmila Majumder at Ohio State University Comprehensive Cancer Centre. Women of African-American background are disproportionately affected by a specific, particularly aggressive subtype of breast cancer. But interestingly, prolonged breastfeeding reduces the risk of this subtype. The integrated research model adopted by Dr Ramaswamy and Dr Majumder directly links epidemiological public health research with laboratory-based research, providing a novel and important example of the tangible benefits of evidence-based public health interventions.

WORLDWIDE CANCER RESEARCH

Cancer presents a global challenge. With over 17 million new cases of cancer worldwide in 2018 and 9.6 million deaths in the same year, better approaches to the prevention, diagnosis and treatment of cancer are urgently required.

Founded in 1979, Worldwide Cancer Research is a UK-based charity dedicated to meeting these needs by starting new cancer cures worldwide. In this exclusive interview, we speak again with Worldwide Cancer Research's Chief Executive, **Dr Helen Rippon**, to hear about their recent efforts and plans for the future.

We last spoke in early 2019. A lot has happened over the past two years! What are the current priorities and focus for Worldwide Cancer Research?

2019 marked our 40-year anniversary and because it was such a significant milestone for us, we wanted to take the opportunity to reflect on what we have achieved since we were founded in 1979, reaffirm our mission as a charity and really focus on our vision for the future.

As a result, we went through a lot of changes as an organisation – we relocated from our original home in St Andrews to Edinburgh, recruited new team members and rebranded to reach new supporters and better represent Worldwide Cancer Research today.

But despite all the changes, our core mission and values have remained the same. We are still the only charity in the UK dedicated to starting new cancer cures by funding discovery research all over the world.

We are still confident in the power of people and the human mind. We know that we can make a real impact when we come together with a single goal in mind – to end cancer.

Each year our panel of scientific experts from around the world come together at our Big Ideas Gathering to decide which new cancer research projects to fund.

And each year we receive an incredible number of applications but we are currently only able to offer funding to around 20% of the applications that could lead to new cures in the future.

That's why our one big priority is to be able to fund more lifesaving cancer research. Specifically, we want to be able to fund £20million of research annually by 2030. Currently, we fund an average of £4million a year, so there's a lot of work for us to do. And we can't do it alone – we need many more people to join us and become Curestarters.

What do you mean exactly when you talk about becoming a Curestarter?

We know that the research journey is a long one and that it can take up to 20 years for an idea to lead to new treatments. But we also know that there can't be an end to cancer if we don't start at the beginning.

And we couldn't start any new cancer cures without our amazing supporters. We don't receive any government funding, so we're entirely reliant on the kindness and generosity of the people who raise much-needed awareness and money for us – whether that's by setting up a Direct Debit, playing our Weekly Lottery, fundraising with family and friends or any number of other options – they are all Curestarters.

It is often asked 'why isn't there a cure for cancer yet?'. What is your response to that?

Cancer is complex and the more we learn about it, the more it becomes clear that there won't ever be a 'magic bullet'. To understand why, the most important thing to know is that cancer is not one disease. Instead, it's an umbrella term for more than 200 distinct diseases. Each broad cancer type also has many subtypes, and they all look and behave differently because they are different on a genetic and molecular level. This is because cancer arises from our own cells, so each cancer can be as different and diverse as people are. And that means that there isn't ever going to be a one-shot cure-all for cancer.

But that doesn't mean that there hasn't been incredible progress made. Thanks to research like the projects funded by Worldwide Cancer Research, UK survival rates for cancer have doubled since the 1970s.

Some cancers, such as leukaemia and testicular cancer, have made particularly impressive jumps. In the 1970s only about 5% of people survived their leukaemia diagnosis for ten years or longer, now almost 50% do. The ten-year survival rate for testicular cancer is now at 91% – an amazing improvement.

But there is still a long way to go. There are still cancers that have seen hardly any improvement in survival rates over the years, including lung, pancreatic and oesophageal cancer.

Because of research, we now know more than ever before about the fundamental biology of cancer. It is this knowledge that is beginning to inform the design and development of brand-new treatments that seek to target the individuality of cancer. Progress so far has been fantastic, and we must push on in order to find ways to close the gap in survival rates for different cancers.

How do you ensure you fund only the most innovative and promising research into cancer around the world?

Since we last spoke, we have refined our Research Strategy to help guide our Scientific Advisory Committee in their decision on what to fund at our annual Big Ideas Gathering. Because we are restricted in how many projects we are currently able to fund, we want to make sure we are picking the best ideas of the bunch. Our remit hasn't changed – to fund the best discovery research into new ways to prevent, diagnose and treat cancer – but we have made it clearer that we are looking for truly innovative and creative ideas that have the potential to transform our understanding of cancer.

This year, our process starts in February, when our annual grant round opens, and we invite applications from scientists all over the world. Once these are in, our Scientific Advisory Committee gets to work, selecting the applications that meet the criteria of our Research Strategy. These projects are then sent out for peer review by external experts in cancer research before the final

decision is made by the Scientific Advisory Committee at our annual Big Ideas Gathering held in October.

What would you say is Worldwide Cancer Research's biggest achievement to date?

We've been funding research for over 40 years so there are many success stories that we know of. The interesting thing is that because we fund truly new ideas, those right at the start of the research journey, it can take years or even decades before we know that breakthroughs made on a project are having a positive impact on the lives of people with cancer.

Out of all of them, I'd say our biggest achievement to date has been our involvement in starting the development of the cancer drug called olaparib – the first-in-class PARP inhibitor. The foundations came from a set of projects we funded with Professor Steve Jackson in Cambridge back in the 1990s and Professor Jackson then took his findings from those projects and went on to develop olaparib.

To this day, olaparib has been used to treat 30,000 people worldwide with certain ovarian, breast, pancreatic and prostate cancers. And research is still ongoing, with the drug now being tested in clinical trials for many different types of cancer.

What are the biggest challenges now facing cancer research and how will Worldwide Cancer Research overcome them?

Peering into a crystal ball when it comes to scientific advances is never easy. The exploratory nature of research means you can never really guess where the next big step is coming from and so it is probably best to resist making any grand predictions! I would guess that the trend towards personalised medicine will continue to gradually figure out which cancer patients will benefit most from which cancer drugs. And that immunotherapy – drugs and cell therapies that harness the power of the body's own immune system to attack cancer – will become a mainstay of treatment for more and more types of cancer.

But those guesses are based on things we already know. Science already done. Worldwide Cancer Research exists to discover the new things, to start new trends and new lines of research that we probably couldn't even guess at yet. History tells us that new ways to prevent, diagnose and treat cancer are rooted in fundamental discoveries made in the lab – often from scientists exploring how cancer works at its most basic level. That's why it's so important that we continue to fund these innovative new ideas. You really don't know where they could lead in the future – and how many lives they could save.

worldwide cancer research

COMMUNITY SCIENCE: STUDYING CANCER IN PETS AND PEOPLE

The presentation and characteristics of cancer in dogs often resemble those seen in people. This observation led **Dr Elinor Karlsson**, based at the University of Massachusetts Medical School and the Broad Institute of MIT and Harvard, to consider whether these pets could be a good model to study the disease in humans. Dr Karlsson, along with an established multidisciplinary team of collaborators, is working to identify the similarities between cancers in humans and dogs and translate this into better therapeutic approaches for both species.

Leading Cause of Death in Dogs

With six million dogs diagnosed with cancer every year in the USA, cancer is the leading cause of death in these pets. Our furry friends are exposed to the same environmental factors as their owners and suffer from many of the same types of cancer. It should not come as a surprise then, that dogs often respond to the same treatments as humans diagnosed with cancer.

However, cancer tends to have an accelerated course in dogs, enabling clinical trials to be conducted more quickly and easily in dogs than in humans. For Dr Elinor Karlsson, this makes canine cancer an ideal model for human cancer. Our pet dogs represent an unparalleled opportunity for researching the risk factors that are involved in cancer and how this disease progresses. 'To fully utilise family dogs as a model, we need to understand in far more detail the similarities and differences between dog and human cancers on both genomic and clinical levels,' explains Dr Karlsson. 'Finding canine and human cancers that share driver genes and pathways will enable development and clinical testing of new targeted genetic therapies in dogs that could help people too.'

A New Way to Detect Cancer in Dogs

While dogs may present as an ideal model to study human cancer, according to Dr Karlsson, this approach only works if research teams are 'able to assemble much larger canine-patient cohorts than currently feasible.' Under normal circumstances, obtaining tumour biopsies from dogs can be extremely difficult, or even impossible, if owners decide on only palliative care or if the dogs are seen at a small veterinary facility with only limited resources.

To overcome this difficulty, Dr Karlsson and her team are using an innovative technique known as 'blood biopsy' that was originally developed for humans and adapting it for use with dogs (more details at https://www.broadinstitute.org/blood-biopsy). Using an approach developed by Dr Karlsson's collaborator, Dr Viktor Adalsteinsson at the Broad Institute's Gerstner Center for Cancer Diagnostics, the team can detect cancer from a sample of blood by analysing

DNA released from dying cells and circulating in the blood. While most of the DNA will be from non-cancer cells, this non-invasive procedure can distinguish the DNA released by tumour cells in a person, or a dog, with cancer.

The technique involves the collection of blood samples in special tubes to avoid damage to the DNA strands. Other than that, it is no different from the blood draws done for other clinical tests, and can be completed easily in any veterinary clinic. The sample is shipped to the Broad Institute Genomics Platform, where the amount of DNA is measured and then sequenced to identify the genetic mutations in the tumour.

‘In the future, we anticipate that the insights gained from studying pet dogs will help us develop more targeted and effective treatments for human cancers.’

Dr Karlsson recently completed a pilot study that demonstrated how this method can be adapted to capture and analyse free DNA circulating in the bloodstream of dogs. In this study, the team collected blood samples from 27 dogs with tumours with a high risk of metastasis, including hemangiosarcoma, lymphoma, and osteosarcoma. They showed that they could find and sequence DNA in every sample, and they identified tumour DNA in over 50% of samples.

Blood biopsy has many promising applications in both the human and veterinary clinic, including as a diagnostic test, and as a non-invasive way to monitor patients undergoing treatment. This method could change the way we treat cancer in dogs and people, although there are still many outstanding questions that the team is working to address in their current research. Dr Karlsson and her team are excited about exploring its potential to reveal whether a given patient is responding to therapy, enabling doctors to evaluate and potentially change therapies quickly. For example, by enrolling dogs undergoing chemotherapy and who are closely followed in the veterinary clinic, blood samples can be taken at controlled intervals, and used to measure whether the blood biopsies accurately predict whether the therapy is working. This approach will also help guide the application of blood biopsy as a diagnostic and monitoring technique in human patients, which is an important goal for the team.

A Better Genetic Understanding

The blood biopsy project started when Dr Karlsson realised that she needed a new approach to studying canine cancer if she wanted to understand the genetics of the disease and develop more effective therapeutics. Two different kinds of genetic mutations can lead to cancer. Somatic mutations are mutations that arise as the tumour grows inside a person or a dog. Germline mutations are genetic mutations an individual inherits from their parents. Some germline mutations may make somatic mutations more likely, which puts an individual at a high risk of cancer. For example, people who inherit germline mutations in the genes *BRCA1* and *BRCA2* have a high risk of somatic mutations that cause breast cancer.

One reason why dogs are of such interest to cancer researchers is that germline cancer mutations are common in some dog breeds. By focusing on these high-risk breeds, scientists can find the germline mutations more easily. The first cancer Dr Karlsson studied was osteosarcoma (bone cancer) when she was a graduate student at Boston University. Some dog breeds are particularly susceptible to osteosarcoma, including greyhounds and rottweilers, a sure sign that germline genetic mutations are involved. Dr Karlsson’s research sought to find those germline risk factors in dogs, since that could provide the first clue to what causes this disease. In both people and dogs, osteosarcoma typically starts at the ends of the long bones in the arms and legs then metastasises to the lungs. Critically, the researchers found that by comparing affected and unaffected dogs in different breeds, they could identify germline risk factors, and common pathways underlying the development of disease.

'Our results highlight how the genetics of angiosarcoma in dogs and humans is similar. Because angiosarcoma is rare in people, genomics is difficult, and dogs are helping us search for new therapeutic options for this terrible disease.'

At this point, though, the research progress slowed dramatically. Even with the high disease rates in dog breeds, cancer is an incredibly complex disease, and many more dogs were needed to take the next steps. This roadblock stymied progress not just on osteosarcoma, but also on two other canine cancers that were promising models – lymphoma and angiosarcoma. For lymphoma, comparing three breeds (boxers, cocker spaniels, and golden retrievers) showed that boxers had more aggressive forms of the disease, whereas golden retrievers had a more treatable version, reflecting different germline mutations between the breeds.

For angiosarcoma (a blood vessel tumour), it turned out that both the canine and the human tumours have multiple mutations in some of the same proteins and mechanisms that are crucial for cancer development. 'We have shown through detailed molecular profiling of canine angiosarcoma that the genetic landscape of these tumours is similar between dogs and humans,' notes Dr Karlsson.

One example is a gene called tumour protein p53 (or *TP53* for short), which was the gene most often mutated in angiosarcomas in golden retrievers. Not surprisingly, *TP53* is also frequently mutated in human angiosarcomas. Known as the 'guardian of the genome', this gene can protect the genetic information in the cells, which means any *TP53* mutations may destroy one of the cells' strongest defence mechanisms.

The researchers also found several mechanisms affected by this type of cancer. One of them was a pathway known as PI3K, which plays an important role in metabolism and immunity. In humans, this pathway is commonly affected in many types of cancer, such as glioblastoma, breast, gastric, colorectal, lung, and endometrial cancers.

Identifying these mutations in osteosarcoma, lymphoma and angiosarcoma is critical for both humans and dogs as it provides new insights into disease pathogenesis for both species. Understanding these mutations may prove key to determine the best treatment options in both humans and dogs. Yet, in each of these cancers, the intriguing initial findings were difficult to follow up on because of the sampling problem. Tumour biopsies are hard to get, and the studies were just too small. Further work is needed with larger sample sizes to offer a more complete comparison between tumours in various locations.

'Our results highlight how the genetics of angiosarcoma in dogs and humans is similar. Because angiosarcoma is rare in people, genomics is difficult, and dogs are helping us search for new therapeutic options for this terrible disease,' summarises Dr Karlsson.

The Future is Community Science

Seeing the opportunity to dramatically increase study sizes while increasing engagement with dog owners, in 2015 Dr Karlsson launched a community science project called Darwin's Ark. To date, her team has enrolled over 28,861 dogs and collected responses to nearly 3,056,323 owner survey questions. The idea is to recruit more dogs into research utilising genomic analyses, while streamlining the collection of detailed information about the dogs' behaviour and health. Embracing modern technology, the project uses a web portal, which allows owners to provide accurate information more readily than any traditional approach, in which typically researchers had to spend considerable time contacting owners or veterinarians for the data required.

Modelling their approach on the successful Count Me In Initiative for direct-to-patient studies of rare human cancers, the team is launching the Darwin's Dogs Cancer Project. The Cancer Project will allow owners to provide information about their dogs' cancer diagnoses and treatments. Some dog owners will also be asked to attach a special type of tag to their dog's collar for a few weeks, as part of a trial testing new technology for measuring what chemicals are in the environment. The team is excited to pilot the enrolment of canine cancer patients via the website for blood biopsy studies and environmental studies. The pairing of a web-based community science approach with a non-invasive sampling technique like blood biopsy could be the key to enabling large-scale genomic studies of canine cancers.

Pets are uniquely placed to help improve our understanding of cancer. Not only do humans and dogs share many similarities, in terms of biology and genetics, but companion animals share our environment and are exposed to the same environmental factors. 'Studying spontaneous cancer in dogs can provide important information that informs subsequent studies in both human and veterinary medicine, ultimately leading to advancements in the care of people and dogs affected by cancer,' Dr Karlsson concludes.

Meet the researcher

Dr Elinor K. Karlsson
Associate Professor
University of Massachusetts Medical School
Worcester, MA
USA

Dr Karlsson completed her PhD in bioinformatics at Boston University, Massachusetts, in 2008. She then completed a postdoctoral position at Harvard University, Massachusetts, and joined the University of Massachusetts in 2014 as an assistant professor. Dr Karlsson remains at the University of Massachusetts where she is now an associate professor, and as well as the Director of Vertebrate Genomics at the Broad Institute of MIT and Harvard, where she manages a team with expertise in comparative genomics. Dr Karlsson has been the recipient of multiple prestigious awards throughout her career, including the NSF Graduate Research Fellowship, the American Cancer Society Postdoctoral Fellowship and the Charles A. King Trust Postdoctoral Research Fellowship. Dr Karlsson leads a team of graduate students and postdoctoral researchers, heading up an impressive number of projects. Dr Karlsson is the founder and chief scientist of Darwin's Ark (https://darwinsark.org/), an innovative approach in which pet owners become active participants in science projects. Current projects, such as Darwin's Ark, combine the study of genetics, behaviour and health to advance the understanding of complex diseases.

CONTACT

E: Elinor.Karlsson@umassmed.edu
W: http://DarwinsArk.org
@eenork

KEY COLLABORATORS

Kate Megquier, DVM PhD
Heather Gardner, DVM PhD
Michelle White, DVM PhD
Cheryl London, DVM PhD
Kerstin Lindblad-Toh, PhD
Viktor Adalsteinsson, PhD
Corrie Painter, PhD

FUNDING

National Institutes of Health

FURTHER READING

K Megquier, J Turner-Maier, R Swofford, et al., Comparative genomics reveals shared mutational landscape in canine hemangiosarcoma and human angiosarcoma, Molecular Cancer Research, 2019, 17(12), 2410–2421.

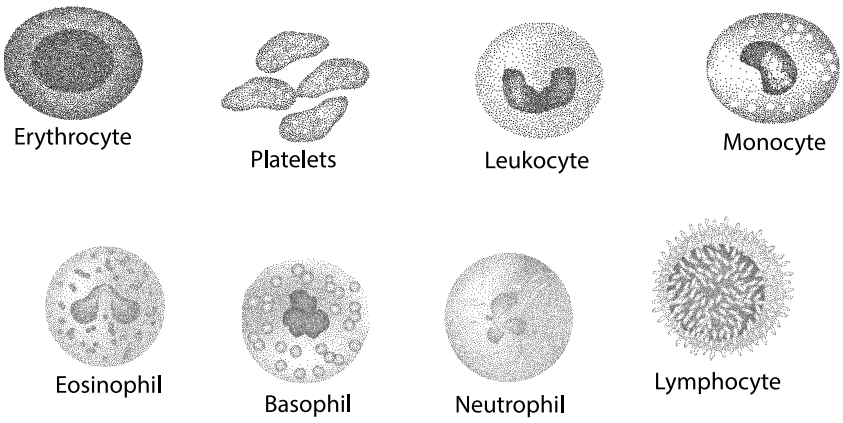
H Gardner, J Fenger, C London, Dogs as a model for cancer, Annual Review of Animal Biosciences, 2016, 4, 199–222.

I Elvers, J Turner-Maier, R Swofford, et al., Exome sequencing of lymphomas from three dog breeds reveals somatic mutation patterns reflecting genetic background, Genome Research, 2015, 25, 1634–1645.

EK Karlsson, S Sigurdsson, E Ivansson, et al., Genome-wide analyses implicate 33 loci in heritable dog osteosarcoma, including regulatory variants near CDKN2A/B, Genome Biology, 2013, 14, R132.

THE FINE MECHANICS OF HAEMATOPOIESIS

Haematopoiesis is the process through which cellular blood components are produced. It starts during embryonic development to ensure the production of blood cells such as erythrocytes (red cells), leukocytes (white cells), and platelets and continues throughout our lives. All blood cells derive from haematopoietic stem cells located in the bone marrow and, unfortunately, blood cancers may occur during this process. Whether blood cells become inefficient or grow excessively, the outcomes are usually devastating. **Dr Chen Zhao** (University of Iowa) and **Dr Qianze Dong** (China Medical University) are exploring the cellular mechanisms of haematopoietic stem cells.



Blood Cells

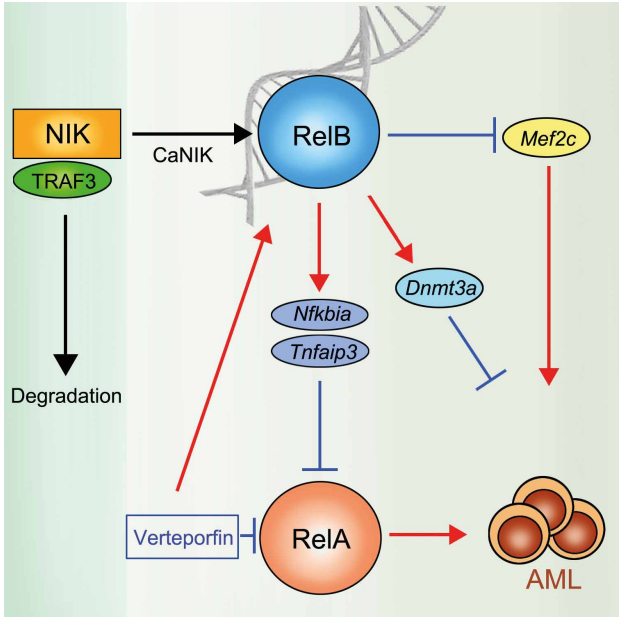
Canonical and Non-Canonical Pathways

Haematopoietic stem/progenitor cells (HSPCs) are highly regulated cells, with the unique ability to self-renew and to differentiate into the different blood cell types. The rate of cell production is controlled by the body's need. For example, more white cells or platelets are respectively produced to compensate for infection or blood loss. One HPSC undergoes a series of transformations to evolve into a definitive cell type. This is controlled by a multitude of signals coming from the bone marrow microenvironment to the cell DNA and influences the cell differentiation via activation or inactivation of genes. This series of signals is known as the cellular signalling pathways. They can be described as 'canonical', referring to traditional pathways specific to cell or tissue or 'non-canonical', where pathways diverge from the classical path. The alternative pathways are less studied but now attracting scientific curiosity.

The Role of the Non-Canonical Pathway in Haematopoiesis

Dr Zhao at the University of Iowa, Dr Qianze Dong at China Medical University, and their colleagues are interested in the well-known NF-κB pathway (nuclear factor kappa-light-chain-enhancer of activated B cells). It includes a collection of different proteins modulating physiological processes such as immune responses, cell proliferation or apoptosis (cell death). NF-κB is expressed in most cells and controls the expression of numerous genes. However, the role of NF-κB signalling to regulate HSPCs is still unclear.

NF-κB is tightly regulated by intermediate signalling molecules, such as the NF-κB inducing kinase (NIK), a crucial kinase of the non-canonical pathways. A kinase is an enzyme allowing the transfer of a phosphate group between two molecules, allowing the signal to spread into the cell from the membrane to the nucleus where the DNA is located. Previous research has established that inactivating NIK impairs the ability of HSPCs to self-renew.



The Role of Non-Canonical Pathways in Acute Myeloid Leukaemia. Credit Chen Zhao.

Whilst the role of the canonical NF-κB pathway is already well described, Dr Zhao and his colleagues are interested in the alternative or non-canonical NF-κB pathway. Its role in normal and pathologic haematopoiesis has been overlooked over the last decade. Dr Zhao previously made the novel observation that non-canonical NF-κB signalling supports HSPC self-renewal and preserves the stem cell pool. Starting from this discovery, Dr Zhao progressed the idea that constant activation of the non-canonical pathway via the permanent activation of NIK could influence haematopoiesis. To test this, Dr Zhao and his team developed a new genetically modified mouse model, where NIK is constantly activated either specifically in the HSPCs or in the whole body.

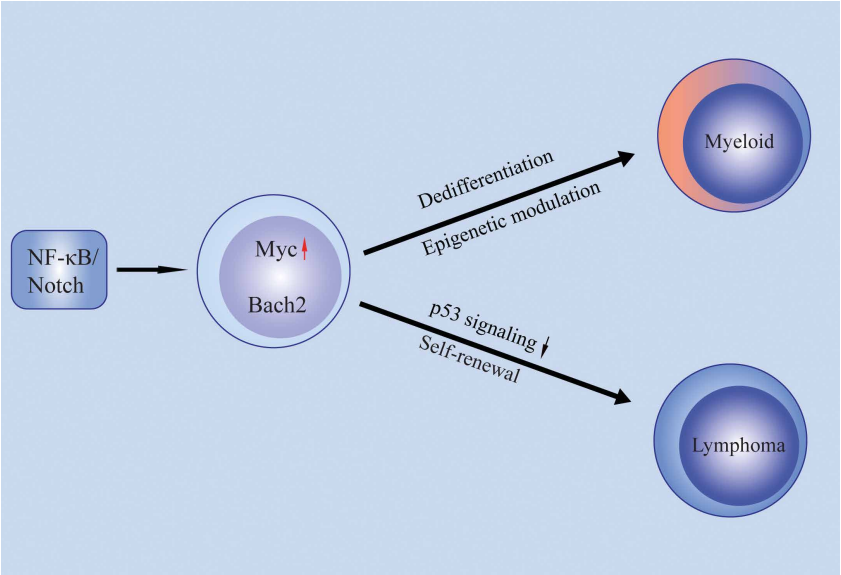
The mutant mice, NIK-inactivated in HSPC, rapidly showed growth defects with reduced body and organ sizes with a life expectancy of 7 days. This was accompanied by reduced levels of erythrocytes, leukocytes, or platelets in the blood. In contrast to Dr Zhao and his team's expectations, a permanent activation of NIK appeared to be detrimental to normal haematopoiesis. They further demonstrated that the activation of NIK compromises the health of HSPCs, increasing inflammation and cell death. These findings highlight the opposite effects of the canonical and non-canonical NF-κB pathways and the role of stable NIK to maintain the healthy production of blood components.

The Role of the Non-Canonical Pathway in Acute Myeloid Leukaemia

Acute myeloid leukaemia (AML) is an aggressive form of blood cell cancer, characterised by the rapid growth of abnormal blood cells, preventing healthy cells from being efficient. It is caused by a small pool of malignant cells, able to self-renew, called leukaemia stem cells (LSCs). Most current treatments involve chemotherapy but understanding the LSC signalling pathways is a promising therapeutic approach aiming to stop them from proliferating. Canonical NF-κB signalling is activated in LSCs and is associated with higher resistance to chemotherapy. It had previously been demonstrated that suppression of the canonical pathway, combined with other therapies, helps to slow down AML development.

Once again, Dr Zhao and his colleagues thought outside the box and explored the role of the non-canonical NF-κB pathway in LSCs. If permanently activating NIK in a healthy context might be detrimental, it might, however, promote the self-renewal of stem cells in pathological conditions such as AML. They used the same mouse model, NIK-activated in the whole body (stabilisation), in combination with the well-characterised MLL-AF9, a genetic recombination mimicking the effects of DNA modifications that frequently result in the formation of human cancer. In this case, MLL-AF9 was used to induce AML.

This study showed that the stabilisation of NIK activated the non-canonical and repressed the canonical pathway. Activation of the NF-κB non-canonical pathway upregulates Dnmt3a and downregulates Mef2c, two genes respectively suppressing and promoting AML. The hypothesis behind this mechanism is that the non-canonical pathway negatively controls the gene responsible for the LSC self-renewal and positively control the genes involved in LSC suppression. Whilst further studies are required to pinpoint the exact mechanisms, this study highlights the ability of NIK stabilisation to suppress AML development and lays the foundation for therapeutic progress.



A New Model of Lymphoma. Credit Chen Zhao.

Identification of Verteporfin to Suppress AML

As the overactivation of NIK impairs the self-renewal of healthy HSPCs, a key challenge is to stabilise NIK only in LSCs. Dr Zhao and his colleagues observed that activating the non-canonical NF-κB pathway via NIK modulates the expression of different genes responsible for the survival and the death of LSCs. A promising therapeutic approach is to identify a drug that targets these specific genes.

The Connectivity Map (CMAP) database is a very useful tool in drug development. It includes a large collection of gene expression profiles from cultured human cells stimulated with various chemicals. The database allows scientists to identify any component responsible for the modulation of genes of interests. Dr Zhao used CMAP, targeting LSC genes, and identified verteporfin as a potential candidate for the treatment of AML. Verteporfin is a well-known treatment that suppresses abnormal vessels responsible for blurred vision in macular degeneration. It was also proven to inhibit tumour growth in various cancer models such as acute lymphoblastic leukaemia, with minimal effects on normal haematopoiesis. Testing verteporfin in vitro was efficient to reduced LSC proliferation and delayed AML development.

A New In Vivo Model to Study Lymphomas and Lymphoma to Leukaemia Conversion

B cell lymphomas are a type of blood cancers affecting the B cells in the lymph nodes. It is sometimes associated with the apparition of myeloid tumours such as AML. The rare conversion of lymphoma to AML is well recognised but the underlying molecular mechanisms are still poorly understood. This is mainly due to the lack of efficient in vivo models. In a recent study, Dr Zhao explained 'Because most patients in whom B-cell lymphoma undergoes conversion to myeloid tumour have a poor prognosis as a result of diagnostic difficulties and lack of standard treatment, it is important to elucidate the biological underpinning of the B-to-myeloid switch and develop new approaches to treat and prevent these uncommon but usually fatal neoplasms.'

Previous studies had already demonstrated that activation of NF-κB or Notch (neurogenic locus notch homolog protein) in B-cells is not sufficient to induce B cell Lymphoma. This is why Dr Zhao and Dr Dong proposed a new mouse model with concurrent activation of NF-kB and Notch signalling in committed B cells. This model is particularly interesting and adequate as coactivation of NF-kB/ Notch signalling in B cells significantly accelerates lymphoma development in mice and has the ability to convert to myeloid lineage, this is the lymphoma to leukaemia conversion similarly observed in patients

After validating the model in vivo, Dr Dong transplanted B cells activated for NF-κB and Notch into healthy mice. Lymphoma to leukaemia conversion occurred in 15% of the mice suggesting that simultaneous activation of both pathways is responsible for B cell lymphoma and conversion to AML. 'Targeting Notch/NF-kB pathways may not only facilitate lymphoma treatment, but also prevent B-myeloid conversion' explains Dr Dong.

Further experiments revealed that DNA methylation, a process by which methyl groups are added to the DNA strands, also plays an important role during the lymphoma-to-leukaemia conversion. DNA methylation regulates the accessibility of the DNA, influencing the gene expression. A drug suppressing DNA methylation effectively reduced converted cells but the underlying mechanisms of this process remain to be clarified.

Dr Zhao's findings represent major scientific breakthroughs. The identification of the role of the non-canonical pathway progressively opens doors to understand the mechanisms of health and poor haematopoiesis in different cancer models such as acute myeloid leukaemia or B cell lymphomas. As such, this early-stage laboratory work holds great promise for the development of alternative anti-cancerous treatments.

Meet the researchers

Dr Chen Zhao
Associate Professor
University of Iowa Hospitals and Clinics
Iowa City, IA
USA

Dr Chen Zhao graduated medical school in 1993 from the China Medical University of Shenyang and completed his PhD in 2002 at Keio University School of Medicine, Tokyo. In 2004, Dr Zhao moved to the USA where he continues his academic path. Having completed a range of clinical and research positions, Dr Zhao is now a tenured Associate Professor at the University of Iowa. With a number of publications in the highest ranking journals including Nature, Dr Zhao has an impressive scientific track record.

CONTACT

E: chen-zhao@uiowa.edu
W: https://zhao.lab.uiowa.edu/

FUNDING

Veterans Health Administration Merit Review Program
National Institutes of Health
University of Iowa and Mayo Clinic SPORE Developmental Research Program Award
American Cancer Society (ACS) Seed Grant of Iowa/American Cancer Society

Dr Qianze Dong
Professor of Pathology
China Medical University and the First Affiliated Hospital of China Medical University
China

In 2009, Dr Qianze Dong completed his medical degree at the China Medical University where he also begun his research career with a PhD in the Department of Pathology. In 2011, Dr Dong obtained title of Assistant Professor followed by Associate Professor in 2013. He is now a Professor in the Department of Pathology, China Medical University and the First Affiliated Hospital of China Medical University. Since 2017, Dr Dong has been a visiting scholar at Dr Zhao's laboratory at the University of Iowa School of Medicine, USA. Together, they are together unravelling the secrets of the cellular signaling pathways involved in haematopoiesis and related blood cancers.

CONTACT

E: dongqianze@mail.cmu.edu.cn

FUNDING

National Natural Science Foundation of China

FURTHER READING

Y Xiu, Q Dong, L Fu, et al, Coactivation of NF-κB and notch signaling is sufficient to induce B-cell transformation and enables B-myeloid conversion, Blood, 2020, 135(2), 108–120.

Y Xiu, Q Dong, Q Li, et al, Stabilization of NF-κB-inducing kinase suppresses MLL-AF9-induced acute myeloid leukemia, Cell Reports, 2018, 22(2), 350–358.

Y Xiu, WY Xue, A Lambertz, et al, Constitutive activation of NIK impairs the self-renewal of hematopoietic stem/progenitor cells and induces bone marrow failure, Stem Cells, 2017, 35(3), 777–789.

GANP: AN IMMUNOACTIVE PROTEIN WITH A KEY ROLE IN TUMOURIGENESIS

Investigating the role of an immune system protein, GANP, and its coding gene, *ganp*, **Dr Yasuhiro Sakai** and **Dr Kazuhiko Kuwahara** (Fujita Health University School of Medicine) have unveiled a potential role for this important protein in tumourigenesis. The scientists apply a multidisciplinary approach to identify potential therapeutic solutions to aid cancer prognosis, a collaboration that occurs in the emerging field of immunopathology. The researchers focus on the differential levels of GANP which appear to correlate with breast cancers (low GANP) and with lymphocytic cancers (high GANP).

Germinal Centre-Associated Nuclear Protein (GANP)

First discovered in 2000 by Dr Kazuhiko Kuwahara, who now belongs to the Fujita Health University School of Medicine, GANP is vital in human health and is associated with multiple functions across cell biology, neurology, immunohaematology and oncology.

On its identification, GANP was linked to B-cell differentiation and the affinity maturation of germinal centres. Affinity maturation requires the hypermutation of variable region genes and class switching of B-cell receptors, which occurs in response to T-cell dependent antigens, a key immune system process that allows antibody production against a vast array of foreign molecules.

Using immunohistochemical methods, Dr Kuwahara and his colleagues identified a molecule in mice that was upregulated in germinal centres and then applied cloning methodologies to identify the gene. The mouse protein product, GANP, is a large, 1971 amino acid protein, and the human form of the protein is similarly sized at 1980 amino acids.

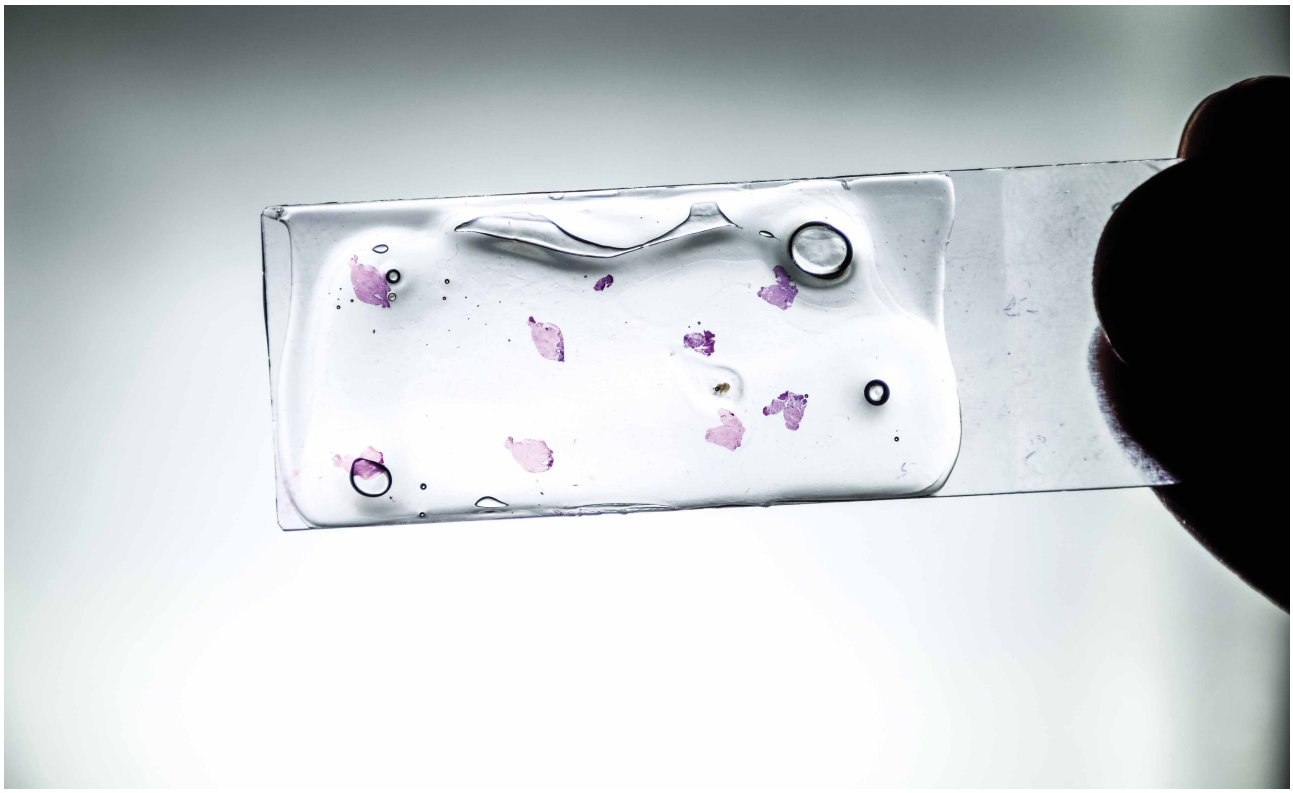
The human form of GANP shows a high degree of similarity to the original mouse protein, and both exhibit some similarity with other proteins, which are related to the functions of this molecule. In the two decades since its discovery, GANP has been found to extend beyond the humoral immune system, with many functions across the entirety of the body tissues now being attributed to this protein.

Functions of GANP

GANP forms a complex with other proteins to produce TREX-2, which is important in mRNA nuclear export, that is, the transfer of messenger RNA (mRNA) from the cell nucleus to the cytoplasm, where the mRNA molecules direct protein synthesis.

Specifically, GANP is a vital part of the complex, which enables the transfer of nuclear RNA export factor 1 (NXF1) binding messenger ribonucleoproteins (mRNPs) to the nuclear pores that provide a transportation route to the cell cytoplasm.

It is important to note that mRNA export occurs by both a GANP-dependent mechanism and another which is GANP-independent. In some circumstances, GANP is associated with cell death (apoptosis). Dr Kuwahara found that *ganp* RNA interference (RNAi) in the HeLa cell line resulted in the cell cycle being stopped, an increase in the abnormal alignment of chromosomes and, ultimately, cell death.



Inhibition of Tumourigenesis

In addition to the NXF1 pathway for mRNA export, there is a secondary specialised pathway known as CRM1, and there is discord amongst scientists with regards to the role that dysregulation of mRNA export plays in the initiation of cancer. However, it is clear that dysregulation in the specialised CRM1 system is apparent in a number of cancers, where overexpression of CRM1 is often reported and, of critical importance, the extent of overexpression is linked to the patients' outcome.

There is building evidence that the bulk export of mRNA, via the NXF1 pathway is also important in cancer initiation, and expression of GANP is frequently altered, either up or down, in cancers.

Increased expression of GANP has been detected in lymphomas (blood cancers) and malignant melanomas (skin cancers), while decreased levels of GANP have been identified in breast cancers and glioblastomas (aggressive cancers of the brain and central nervous system).

GANP and Breast Cancer

Dr Yasuhiro Sakai (also at Fujita Health University School of Medicine) and Dr Kuwahara have studied the biological indicators of breast cancer resulting in cutting-edge findings. The BReast CAncer (BRCA) genes have developed, even within the general population, a strong connection to breast cancer, with mutations in these genes being associated with hereditary cancer of the breast. While screening for the presence of BRCA mutations does assist some potential breast cancer sufferers, the vast majority of breast cancer cases arise sporadically. In other words, there is no family link that can be determined via BRCA screening.

Sporadic breast cancer is a complex condition associated with a myriad of biological processes likely combining to implement its initiation. Dr Sakai and Dr Kuwahara have investigated GANP and how it affects the development of sporadic breast cancers. We know that GANP has an important immunological role. However, the two researchers understand that the role that GANP plays in DNA damage may contribute to the initiation of a number of malignancies, including breast cancer tumourigenesis. Dr Sakai and Dr Kuwahara are combining their specialist areas of tumour pathology and immunology in the quest for clarity in understanding the progression of sporadic tumours and the role of GANP.

Using a mouse model, Dr Sakai and Dr Kuwahara have shown that a deficiency in GANP results in the development of mammary tumours. They note that although there are clear differences between the mouse and human models, this work is a vital step towards a novel therapeutic for breast cancer tumours.

One of the current aims of Dr Sakai and Dr Kuwahara is to determine the molecular processes that result in lowered expression of the *ganp* gene. It is largely accepted that excess oestrogen plays a key role in carcinogenesis of breast tissue, via damaging cellular DNA. GANP appears to operate as a tumour suppressor in these circumstances, and maintaining the expression of GANP may be pivotal in reducing the DNA damage induced by oestrogen in the breast tissue which

results in tumour initiation. It appears that GANP has an antioncogenic effect on breast carcinogenesis.

The Immune System Role of GANP

GANP is well known for its role in B-cell affinity maturation, including somatic hypermutation and double-stranded DNA break formation in the variable regions of antibodies. Somatic hypermutation is the mechanism by which the immune system changes to manage its response to novel antigens.

A further immune system role for GANP is in inducing B-cells to change to macrophages, a process called transdifferentiation or producing a mixed phenotype cell that combines B-cell and macrophage characteristics. This is important in some cancers such as Hodgkin lymphoma which shows a B-cell/macrophage biphenotype and where GANP is overexpressed.

Hodgkin Lymphoma Initiation and GANP

Hodgkin lymphoma is a cancer of the lymphoid system in which B-cells change to larger lymphocytes with altered characteristics. In some cases the B-cells lose their markers and can simultaneously acquire characteristics of macrophages, such as secreting macrophage-specific cytokines (specialised proteins of the immune system) and becoming phagocytic, that is to say, develop the ability to engulf foreign materials.

Dr Sakai and Dr Kuwahara's work using mouse models shows that GANP regulates the switching process between B-cells and macrophages, and that overexpression of GANP may result in Hodgkin lymphoma, which shows the mixed B-cell/macrophage phenotype.

Development of a Murine Model of Hodgkin Lymphoma

Hodgkin lymphoma is difficult to research from a cellular pathology perspective due to a lack of suitable animal models. While Hodgkin lymphoma is thought to originate from lymphoid germinal centre B-cells, the cellular characteristics of the tumour cells demonstrate the characteristics of both B-cells and macrophages.

In a *lyn*-deficient mouse model, similar B-cell/macrophage cells have been identified. The *lyn* gene product is involved in transferring signals from some important B-cell molecules. GANP is a target for *lyn*-mediated signalling in germinal centre B-cells for the maturation of high affinity antibodies.

Their work shows that GANP has a pivotal role in the B-cell changes which result in the B-cell/macrophage biphenotype that corresponds to Hodgkin lymphoma. Dr Sakai and Dr Kuwahara have identified the putative region of the *ganp* gene that is targeted by *lyn*, the PU.1 binding site. Critically, this PU.1 region regulates both B-cell and macrophage differentiation, and consequently, PU.1 may exert control over the reprogramming that occurs to produce the B-cell/macrophage mixed phenotype cells. They found that a low concentration of PU.1 leads the B-cell/macrophage precursor cells down the B-cell pathway, whereas a high concentration of this product promotes macrophage differentiation.

Overexpression of GANP is found in a number of human blood and lymphoid cancers, including cells of Hodgkin lymphoma. This indicates a regulatory role for GANP in the 'reprogramming' of B-cells to macrophage-like cells in these cancers, and GANP is detectable in human Hodgkin lymphoma cells.

For the mouse model, the researchers found that *Ig-ganp* transgenic mice spontaneously develop Hodgkin lymphomas, which show similar properties to human B-cell/macrophage biphenotypic cells, making this novel murine model an excellent option for studying cytopathology of Hodgkin lymphoma.

Future Work

In addition to the well-characterised role in immunohaemotology for GANP, Dr Sakai and Dr Kuwahara have demonstrated a role for GANP in tumourigenesis. Using animal models, some of which they have developed for this purpose, they show that GANP expression is upregulated in some tumours, and that it may play an important role in inducing tumourigenesis.

It is critical to note that, depending on tumour type, GANP may be either up- or downregulated. Given this situation, Dr Sakai and Dr Kuwahara stress the absolute requirement to define the differential effects of GANP on tumourigenesis of blood cell malignancies, where it is overexpressed, and in breast cancer and glioma, where it is underexpressed.

In conclusion, the in-depth analysis of the molecular pathways and interactions of GANP and its regulators by Dr Sakai and Dr Kuwahara has allowed the identification of areas of key interest with the potential to inform future therapeutic options for cancer.

Meet the researchers

Yasuhiro Sakai, MD, PhD
Senior Assistant Professor
Department of Diagnostic Pathology
Fujita Health University School of Medicine

Dr Yashiro Sakai, a board certified pathologist, completed his MD in 2009 and then undertook a PhD at the Shinshu University Graduate School of Medicine. Upon completion of his PhD, he completed a residency in surgical pathology at the University of Fukui Hospital, and is now a Senior Assistant Professor in the Department of Diagnostic Pathology, Fujita Health University School of Medicine. Dr Sakai has a wide range of interests, including the role of artificial intelligence in pathology. Dr Sakai's major focus is currently in a collaborative study, incorporating both pathology and immunology, to investigate the role of GANP and associated molecules, with a focus on the molecular and biological pathways that contribute to the regulation of tumourigenesis in haematological and other tumour types.

CONTACT

E: ya-sakai@fujita-hu.ac.jp
W: https://researchmap.jp/7000023712?lang=en

Kazuhiko Kuwahara, MD, PhD
Assistant Professor
Department of Diagnostic Pathology
Fujita Health University School of Medicine

Dr Kazuhiko Kuwahara completed his MD in 1989 at the Saga Medical School, and thereafter undertook a series of research positions, culminating in an Assistant Professorship at the Fujita Health University School of Medicine. Dr Kuwahara's early work focused on immunology research, and he originally identified the protein GANP as a B-cell modulator, which is now the focus for a collaborative tumourigenesis project. The collaborative project has shown that GANP is both up- and down-regulated in various cancers, and the focus of the work is now investigating the balance necessary to aid in the treatment of breast cancer while ensuring that lymphocytic cancers are not induced.

CONTACT

E: kazukuwa@fujita-hu.ac.jp
W: https://researchmap.jp/read0068817?lang=en

FURTHER READING

Y Sakai, S Phimsen, S Okada, K Kuwahara, The critical role of germinal center-associated nuclear protein in cell biology, immunohematology, and hematolymphoid oncogenesis, Experimental Hematology, 2020, 90, 30–38.

Y Sakai, A Rezano, S Okada, et al, A novel cytological model of B-cell/macrophage biphenotypic cell Hodgkin lymphoma in *ganp*-transgenic mice, Cancers, 2020, 12, 204.

S Fujimura, Y Xing, M Takeya, et al, Increased expression of germinal center-associated nuclear protein RNA-primase is associated with lymphomagenesis, Cancer Research, 2005, 65, 5925–5934.

FUJITA HEALTH UNIVERSITY

THE MIT SUPERFUND RESEARCH PROGRAM: STUDIES ON CLEANING UP GENES AND THE ENVIRONMENT

In the United States, there are thousands of industrial sites contaminated by the irresponsible disposal of chemical waste. The higher than expected frequency of cancer cases near these sites has caused alarm, since many of the chemical contaminants found at these sites have been linked to the development of long-term health problems, including cancer. As leaders of the Massachusetts Institute of Technology Superfund Research Program, **Dr Jennifer Kay** (Research Scientist and Research Translation Director) and **Professor Bevin Page Engelward** (Program Director) are using their expertise to investigate the genetic factors that influence susceptibility to adverse health outcomes following exposure to environmental chemicals.

Widespread Contamination

The improper storage or dumping of hazardous waste from industry and mining has led to thousands of contaminated sites across the United States. Lead, asbestos, and a host of other harmful chemicals have been detected at these sites; in many cases, these chemicals have entered the air or water supply, potentially impacting the health of citizens in the surrounding area.

In 1980, the United States Congress passed the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), creating the Superfund Cleanup Program. The ongoing program aims to clean up sites heavily contaminated with hazardous materials across the US, eventually returning these sites to productive use.

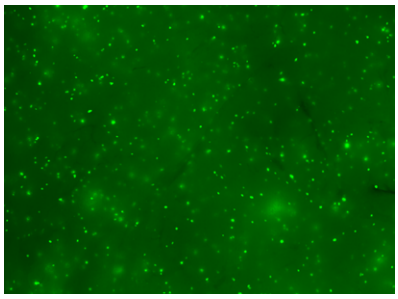
To support these cleanup efforts, the National Institute of Environmental Health Sciences started the Superfund Research Program in 1987, with the aim to support research into developing methods to detect hazardous substances, assessing the impact of these substances on health, and mitigating the risk that these chemicals pose to the general population.

Novel Solutions

Dr Jennifer Kay (Lead Scientific Researcher and Director of Research Translation) and Professor Bevin Page Engelward (Director) have played key roles in the Massachusetts Institute of Technology (MIT) Superfund Research Program, a program set up in 2017 to bring together multidisciplinary researchers from nearly a dozen MIT laboratories to address the issue of Superfund sites in Massachusetts

and Maine. Both researchers have an extensive background in studying DNA damage and cancer, and together with Professor Leona Samson, they lead a project within the program to investigate the interaction of these chemicals with the genome.

The MIT Superfund Research Program is made up of projects that use cutting edge biological research and engineering to tackle the problems associated with local Superfund sites. Other major projects in the program include measuring point mutations



Mutant cells fluoresce green in a RaDR-GFP mouse pancreas. Credit Jennifer Kay. The cover image features yellow fluorescent mutant cells.

and studying systems-level responses to environmental contaminants, creating sensors for measuring levels of contaminants in air and water, and studying flux between sediments and water to gauge how these chemicals can travel through the ecosystem.

MIT also engages with affected communities and local, state and national agencies. The areas around some of these sites include marginalised and underprivileged communities – thus, addressing the problems caused by these chemicals represents a matter of environmental justice.

Cancer Chemicals

The MIT Superfund Research Program is focused on two classes of chemical contaminants detected at a number of contaminated sites: *N*-nitrosamines, particularly *N*-Nitrosodimethylamine (NDMA), and polycyclic aromatic hydrocarbons (PAHs). The International Agency for Research on Cancer has classified several PAHs as Group 1 known human carcinogens, and has classified NDMA as Group 2A probable carcinogen as there is strong evidence that it causes cancer in animal models and is expected to do the same in humans.

PAHs have been found in the Loring Airforce Base Superfund site located near Native American Tribes living in Maine, and both NDMA and PAHs are present in Superfund sites adjacent to communities in the Mystic River Watershed, north of MIT's home city of Cambridge.

People are exposed to PAHs and NDMA not only as a consequence of contamination of the environment, but also through other routes. Of striking importance is the recent discovery that NDMA is present in commonly used drugs, including Zantac (for acid reflux) and Valsartan (a blood pressure medication). The levels in these medications are quite a lot higher than the levels in the environment, raising concerns about the long term health consequences of exposure to NDMA via commonly used drugs.

Shedding Light on the Problem

Previous studies have shown that NDMA can cause changes to DNA, the genetic code of the cell. Most of the time, proteins in the cell will repair this damage, or the cell will undergo a process called apoptosis, which kills the cell in a controlled manner. However, multiple mutations, especially in genes that control cell division or cell lifespan, can cause the cell to divide in an uncontrolled manner, forming the basis of cancer.

Dr Kay and Professor Engelward hypothesise that the expression of two DNA repair proteins, the alkyladenine DNA glycosylase (AAG) and the ^{06}-methylguanine DNA methyltransferase (MGMT), modulate the ability of NDMA to produce cancers in the liver. It is estimated that over 80% of NDMA-induced DNA damage is repaired by these two proteins.

To understand the role of AAG and MGMT in mitigating the effects of NDMA, the Engelward laboratory created a line of mice genetically modified to contain a DNA reporter known as RaDR-GFP. RaDR-GFP acts as a sensor to let the researchers see if the mouse's DNA has been damaged and improperly repaired. It contains a truncated version of a gene for a green fluorescent protein; in cases where the DNA in the RaDR-GFP reporter has been broken, proteins in the cell will try to repair the break and realign each strand in a process known as homologous recombination.

As the gene contains two repeated sections, the recombination process will sometimes misalign the DNA strands, altering the genetic sequence and producing a complete copy of the gene. This completed copy then allows the cell to produce a green fluorescent protein that can be detected in tissues by microscopy, with fluorescent green dots in the tissue showing where these repair events have taken place – the more DNA breaks and subsequent recombination events, the more spots of green in the tissue. Since green cells have undergone a permanent rearrangement of their genetic sequence, fluorescent cells are by definition mutated, allowing researchers to compare the frequency of these types of mutations in different mice. In addition, the progeny of RaDR-GFP mutant cells also contain the full gene for green fluorescence, and so these animals also allow for analysis of proliferation of mutant cells.

Professor Samson's laboratory, with support from Bevin Engelward as a graduate student, also developed

genetically modified mice that lack either Aag or Mgmt. The Samson laboratory also created mice that contain additional genes for Aag that cause overexpression of the protein (AagTg). By using RaDR-GFP mice with these genetic backgrounds, the team will be able to determine how the levels of Aag and Mgmt affect the capability of these cells to repair the genetic damage caused by NDMA.

So far, the group has begun looking at the short-term and sustained responses of these animals to NDMA, and they are in the process of monitoring animals for a 9 to 12-month period in order to study the susceptibility of the Aag, Mgmt and AagTg animals to cancer. Through partnering with other researchers in the Superfund Research Program, the group will also analyse tissue from these mice using proteomics and transcriptomics techniques to understand how NDMA exposure and consequent mutations might change gene expression across the whole genome, and how this might drive cancer. Ultimately, the goal is to be able to identify people who are at an increased risk of cancer if exposed to chemicals that damage DNA.

Measuring DNA Damage

Professor Engelward's group has had success using a similar mouse model to study links between inflammation and cancer. In a study published in *PLOS Genetics* in 2015, the group used an analogous fluorescent mouse model based on an equivalent genetic construct for detecting DNA damage-induced homologous recombination. In this case, the cells of the mice contained truncated copies of a gene for a yellow fluorescent protein rather than green.

The group found that although acute inflammation did not increase the instances of homologous recombination, the period after inflammation where cells proliferate and replace damaged tissue carries with it an increased risk of mutations. Simultaneous inflammation events during this proliferation period can cause double-stranded DNA breaks and homologous recombination; this has significance for people with chronic inflammatory conditions in which the risk of cancer is higher.

In this study, the team also found more fluorescent mutant cells in the pancreas of mice treated with the carcinogen N-nitros-N-methylurea (MNU), an alkylating agent closely related to NDMA. Interestingly, cell proliferation following inflammation significantly potentiated the mutagenic effects of MNU. This suggests that chemicals that can cause both inflammation and DNA damage, a group that includes NDMA and other Superfund chemicals, may be particularly adept at causing mutations and initiating cancer.

Additional Tools

While these mice can provide the researchers with information on the frequency and location of these mutations, the group also wishes to understand the effect of NDMA on the overall health of the cells. In order to do this, Professor Engelward's team has developed a novel toxicity assay in the form of the MicroColonyChip, or uCC.

The μCC is a gel-based chip containing thousands of tiny microwells, microscopic indents that small colonies of cells can be grown in. Through treating the colonies of cells with chemicals and imaging them using a fluorescent microscope, the platform allows the researchers to closely study the reaction of small colonies of cells to environmental chemicals *in vitro*.

This novel technique is a significant advance for measuring toxicity. It is more sensitive than commonly used toxicity assays, and less susceptible to artefacts and errors than other high-throughput methods. The assay measures the size of cell colonies and uses the distribution of colony size to measure cell survival, the first time this readout has been used as a high-throughput measure of cytotoxicity.

In addition to the uCC, the Engelward laboratory has also developed technologies for detecting and quantifying DNA damage. Specifically, the CometChip and the HepaCometChip enable the detection of DNA strand breaks and bulky lesions, respectively. The CometChip is now being broadly distributed by Trevigen, which is part of BioTechne, Inc.

Looking to the Future

Having developed these cutting-edge tools and started work on looking at the short-term effects of NDMA exposure on cell proliferation and DNA damage, the group hopes to study the long-term impact of Aag and Mgmt on cancer susceptibility. The findings from Dr Kay and Professor Engelward are consistent with previous work showing that NDMA and related compounds are potent mutagens, and future work will identify genetic predispositions and genome-wide changes caused by this compound.

With contemporary studies showing traces of NDMA in food, medications, and in contaminated water (in some cases adjacent to Superfund sites), Dr Kay and Professor Engelward's research will be important in identifying at-risk populations, and understanding the negative effects of NDMA on human health. Importantly, ongoing research is also focused on mitigating the impact of previous exposure to NDMA, with the goal of suppressing the risk of cancer for people who have been exposed to NDMA. For all of their projects, results will be shared with those communities affected by these chemicals to inform those who will benefit from the research the most. Ultimately, Dr Kay, Professor Engelward, and their colleagues in the MIT Superfund Research Program aim to impact public health via disease prevention and disease mitigation, as well as by contributing to public health via engagement with their local communities.

Meet the researchers

Dr Jennifer Elizabeth Kay
MIT Superfund Research Program
Department of Biological Engineering
Massachusetts Institute of Technology
Cambridge, MA,
USA

Dr Jennifer Elizabeth Kay completed her PhD in Professor Engelward's lab at the Massachusetts Institute of Technology in 2017, where she is now a postdoctoral research fellow. From 2017, she has also been the Research Translation Core Leader at the MIT Superfund Research Program. Her work with the translation core involves partnering with government agencies and communicating scientific findings from MIT Superfund projects to the wider community. Dr Kay's research focuses on analysing genetic susceptibility to N-nitrosamine-induced DNA damage and studying short and long-term consequences of chemical exposure in mice with modified DNA repair mechanisms.

CONTACT

E: jekay@mit.edu
W: http://superfund.mit.edu/people/Jennifer-kay
@MIT_SRP, @justjkay

FUNDING

National Institutes of Health, National Institute of Environmental Health Sciences
Superfund Basic Research Program, National Institute of Health, P42 ES027707
MIT Center for Environmental Health Sciences, P30-ES002109

Professor Bevin Page Engelward
Director, MIT Superfund Research Program
MIT Center for Environmental Health Sciences
Department of Biological Engineering
Massachusetts Institute of Technology
Cambridge, MA
USA

Professor Bevin Page Engelward graduated from Yale University and then received her doctoral degree from the Harvard School of Public Health. In 1997 she became an Assistant Professor in the Division of Bioengineering and Environmental Health at the Massachusetts Institute of Technology. She is now a Professor in the Department of Biological Engineering and the Director of the MIT Superfund Research Program. Professor Engelward's research focuses on how genetics and DNA repair mechanisms modulate disease susceptibility, with an emphasis on the development of novel tools for studying exogenously-induced genetic changes in animals and human cells.

CONTACT

E: bevin@mit.edu
W: http://engelward-lab.mit.edu
@MIT_SRP

PUBLIC HEALTH ADVOCACY IN THE FIGHT AGAINST BREAST CANCER

Health advocacy is an often-overlooked part of the work of public health specialists, but in many ways, it can be argued to have the greatest long-term impact on the population's health. Here we look at the impressive work of **Professor Janet Gray** (Vassar College) and Public Policy Strategist **Ms Nancy Buermeyer**, working with colleagues at Breast Cancer Prevention Partners, the leading USA science-based policy and advocacy organisation to focus on preventing breast cancer by eliminating exposure to toxic chemicals and radiation linked to the disease.

Public Health Advocacy and Disease Prevention

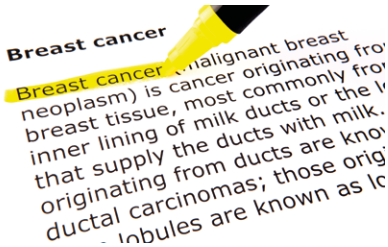
The discipline of public health is focussed on population health and has social justice at its heart: working to address the fundamental determinants of poor health through reducing health, social and economic inequalities; improving access to healthcare and healthy environments; and encouraging everyone from individuals to governments to 'do the right thing' to ensure people have health and well-being as fundamental rights.

The role of public health advocacy is, therefore, to be a proponent for vital health issues, new research, or to highlight risks to health, often as part of a political or legislative process. However, it may also be educating the public about community, social or environmental factors that may compromise health-related conditions, and working to mitigate those factors.

Public health advocates are skilled in utilising and communicating about complex health and research information, alongside knowledge of policy procedures to shape future legislative and regulatory approaches. As such, public health advocacy has delivered some of the largest stepwise improvements in the public's health in the past 150 years, including the introduction of urban sanitation (started in London to reduce cholera in the mid-19th century), workplace health and safety legislation, and the banning of smoking in public places in the 1990s. These are all good examples that have changed established social norms and values, and significantly prevented illness and death.

Breast Cancer Prevention Partners (BCPP)

Professor Janet Gray (Vassar College) is a scientific advisor for and Senior Policy Strategist Ms Nancy Buermeyer works for Breast Cancer Prevention Partners (BCPP). Founded in 1992, BCPP is the USA's leading science-based non-profit organisation dedicated to the prevention of breast cancer by working to eliminate our exposures to toxic chemicals and radiation. In its health advocacy model, BCPP works to educate the public about links between



environmental toxicants, many of which are found in common products used daily, and risk for developing breast cancer. It also engages in both market-based and policy initiatives aimed at eliminating exposures to harmful chemicals.

In the USA, breast cancer incidence has risen by 40% since the 1970s and affects one in eight women during their lifetimes. The rise cannot be explained by genetic factors alone, and therefore BCPP's mission is to raise awareness of, and take action to reduce exposure to, the many environmental factors, specifically toxic chemicals and radiation, that are contributing to breast cancer. Here we explore some of the contributions by Professor Gray and Ms Buermeyer to the scientific understanding of these issues and BCPP's advocacy work, including

its ground-breaking breast cancer prevention policy agenda.

State of the Evidence

Professor Gray is a leading academic figure in breast cancer prevention, compiling the scientific evidence that provides the foundation for advocacy for progressive chemical policy reform to address chemical exposures to carcinogens and other toxicants in consumer products and our living and working environments.

Working with BCPP colleagues, Professor Gray led the team that published the latest version of the BCPP's *'State of the Evidence 2017: an update on the connection between breast cancer and the environment.'* The regularly updated review is a centrepiece of BCPP's work to raise awareness of their agenda and examines the continually growing and compelling scientific evidence linking radiation and chemical exposures to the increase in breast cancer incidence. The findings are both impressive and alarming.

The evidence cited leads to the inescapable conclusion that many common, everyday products and their by-products contain toxicants that can increase the risk of breast cancer. The report focuses on seven major areas of concern for BCPP: 1) hormones in pharmaceutical agents and personal care products; 2) endocrine disrupting compounds (EDC); 3) hormones in food (natural and additives); 4) non-EDC industrial chemicals; 5) tobacco smoking (active and passive); 6) shift work (light-at-night and melatonin); and 7) radiation.

There is a particular focus on reducing exposures from gestation through to early adulthood, as exposure-induced changes in genetic, epigenetic (relating to non-genetic influences on gene expression) and physiological processes in the developing mammary system can lead to a greater risk of developing breast cancer in later life.

Advocacy in Action and Policy Successes

As a Senior Policy Strategist at BCPP, Ms Buermeyer plays a major role in developing and implementing the public health policy and advocacy programmes for the organisation. This begins with utilising the evidence produced from the research described previously, to educate and inform the public and policymakers of the realities of breast cancer risks.

The comprehensive BCPP website (https://www.bcpp.org/about-us/) is a fantastic resource for information and guidance to support anyone interested in the subject (whether professionals, policymakers or members of the general public), to understand the issues and to be empowered to 'take action' for change. Accurate knowledge, when in the public arena, can be a powerful tool to change public opinion, to put pressure on policymakers and to counter the misinformation and misleading claims of those with a vested interested in maintaining the status quo.

An excellent example of advocacy in practice is the 2018 BCPP report *'Right to know: exposing toxic fragrance chemicals in beauty, personal care and cleaning products.'* In this report, BCPP set out to expose the hidden

toxic chemicals in products in daily use, a situation enabled by the lack of USA consumer-protection legislation requiring the labelling of ingredients for cleaning products or fragrance in personal care products and reliance on 'self-regulation' by the manufacturers.

The testing of well-known products, especially those marketed at vulnerable populations (e.g., women of colour, children), endorsed by celebrities or marketed as 'good for the environment' or 'green' products, were featured to capture the greatest attention. A 'top 10' list of named commercial products, ranked as the most hazardous (defined as containing 'the highest number of chemicals linked to cancer, hormone disruption, developmental and reproductive toxicity and respiratory effects') was topped by a children's shampoo marketed to children of colour, and included products endorsed by celebrities Taylor Swift and Jenifer Lopez, and designer Marc Jacobs. The report highlights the weak USA Food and Drug Administration laws and oversight that leave consumers largely unprotected.

For the companies exposed, the report and subsequent widespread news coverage were highly embarrassing and damaging to their reputation, undermining the carefully crafted deceits of their products being 'gentle', 'natural' and 'sustainable', for example. This information, leading to public awareness and action, can ultimately lead companies to reformulate their products to remove toxic ingredients.

In addition to press coverage, evidence from this report was important for the passage of the California Cleaning Product Right to Know Act, a bill that was shepherded through the California legislature by BCPP, in partnership with its allies. This is currently the only law in the world that requires manufacturers of cleaning products to disclose the chemicals in their products, on product websites this year and on the product packaging by next year. In an example of ongoing work at the governmental level, in June 2020, BCPP and co-sponsors Black Women for Wellness, CALPIRG and the Environmental Working Group were successful in lobbying the California State Assembly to approve the historic 'Toxic-Free Cosmetics Act' to ban a dozen highly toxic chemicals which are already banned in the EU, from personal care products sold in the state. If approved by the state senate, it will be the first such law passed in the USA.

Moving Forward: Combining Science, Community Wisdom and Advocacy

Over the past 4 years, Ms Buermeyer led the BCPP team in developing the ground-breaking *'Paths to Prevention: the California Breast Cancer Primary Prevention Plan',* which was launched in September 2020. With input from academics, government regulators, non-profit organisations and impacted communities, Ms Buermeyer describes the 'first of its kind' report as, 'a labour of love that combined science and community wisdom to identify 23 factors impacting breast cancer risk and provide recommendations on research to better understand them and, most importantly, policy interventions to reduce breast cancer risk in California.'

Paths to Prevention is unique in several ways:

- Focuses exclusively on primary prevention – stopping the disease before it starts.
- Calls for systemic solutions to decrease breast cancer risk factors rather than focusing on individual actions.
- Weaves together the wisdom of local communities and scientific evidence.
- Considers these issues and their potential solutions through a social justice lens to ensure everyone benefits from policy change.

A key element of developing the Prevention Plan involved BCPP staff travelling across the state to meet with members of marginalised, environmental justice communities who bear a disproportionate burden of toxic exposures. The wisdom and learnings from these community meetings are deeply embedded in the Prevention Plan.

The *Paths to Prevention* will provide a blueprint for BCPP's work going forward. While focused on the state of California, the Prevention Plan will hopefully become a model for states across the USA. While focused on breast cancer prevention, the recommended policy interventions will reduce the risk of numerous other health issues. This is fantastic evidence that the work of public health advocates such as Professor Gray and Ms Buermeyer is bringing about meaningful population-level changes to reduce the unnecessary deaths and illness caused by breast cancer.

Meet the researchers

Professor Janet Gray
Vassar College
Poughkeepsie
New York, NY
USA

Professor Janet Gray began her scientific career with a doctorate in Behavioural Neuroscience at the University of Massachusetts at Amherst in 1980. Since then, she has been at Vassar College, first as a National Institute of Mental Health post-doctoral fellow in the Department of Biology and then as a member of the faculty in the Department of Psychology. She is now is Professor Emerita in Psychology/Neuroscience. For the past 20 years, she has focused her non-teaching work on the relationships between environmental toxicants and risk for developing breast cancer. For ten years, she has served on the National Board of Directors for the Breast Cancer Prevention Partnership and was a founding member of its Science Advisory Panel. Professor Gray currently serves on the board of Clean and Healthy New York.

CONTACT

E: grayj@vassar.edu
W: https://www.vassar.edu/

Ms Nancy Buermeyer
Breast Cancer Prevention Partnership
San Francisco, CA
USA

Ms Nancy Buermeyer graduated from the University of Pittsburgh with a Bachelor of Science in Biology and earned a Master's degree in Biological Oceanography from the University of Connecticut. She now works as the Senior Policy Strategist at the Breast Cancer Prevention Partnership (BCPP) at a state and federal level to advance public policy to reduce exposures to toxic chemicals, including ingredient transparency, funding for environmental health programs and updates to the Toxic Substances Control Act. Before joining BCPP, Ms Buermeyer spent over 20 years in Washington DC advocating for numerous causes, including civil rights for women and the LGBT community.

CONTACT

E: nancy@bcpp.org
W: https://www.bcpp.org/about-us/

FUNDING

California Breast Cancer Research Program (CBCRP)
Generous individual donors to the Breast Cancer Prevention Partnership's Science Leadership Circle

FURTHER READING

N Buermeyer, C Engel, J Nudelman, S Rasanayagam, H Sarantis, Paths to Prevention: The California Breast Cancer Primary Prevention Plan, Breast Cancer Prevention Partners, September 2020, Available at https://www.bcpp.org/resource/california-breast-cancer-primary-prevention-plan/

MC White, MMHE Kavanaugh-Lynch, S Davis-Patterson, N Buermeyer, An Expanded Agenda for the Primary Prevention of Breast Cancer: Charting a Course for the Future, International Journal of Environmental Health Research, 2020, 17(3), 714, doi:10.3390/ijerph17030714.

J Gray, S Rasanayagam, C Engel, J Rizzo, State of the evidence 2017: An update on the connection between breast cancer and the environment, Environmental Health, 2017, 16, 94.

Breast Cancer Prevention Partners
Exposing The Cause Is The Cure

BREASTFEEDING TO REDUCE BREAST CANCER RISK, ESPECIALLY IN AFRICAN-AMERICAN WOMEN

Breastfeeding is widely accepted as being the best option for babies' health and development. **Dr Bhuvaneswari Ramaswamy**, working with **Dr Sarmila Majumder** at Ohio State University Comprehensive Cancer Centre, USA, notes that breastfeeding also has long-term benefits for mothers, particularly in reducing their future breast cancer risk. Here, we explore their work to understand the connection between breastfeeding and cancer and how to reduce the risks for a particularly vulnerable population.

African-American Women: A High-Risk Population

Dr Bhuvaneswari Ramaswamy is a breast medical oncologist and physician-scientist at the Ohio State University Comprehensive Cancer Centre, Columbus, USA. Working alongside Research Scientist Dr Sarmila Majumder, their research interests are focussed on improving the lives of women with breast cancer, and particularly those from an African-American background, who are disproportionately affected by an aggressive subtype of breast cancer and face higher mortality from breast cancer than the general population.

Epidemiological evidence from the USA has identified clear differences in the incidence, death rates and length of survival after treatment of breast cancer among different ethnic groups. Although the overall incidence of breast cancer is lower among African-American women when compared to Caucasian women, the proportion of younger African-American women affected with breast cancer is almost double that of Caucasian women. The five-year survival rate for African-American women (77%) is significantly lower than that for Caucasian women (90%), and the age-adjusted mortality rate for African-American women is the highest for any ethnic group studied.

Breast cancer in African-American women has different characteristics compared to Caucasian women. In general, the cancer has an earlier onset, a poorer clinical outcome and an aggressive tumour phenotype, a three times higher frequency of the aggressive triple-negative breast cancer (TNBC), so-called because the cancer cells lack oestrogen, progesterone and HER-2 receptors and will not respond to hormone therapy.

Drs Ramaswamy and Majumder, along with their team, are investigating the underlying biology of how lifestyle factors impact breast cancer rates and outcomes.

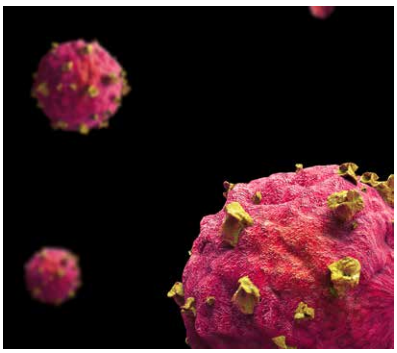
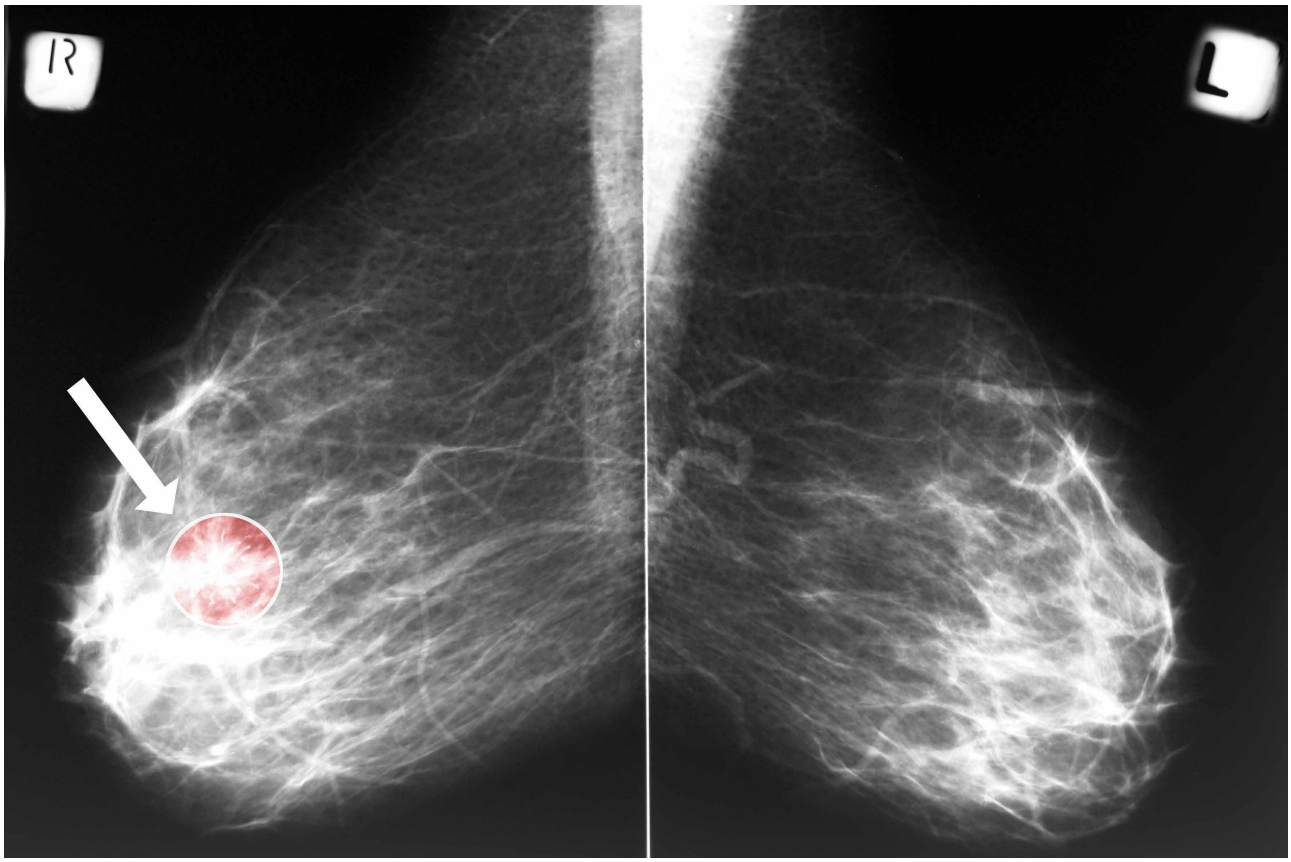


Illustration of Cancer Cells

Lifestyle Determinants of Breast Cancer

A number of lifestyle behaviours are known to increase breast cancer rates. Access to health care, obesity and breastfeeding are well-established determinants that are connected epidemiologically with ethnic differences in behaviour and characteristics.

An interesting aspect of breastfeeding is that prolonged breastfeeding reduces the risk of TNBC. This is the subtype of breast cancer that is more common in



Mammogram of Breast Cancer

African-American women and is partly responsible for the higher mortality faced by these women. Estimates suggest that breastfeeding prevents 20,000 breast cancer deaths annually based on the current breastfeeding rates. Critically, however, African-American women have significantly lower rates of breastfeeding and a higher prevalence of obesity than the Caucasian population.

The links with obesity and breast cancer risk have been well-established through multiple meta-analyses. Women with a BMI >30kg/m2 are at significantly higher risk of developing premenopausal TNBC and basal-like breast cancer. Early research findings by the Ohio team suggest that diet-induced obesity may accelerate precancerous changes upon short-term breastfeeding and this is discussed in more detail below.

To better understand the behaviour and prior knowledge of breastfeeding mothers on this issue, Dr Ramaswamy's team performed a national survey of 724 women regarding the information they had received about breast cancer risk reduction during antenatal breastfeeding counselling, and whether this influenced their decision to breastfeed their child. Over half of the women (56%) were aware of the links to reduced breast cancer risk, and a third were influenced by this knowledge to breastfeed and sustained breastfeeding for a longer period. It was concerning, however, that only 16% of women were given this information by a health professional, and so an opportunity to inform women that prolonged breastfeeding was also a benefit to their own health, as well as the baby, was missed.

Confirming earlier findings, more Caucasian women in the survey reported breastfeeding a child for more than 6 months compared to African-American women. Research by the US Centers for Disease Control and Prevention has suggested that there is a lack of social and cultural acceptance of breastfeeding for black women in their communities and workplaces and inadequate support from healthcare professionals. Such findings make it essential that public health interventions to influence modifiable risk factors, such as breastfeeding, are used to change social and individual barriers.

How Does Prolonged Breastfeeding Reduce Cancer Risk?

Dr Ramaswamy is determined to understand the underlying mechanisms by which lifestyle factors, such as lack or short duration of breastfeeding and obesity in African-American women, can increase the risk of breast cancer, and how that risk could be reduced. She has set out the long-term objective of the research team 'to reduce the risk of developing aggressive TNBC, especially for African-American women, by discovering how prolonged breastfeeding protects the breast from this risk, to ultimately address the disparity of breast cancer outcomes among these patients.'

Dr Sarmila Majumder, a molecular biologist and biochemist, is one of the co-investigators and has a specific research interest in the factors regulating breast cancer tumour initiation and progression, metastasis and drug resistance. It is already known that even women who carry a *BRCA1* gene mutation can reduce their risk of developing TNBC if they breastfeed for longer than a year, but why this occurs is unknown and is being explored by this team.

The duration of breastfeeding is clearly a key factor. It is part of the pregnancy-lactation-involution cycle, with involution being the return of the breast tissue to its pre-pregnancy state following its adaption to lactation. Studies of lactation in hunter-gatherer societies suggest that the normal duration of lactation in women is three to four years, and weaning to solid foods is prolonged and gradual (that is, child-led). Changes to the mammary glands are gradual, and the epithelial (surface cell) structures atrophy slowly.

Breast tissue responds differently when breastfeeding involution is abrupt, as opposed to more gradual. Following early studies using mice, the team noticed distinct histological and molecular changes in the mammary glands following abrupt involution. In discussing their initial findings, the team noted: 'We have shown dramatic shifts in the cellular composition of the mammary epithelial cell compartment and global changes in inflammatory markers and, importantly, we have observed precancerous hyperplastic lesions within 120 days postpartum in the [abrupt involution] glands.'

The Response to Abrupt Breastfeeding Involution

Following abrupt involution (after short-term breastfeeding), mammary glands were shown to display significant changes. Luminal progenitor (LP) epithelial cells were seen to increase significantly, coinciding with an increase in cell proliferation, deposition of collagen, the enrichment of oestrogen signalling pathway genes, elf5 gene expression, and a more intensive inflammatory response in the glands that showed signs of precancerous hyperplastic lesions.

Taken together, these changes appear highly significant. The expansion of the LP population and raised levels of elf5 mRNA and proteins are a key finding. All are believed to be closely linked to *BRCA1* associated basal-like breast cancer and TNBC. These cells appear to enrich cellular pathways that sustain cancer stem-cell survival.

Increased stromal collagen is one of the strongest independent risk factors for developing breast cancer and is associated with regions of high breast density. Collagen is a mediator of inflammation, the proliferation of breast epithelial cells and signalling factors known to stimulate the growth of specific cancer progenitor cells, creating a pro-tumorigenic environment.

Following these findings, the research team hypothesise that abrupt involution changes the mammary epithelial cells, and the subsequent prolonged expression of oestrogen receptors and Elf5 in an inflamed cellular environment mediates an increased cancer risk. To further explore these mechanisms and to find new treatment approaches for the prevention of breast cancer, Dr Ramaswamy, Dr Sarmila Majumder and the wider team are pursuing several research strands.

Further Investigation

Gradual involution and weaning clearly provide a protective effect on developing breast cancer. The team are now investigating whether blocking the raised oestrogen signalling, observed in abrupt involution, will block the hyperplastic changes.

The role of Elf5 is also being further elucidated. It is now known that Elf5 is highly expressed in luminal progenitor cells, which are the cell of origin for TNBC, and a higher population of luminal progenitor cells is linked to more aggressive basal cancers. The team are investigating whether the absence of Elf5 impacts on the functions of luminal progenitor cells and the wider precancerous changes induced by abrupt involution. Although it is too early to draw conclusions from the findings so far, the general scientific consensus suggests Elf5 could have a role in the aberrant expansion of the cell type which is the potential cell of origin for the aggressive TNBC subtype.

Finally, the team has begun to validate the histological and molecular changes that they have observed in mice models, using human mammary tissue from women whose breastfeeding ended either abruptly or gradually.

A Clinical and Public Health Collaboration

The in-depth and wide-reaching work led by Dr Bhuvaneswari Ramaswamy and Dr Sarmila Majumder provides a fantastic and novel example of an integrated research model that directly links epidemiological public health research with lab-based research, and aims to find specific causes and treatment solutions that can be translated to humans. Demonstrating clinically that modifiable behaviour, such as prolonged breastfeeding and obesity, can reduce the risk of developing breast cancer, especially for the highly vulnerable African-American population, may provide the incentive for health professionals to more actively encourage and support breastfeeding as a protective measure with a more targeted and focussed approach.

Meet the researchers

Dr Bhuvaneswari Ramaswamy
Ohio State University
Columbus, OH
USA

Dr Bhuvaneswari Ramaswamy is the Professor of Internal medicine at Ohio State University. She was educated in India and England and worked as a registrar in the UK between 1990–1995. She completed her postdoctoral oncology fellowship at Ohio State University, USA, before becoming Assistant Professor of Internal Medicine at Ohio State University in 2006. Dr Ramaswamy is an NIH-funded investigator and extremely active in clinical and translational research on breast cancer and the Principal Investigator for several cooperative groups and investigator-initiated studies investigating the biology of breast tumours.

CONTACT

E: Bhuvaneswari.Ramaswamy@osumc.edu
W: https://cancer.osu.edu/for-cancer-researchers/research/research-labs/ramaswamy-lab

Dr Sarmila Majumder
The Comprehensive Cancer Center
College of Medicine
Ohio State University
Columbus, OH
USA

Dr Sarmila Majumder is a Research Scientist in the Department of Internal Medicine at Ohio State University-Comprehensive Cancer Center. After obtaining her Doctorate at Calcutta University, India in 1990, she joined Ohio State University in 1997. Dr Majumder has a broad background in molecular biology and biochemistry, with specific training in studying gene expression regulation in cancer.

CONTACT

E: Sarmila.majumder@osumc.edu

Mustafa Basree MS (graduate student currently in medical school at the University of Pikeville) and Neelam Shinde MS (senior research assistant). Credit Bhuvaneswari Ramaswamy.

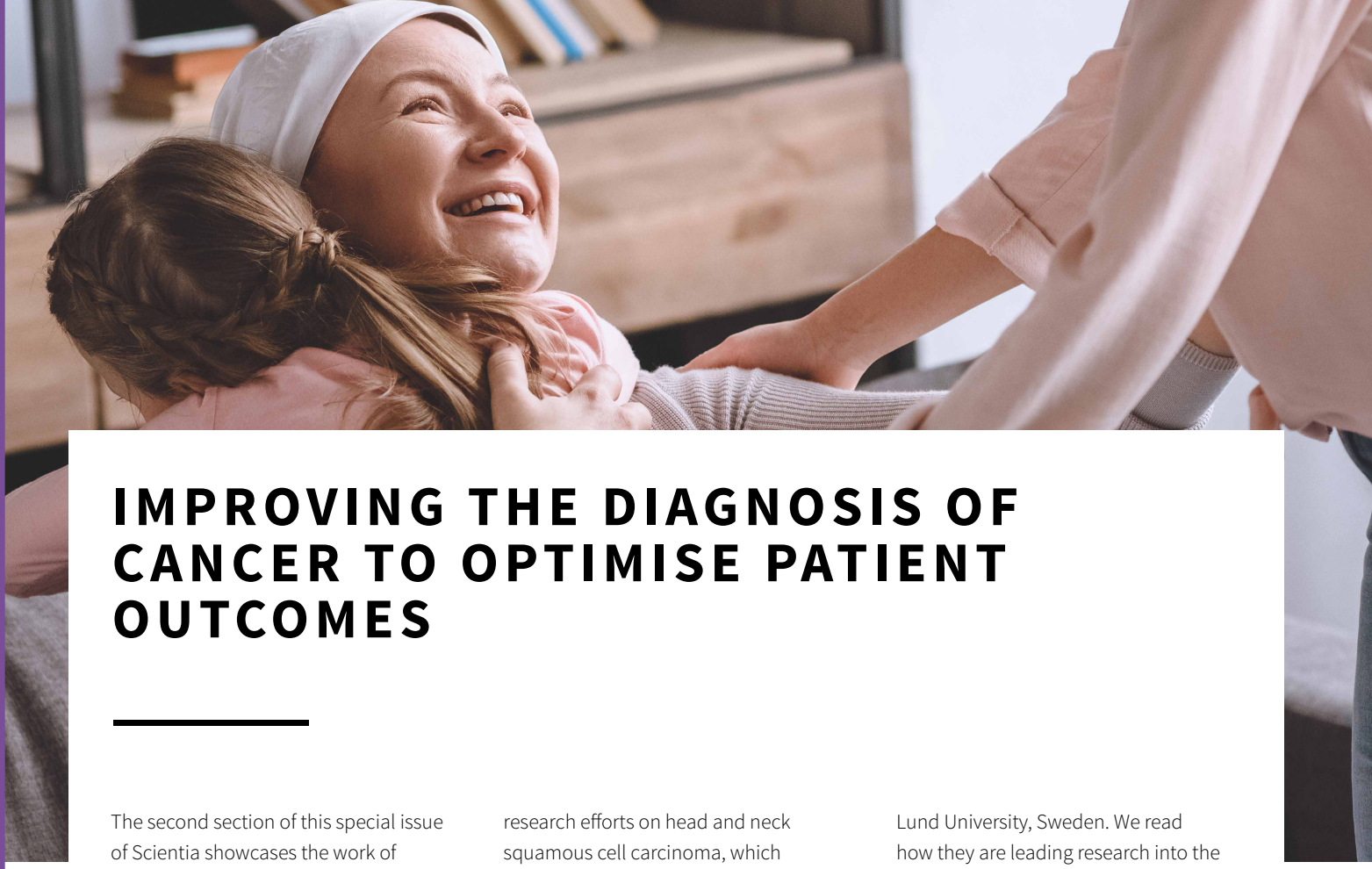
FURTHER READING

MM Basree, N Shinde, C Koivisto, et al, Abrupt involution induces inflammation, estrogenic signaling, and hyperplasia linking lack of breastfeeding with increased risk of breast cancer, Breast Cancer Research, 2019, 21, 80.

A Ganju, A Suresh, J Stephens, Learning, Life, and Lactation: Knowledge of Breastfeeding's Impact on Breast Cancer Risk Reduction and Its Influence on Breastfeeding Practices, Breastfeeding Medicine, 2018, 13(10), doi:10.1089/bfm.2018.0170.

THE OHIO STATE UNIVERSITY

DIAGNOSING CANCER



IMPROVING THE DIAGNOSIS OF CANCER TO OPTIMISE PATIENT OUTCOMES

The second section of this special issue of Scientia showcases the work of researchers dedicated to the accurate diagnosis of cancer at the earliest possible stage. This is an absolutely critical area of research because we know that survival rates are higher when cancer is diagnosed earlier. Sadly, it remains the case that most cancers are detected at later stages of development, with the impact of survival rates being much lower.

We open this section by meeting Dr Anton Iliuk at Tymora Analytical Operations, USA. Traditionally, the diagnosis of cancer has relied on invasive and painful procedures, such as tumour biopsies. We read how Dr Iliuk and his team have optimised a method that allows the isolation of extracellular vesicles (particles released from cells) from plasma, urine, saliva and other biological fluids to allow effective analysis and identification of disease markers that were previously undetectable. This low-cost, non-invasive procedure has exciting applications for the detection of novel biomarkers in cancer as well as other diseases.

Dr Muy-Teck Teh, from Queen Mary University of London, focuses his research efforts on head and neck squamous cell carcinoma, which constitutes around 90% of all head and neck cancers. We read how by better understanding the factors leading to cancer, Dr Teh is leading the development of novel and less invasive detection methods. One of these is the highly accurate and rapid 'quantitative Malignancy Index Diagnostic System', the first diagnostic test for the early detection of oral cancers.

Dr Chengyu Liang, from The Wistar Institute in Philadelphia and her collaborators from the University of Southern California are working to improve the diagnosis of skin cancer (melanoma). Although ultraviolet (UV) radiation from sunlight has been identified as a key risk factor for the development of melanoma, the underlying mechanisms are poorly understood. We read how Dr Liang has identified the function of the UV-irradiation resistance associated gene known as UVRAG and the utility of this as a novel prognostic and predictive biomarker in melanoma.

Professor Carl Borrebaeck and Dr Ulrika Axelsson are Director and Deputy Director, respectively, of the CREATE Health Translational Cancer Centre, Lund University, Sweden. We read how they are leading research into the fascinating topic of whether cancer patients' psychological resilience after their cancer diagnosis may be linked to biomolecular processes, suggesting a mind-body link between the ability to cope psychologically and its impact on cancer prognosis.

Remaining on the topic of breast cancer, we turn to the work of Dr Roy Jafari at the University of Redlands. We read how he is utilising a machine-learning subset of artificial intelligence known as an artificial neural network in his research dedicated to facilitating more improved diagnosis decisions for patients. More specifically, Dr Jafari is developing algorithmic approaches to decision-making that are both data-driven and equitable with the intention of reducing stress on patients and creating a better care experience.

We conclude this section with an exclusive interview with Richard Bahu, Chair of Trustees at the UK research charity Against Breast Cancer. We read of their focus on funding research into the prevention and detection of breast cancer and commitment to the development of much-needed new therapies.

A NON-INVASIVE, LOW-COST PROCEDURE TO DETECT CANCER BIOMARKERS FROM BIOLOGICAL FLUIDS

Liquid biopsies have recently gained attention as sources of non-invasive diagnostic cancer biomarkers. Traditional biofluid sampling methods, however, are onerous and time-consuming and present many limitations including poor sensitivity for biomarkers in low concentrations. Dr Anton Iliuk and his team at Tymora Analytical Operations, West Lafayette, USA, have developed a way to isolate extracellular vesicles from plasma, urine and other biofluids. They now aim to grow their network of collaborators to use their platform for the discovery of protein biomarkers from several cancers, neurodegenerative diseases, and other conditions.

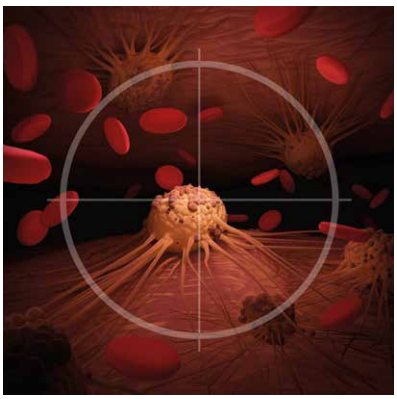
The Case for 'Liquid Biopsies'

The analysis of biological fluids such as plasma, serum, urine, tears, or saliva, have recently emerged as a source of diagnostic biomarkers. Traditionally, the analysis of altered tissue in cancer and other diseases relies on invasive and painful procedures, such as tumour biopsies, which involve the removal of solid tissue samples that are processed for further testing. The use of 'liquid biopsies' offers several advantages, including low costs, faster processing times, their non-invasive nature and the ability to aid early diagnosis. However, they also present some limitations in terms of sensitivity towards the lower biomarker concentrations in biofluids.

Dr Anton Iliuk and his team at Tymora Analytical Operations, West Lafayette, USA, have optimised a method that allows the isolation of extracellular vesicles (EVs) from plasma, urine, saliva and other biological fluids. Their procedure involves subsequent analysis of the enriched EV proteins by mass spectrometry, allowing identification of the disease markers that were previously undetectable. Their method will allow clinical scientists around the world to discover and validate protein biomarkers for diagnostic and monitoring purposes. Dr Iliuk's team has successfully used the platform to discover new EV protein markers from several cancers and other conditions, such as neurodegenerative diseases and diabetes.

Overcoming the Limitations of Biofluid Analyses

A major limitation of methods relying on the handling of biofluids is their poor sensitivity towards the relatively low abundance of biomarkers in the blood, especially at the early stages of the disease. Many techniques currently focus on genetic information, especially gene mutations. While



genome information can be helpful as a diagnostic tool, its interpretation is often complex, as there are many layers of regulation that exist between DNA, RNA and the expression of proteins in health and disease. Protein analysis, on the other hand, provides direct, real-time information about the physiological status of the organism and disease progression. Compared to gene testing, protein analysis also offers a simpler setup and is relatively inexpensive.

Dr Iliuk and his collaborators have recently developed a separation technique that is especially adapted for the identification and detection of new protein biomarkers. By using cell-secreted EVs, also called exosomes, they have found a way to bypass the challenges typically associated with the analysis of biological fluids. EVs formation is initiated by cellular membranes, which shed the vesicles into every biological fluid. The EV membranes protect the proteins inside from degradation by external enzymes, preserving the integrity of the biomarkers that could be detected in diagnostic tests. Interestingly, EV-associated proteins and peptides can be identified well before the onset of symptoms, making them promising disease biomarker candidates for detecting early-stage cancer and other pathological conditions.

Tracking Protein Phosphorylation as a Diagnostic Strategy

EVs provide scientists with a quick snapshot and a good representation of the protein and nucleic acid composition of their parent cells. Among others, one very effective way to monitor disease progression through EV sample testing would involve the analysis of protein phosphorylation, as a direct marker of cellular signalling during disease.

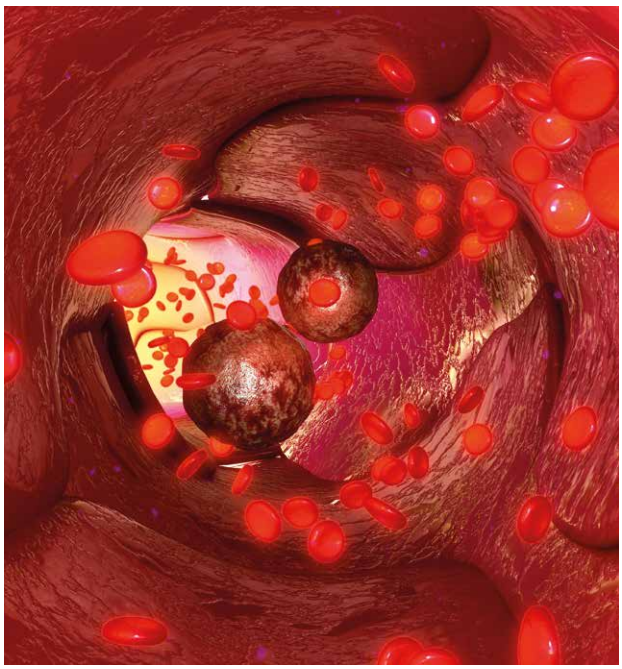
The physiological process of phosphorylation involves modification of proteins by enzymes that aid the attachment of a phosphate group onto the protein structure, also resulting in a modification of its functions. Protein phosphorylation is a key control mechanism for the regulation of cellular pathways. The detection of changes in phosphorylation is of fundamental importance in the understanding of how signalling networks interact and how they are misregulated as a result of disease.

Dr Iliuk proposes that phosphorylation analysis in EVs can be used in two ways: either to detect changes in cancer-induced phosphorylation in EV proteins; or as a tag, whereby phosphorylation acts as an enrichment marker of low abundant proteins that otherwise would not be detectable by traditional methods.

EVtrap: A Novel Method to Detect Multiple Disease Biomarkers

Dr Iliuk and his team have been focusing on the development of a non-invasive and inexpensive approach in the detection and monitoring of bladder cancer, a disease that affects more than 530,000 patients in the USA alone. Their initial goal was to develop an effective disease-monitoring urine test that can be used to examine bladder cancer patients who underwent treatment and require monitoring for cancer recurrence. The monitoring would look at the direct output of cancer cells through their EV shedding in the urine, with the aim of detecting any recurrence in malignancy at an early stage, allowing all necessary follow-up interventions to be carried out rapidly to stop the disease progression.

After years of hard work and determination, Dr Iliuk and his colleagues recently published a study introducing a rapid EV isolation method called EVtrap (extracellular vesicle total recovery and purification). The technique uses magnetic beads that are able to capture the vesicles and allow their separation by the action of a magnetic field. The EVtrap method enables the full screening of significantly higher levels of EV markers, compared to other common approaches of vesicle sampling. Given the high recovery rate of the technique, the isolated vesicles can be used for several types of follow-up analyses. Additionally, the EVtrap approach is fast and simple, allowing EV capture in 10 to 30 minutes, instead of the 6 to 22 hours needed for traditional EV separation methods based on centrifugation.



Direct Biomarker Detection from Urine and Plasma

The team believes that the EVtrap method could soon be widely used by medical practitioners worldwide for the efficient capture of extracellular vesicles from unfiltered human urine samples. The data in the study showed that close to 2,000 unique phosphopeptides could be identified from more than 860 unique phosphorylated proteins using just a 10 mL sample of urine. These data offer hope that urine EV phosphorylated proteins could be used not only for early stage cancer detection, but also as molecular targets in companion diagnostic procedures for the targeted treatment of several types of cancer.

The diagnostic potential of the technique is not limited to the analysis of urine. Dr Iliuk and his collaborators successfully adapted the EVtrap method for the protein phosphorylation analysis of EVs from human plasma. Strikingly, by using EVtrap, they reported being able to identify over 5,500 unique phosphopeptides representing almost 1,600 phosphorylated proteins by using samples of only 1 mL of plasma. This allowed them to analyse plasma samples from patients diagnosed with chronic kidney disease or kidney cancer, identifying dozens of phosphoproteins capable of distinguishing disease states from healthy controls.

The team believes that the EVtrap method can similarly be adapted for DNA/RNA examination. The approach can also be easily automated for high-throughput screening assays and hands-off analyses. EVtrap will allow clinical practitioners to be able to uncover plasma biomarkers even at very low levels. The study paves the way for the development of non-invasive detection of renal cell carcinoma from plasma, even at early stages of the disease.

Financial Considerations and Future Plans

In addition to the advantages outlined above, the EV capturing method is set to offer several steps forward also in terms of financial considerations. Taking bladder cancer as an example, due to the requirements for ongoing monitoring and a large rate of re-occurrence of the disease, more than a million diagnostic biopsies are booked every year in the USA alone, putting a significant financial strain on the healthcare system. Dr Iliuk's EVtrap method offers a new cost-effective approach for the early diagnosis and follow-up monitoring of cancer and other diseases.

The team hopes to scale up the production of multi-biomarker tests, based on the use of 2 to 6 protein biomarkers detectable in the urine EVs of bladder cancer patients. The team argues that using multiple markers in a single test offers a better strategy for disease diagnostics, especially in cancer, given the complexity of the cellular pathways occurring in the disease. The multi-marker approach is expected to increase the sensitivity of disease detection over other traditional approaches. By using a high-sensitivity approach, clinicians can further refer positive results for further biopsy analysis, while using a negative test result as the point of elimination.

Urinary EV biomarkers can also be used for the successful diagnosis of kidney and prostate cancers. Dr Iliuk now aims to extend the scope of the EVtrap technique to isolate vesicles from other biological fluids, such as plasma, tears and saliva. The platform offers the capability of discovering protein biomarkers from several other diseases aside from cancer, including diabetes and neurodegenerative conditions. The team hopes to expand their network of collaborators to include clinicians and researchers that wish to discover novel biological markers for their disease of interest.

Meet the researcher

Dr Anton Iliuk, PhD
Tymora Analytical Operations
West Lafayette, IN
USA

Dr Anton Iliuk is the co-founder, president and chief technology officer of Tymora Analytical Operations, West Lafayette, USA. He obtained his PhD in biochemistry in 2011 from Purdue University, Indiana. His research has focused on the development of novel techniques for non-invasive biomarker discovery, proteomics and phosphorylation analysis. Through these efforts, he published over 35 manuscripts and book chapters and submitted 6 patent applications. Dr Iliuk has given oral presentations, showcasing his research, at multiple international conferences. At Tymora, he is the co-inventor of several technologies and applications, including PolyMAC, pIMAGO, EVCISE, and EVtrap.

CONTACT

E: anton.iliuk@ty mora-analytical.com
W: www.tymora-analytical.com

KEY COLLABORATORS

Multiple academic institutions, cancer centres, hospital systems, pharmaceutical and diagnostic companies.

FUNDING

Funding from NIH and NSF (SBIR and STTR grants), the Michael J. Fox Foundation and Indiana Matching Fund.

FURTHER READING

A Iliuk, X Wu, L Li, J Sun, M Hadisurya, RS Boris, WA Tao, Plasma-Derived Extracellular Vesicle Phosphoproteomics through Chemical Affinity Purification, Journal of Proteome Research, 2020, 19(7), 2563–2574.

X Wu, L Li, A Iliuk, WA Tao, Highly Efficient Phosphoproteome Capture and Analysis from Urinary Extracellular Vesicles, Journal of Proteome Research, 2018, 17(9), 3308–3316.

TYMORA
Analytical Operations, LLC

A NOVEL DIAGNOSTIC TOOL FOR CANCER DETECTION

Head and neck squamous cell carcinoma (HNSCC) constitutes around 90% of all head and neck cancers. Millions of individuals are diagnosed across the globe every year, very often too late and with poor prognosis. Among other factors, alcohol consumption and smoking increase the risk to develop HNSCC. **Dr Muy-Teck Teh**, from Queen Mary University of London, is driving forward our understanding of the factors leading to cancer, leading the development of novel less invasive detection methods, and progressing better therapeutic options.

Early Detection is Key

The early detection of precancerous lesions remains the most efficient way to prevent cancer development and minimises the risk of intensive surgery. This is important, because surgical treatments may lead to physical disfiguration or functional handicaps, such as impaired swallowing or breathing, both of which can significantly impact on the patient's overall quality of life.

The current method of identifying a cancerous, malignant lesion is based on the microscopic observation of the tissue. This histopathology is a costly, time-consuming and unreliable procedure for detecting an early tumour. It requires invasive biopsies to obtain a tissue sample 5–20mm in size, large enough so that the pathologist can observe the difference between the malignant and healthy cells. It often requires suturing, causing significant pain to the patient. The accurate observation of the malignant cells is highly dependant on the pathologist's skills and the preparation of the sample. Furthermore, the diagnostic report can take up to a week to complete, and this waiting may cause extreme stress to the patient. There is an urgent need for rapid and reliable detection methods of early cancer to improve patient outcomes and reduce public healthcare costs.

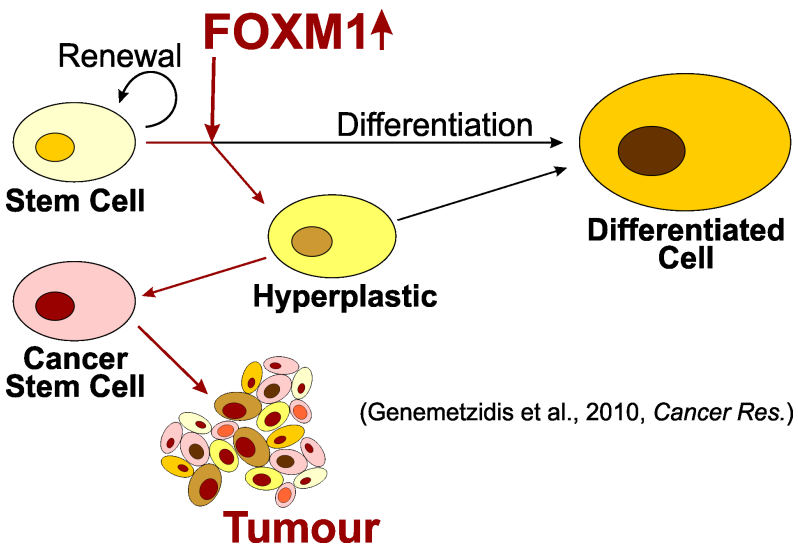
Dr Teh at Queen Mary University of London is a real-life cancer detective with a career spanning over 20 years. He is committed to understanding the mechanisms underlying the transformation of an abnormal cell growth into cancer, a process known as oncogenesis. In 2019, together with his team, Dr Teh patented the 'quantitative Malignancy Index Diagnostic System' (qMIDS), the first diagnostic test for the early detection of oral cancers. Being 90% more accurate than conventional tests and providing results as quickly as in 90 minutes, qMIDS represents a significant step forward in the early detection of cancer. Work by Dr Teh is ongoing to take this even further.

FOXM1: Molecule of the Year

Most cancers occur as a result of DNA alterations such as mutations, amplifications or deletion of genetic material leading to uncontrolled cell proliferation. Over the last decades, scientists have identified the genes

and proteins that are expressed during the different phases of tumour development. However, the clinical use of these data remains a scientific challenge. Although biomarkers for certain types of cancer have already been identified, head and neck cancers remain very difficult to diagnose.

Dr Teh and his colleagues concentrate their research on the Forkhead box protein M1 (FOXM1), a human protein coded by the gene FOXM1 and influences cell fate such as division or death, therefore playing an important role in regulating cell's ability to proliferate. In fact, Dr Teh and his group were the first to provide evidence for the role of FOXM1 in human cancer. Since their seminal work published in 2002, the field of FOXM1 expanded exponentially and it is now a key oncogene that is found to be driving cancer progression in almost all human cancer types.



A key role for FOXM1 in the regulation of stem cell renewal was unveiled by Dr Teh. Abnormal activity of FOXM1 leads to excess stem cell renewal and subsequently promoting tumour initiation. Credit Dr Teh.

FOXM1 belongs to a group of proteins called transcription factors, able to bind DNA and regulate the transcription of a DNA sequence into messenger RNA (mRNA). mRNA then exits the cell nucleus to be translated into proteins. FOXM1 is particularly relevant in cancer research as it regulates numerous genes involved in different stages of the disease, from initiation to metastasis. FOXM1 was designated Molecule of the year in 2010 by the International Society for Molecular and Cell Biology and Biotechnology Protocols and Research for its potential in cancer research. Dr Teh and his colleagues use FOXM1 as a 'molecular gauge' to quantify the progression of cancer in single tissue biopsy.

Quantifying Tumour Progression

Cancers are, unfortunately, very complex diseases and one marker alone would not be sufficiently reliable or accurate for diagnosis. Initially testing 200 potential genes, Dr Teh and colleagues identified 14 relevant FOXM1 associated genes that were expressed differently during cancer and two reference genes, expressed at constant levels. Using real-time polymerase chain reaction, a very reliable and easy technique widely used in laboratories to quantify gene expression, they computed the results into an algorithm to generate a 'qMIDS malignancy index scoring system'. The score, based on the expression of the 16 genes, is correlated with tumour progression. 'The qMIDS assay objectively measures the malignancy status of a biopsy tissue sample using molecular signatures of multiple FOXM1-orchestrated biomarkers' explains Dr Teh.

To demonstrate proof of concept, the accuracy of qMIDS was tested in benign and malignant biopsies from two cohorts of patients from the UK and Norway. The high sensitivity of the test prompted Dr Teh and his colleagues to further investigate if qMIDS could be used to further characterise the tumour. They performed macro dissection of larges tumours and precancerous lesions to compute information and create a malignancy 'heat map' based on the molecular information. The heterogeneity of the tumour and the clinical significance of the molecular patterns warrant further investigations, but heat maps allow the simultaneous detection of tumour progression and tumour margin (where the tumour stops), which is highly relevant for surgery.

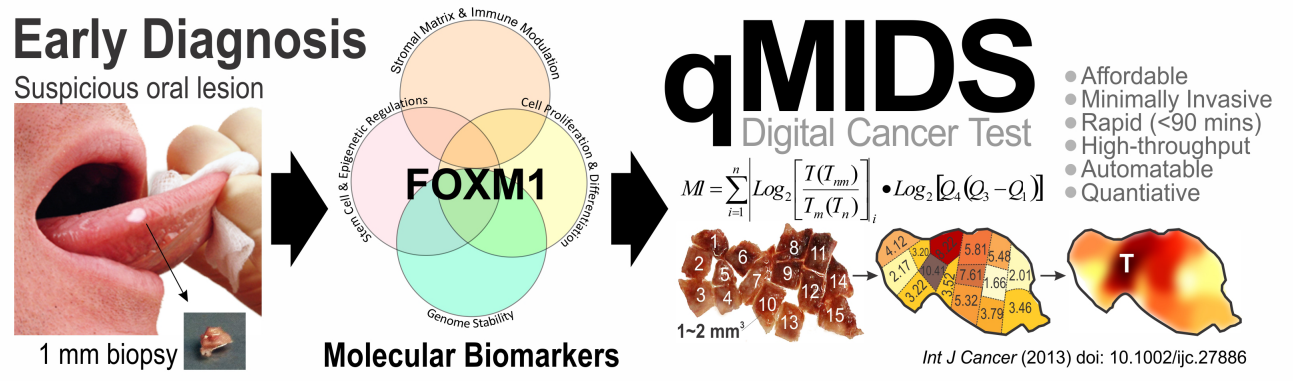
The next step was to validate the diagnostic in a larger non-European cohort. This was essential as the genetic expression can differ between different ethnic groups. Previous studies have reported that sociodemographic factors can influence the genetic background of HNSCC. Dr Teh and his team tested qMIDS in a Chinese cohort and examined the correlation between qMIDS score and progression to cancer. The study, published in 2016, revealed identical datasets between the European and Chinese populations and further demonstrated the robustness of qMIDS in accurately diagnosing HNSCC in different ethnic groups.

Dr Teh's additional collaborations with India and Pakistan further provided independent evidence that the pathophysiology of OSCC was molecularly indistinguishable between the Asian and European specimens. The qMIDS test robustly quantifies a universal FOXM1-driven oncogenic program in OSCC which transcends ethnicity, age, gender and geographic origins

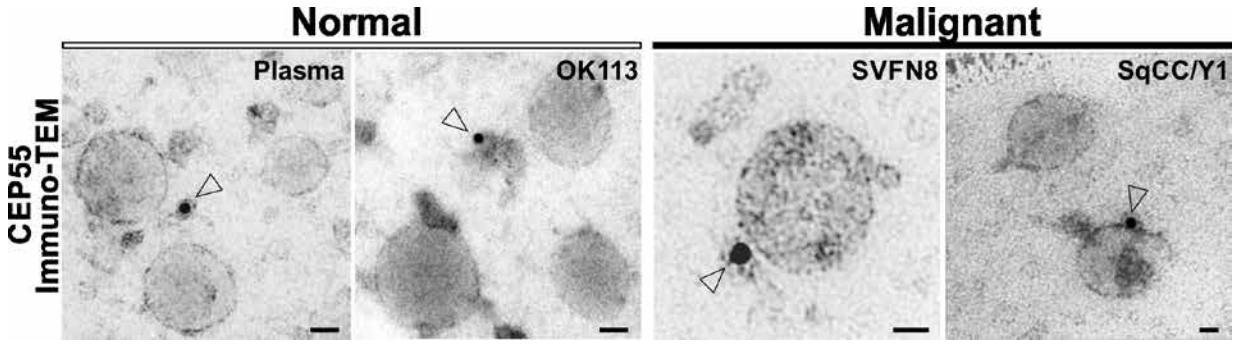
Dr Teh and his colleagues continue their optimisation of qMIDS and are now investigating whether qMIDS can also diagnose other types of cancer. They also have promising evidence that qMIDS could be used for the detection of vulva and skin cancers.

Cancer Biomarkers Hidden in Body Fluids

Recent studies suggest that exosomes contribute to the development of tumours. Exosomes are very small vesicles formed and released by all cell types. Among other important functions, they transport information from a cell to another. The possibility that cancer cells may use exosomes to send reprogramming signals to other cells contributing to the development of tumour and cancer spread, caught the attention of Dr Teh. The presence of exosomes in body fluids such as blood or saliva represents a promising approach to develop non-invasive diagnostics and therapeutics.



The novel, affordable, high-throughput, quantitative Malignancy Index Diagnostic System (qMIDS) can accurately differentiate between low and high-risk oral lesions. Credit Dr Teh.



CEP55 protein localisation using immunogold transmission electron microscopy on exosomes derived from normal human plasma, OK113, SVFN8 and SqCC/Y1. Credit Dr Teh.

Saliva is a complex body fluid, and the challenge was to identify a single protein that can be used as an exosomal biomarker. Based on their previous observation that the CEP55 protein is regulated by FOXM1, Dr Teh and his colleagues demonstrated that CEP55 is exclusively found in the exosomes of malignant cell culture but is absent in healthy cultures. Further in vivo validations of the results in clinical samples are required but these results, published in 2018, provide confidence that CEP55 up-regulation could be used as an exosomal cancer biomarker.

Personalised Therapeutics and Future Care

Unfortunately, progress in the treatment of HNSCC is held back by the heterogeneity of tumours and the complexity of the structures they affect. Despite the numerous on-going clinical trials and therapeutic advancements, the survival rate for patients with HNSCC remains too low. Unlike other types of cancer such as breast or lung cancers, HNSCC cancers are treated with a standard combination of treatments regardless of the genetic biomarkers. It is therefore essential to classify HNSCC patients and propose a more tailored plan of intervention. This can prevent unnecessary and aggressive treatments for some patients and alleviate the intervention cost.

In 2019, Dr Teh and his colleagues conducted a retrospective analysis linking sociodemographic and clinicopathological data, allowing the identification of two subgroups of HNSCC patients which were molecularly and clinically distinct. The two opposite molecular signatures (+q6 and -q6) match two well-studied high-risk groups in the UK population, statistically differing in age, sex, ethnicity and lifestyle. For example, the group +q6 had a higher alcohol consumption that the -q6 group. Although further investigations are needed to link the data with tumour progression, the identification of the two subgroups represents a significant step towards personalised molecular-signature-guided treatments for HNSCC patients.

It should be noted that although FOXM1 expression is a powerful tool that can be utilised for diagnostic and therapeutic aims, many individual factors remain to be overcome. FOXM1 can be expressed in at least five confirmed variants and a further seven predicted variants have been identified. Although most people study FOXM1B and FOXM1 C in cancer aetiology, other isoforms are worthy of exploration.

Over 20 years, Dr Teh has made tremendous strides forward in the detection and treatment of HNSCC. Looking to the future, he envisages that patient care will involve combinations of non-invasive oral cancer detection (such as using saliva or blood) to screen asymptomatic patients, and then non-invasive optical or imaging approaches to inform as to the best sampling location. This could then be followed by molecular and histopathological analysis methods to determine an accurate diagnosis and to tailor the most appropriate treatment intervention for patients.

Meet the researcher

Dr Muy-Teck Teh
Senior Lecturer
Barts & the London School of Medicine & Dentistry
Queen Mary University of London
London
UK

Dr Muy-Teck Teh obtained a BSc (Hons) in Biomedical Science in 1996, followed by a PhD in Physiology, from King's College London, in 2000. He undertook two postdoctoral research positions, funded by the Wellcome Trust and then Cancer Research UK. Dr Teh is now a Senior Lecturer in Head and Neck Cancer at Barts & the London School of Medicine & Dentistry, Queen Mary University of London. As part of his outstanding research career to date, Dr Teh pioneered the identification of FOXM1 as a key driver in human cancer initiation which was awarded 'Molecule of the Year' in 2010 by the International Society for Molecular and Cell Biology and Biotechnology Protocols and Research. He leads a research group investigating cancer biomarkers and novel diagnostic methods with the overarching aim of personalising cancer treatment based on individual molecular signatures. In 2019, he patented the world first FOXM1-based digital molecular cancer test 'quantitative malignancy diagnostic system (qMIDS)' for the early detection of oral cancer. Dr Teh has numerous international collaborators across the world and has published over 60 papers in prestigious journals.

CONTACT

E: m.t.teh@qmul.ac.uk
W: http://www.dentistry.qmul.ac.uk/people/profiles/drmuyteckteh.html
https://twitter.com/FOXM1B
ORCID: https://orcid.org/0000-0002-7725-8355
Laboratory: https://sites.google.com/view/qmids/home

KEY COLLABORATORS

Edward W Odell, King's College London, UK
Allan Hackshaw, University College London, UK
Eric Lam, Imperial College London, UK
Christain Simon, University of Lausanne, Switzerland
Daniela E. Costea, University of Bergen, Norway
Dipak Sapkota, University of Oslo, Norway
Bengt Hasséus, University of Gothenburg, Sweden
Monica C Solomon, Manipal University, India
Akhilanand Chaurasia, King George's Medical University, India
Malik Waqar Ahmed, Comsats University, Pakistan
Hong Ma, Guizhou Medical University, China
Hao Chen, Guangzhou Medical University, China
William A. Yeudall, Augusta University, USA
W. M. Tilakratne, University Malaya

FUNDING

Medical Research Council
Wellcome Trust
Saving Faces
The Rosetrees Trust
State Administration of Foreign Experts Affairs, China
Guizhou Medical University
Union for International Cancer Control
Pakistan Higher Education Commission
Guangzhou Medical University, Cancer Hospital and Institute
British Council

FURTHER READING

F Qadir, A Lalli, HH Dar, et al, Clinical correlation of opposing molecular signatures in head and neck squamous cell carcinoma, BMC Cancer, 2019, 19, 830.

F Qadir, MA Aziz, CP Sari, et al, Transcriptome reprogramming by cancer exosomes: identification of novel molecular targets in matrix and immune modulation, Molecular Cancer, 2018, 17, 97.

MT Teh, Oral Cancer Biomarkers: Is it a Meaningless Game? The Open Dentistry Journal, 2016, 3, e1–e3.

H Ma, H Dai, X Duan, Z Tang, et al, Independent evaluation of a FOXM1-based quantitative malignancy diagnostic system (qMIDS) on head and neck squamous cell carcinomas, Oncotarget, 2016, 7, 54555–54563.

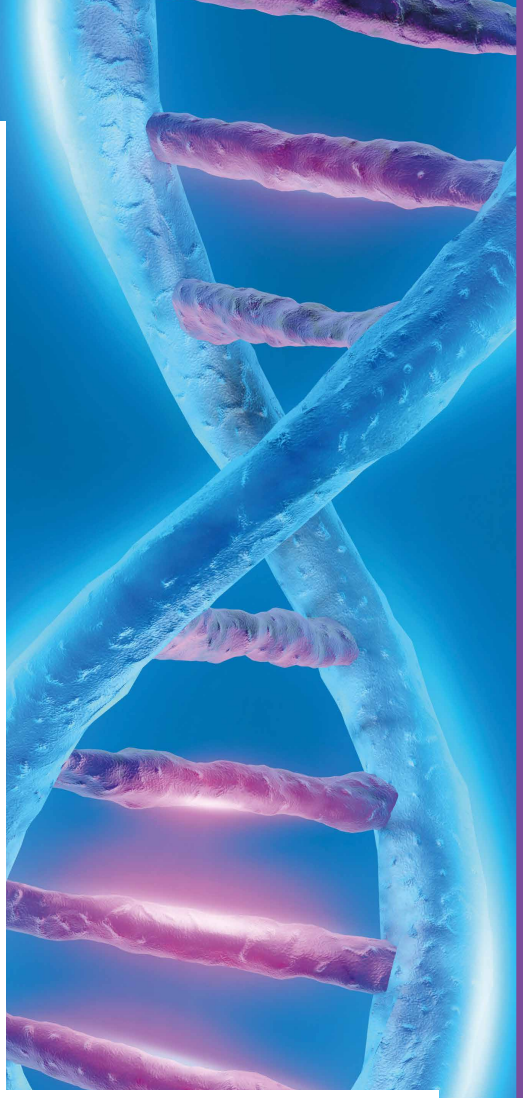
MT Teh, Can Challenges of Oral Cancer Diagnosis be Resolved? Dental Health Current Research, 2015, 1.

Teh MT, Is salivary exosome the answer to early detection of oral cancer? The Open Dentistry Journal, 2015, 2, e3–e4.

MT Teh, IL Hutchison, DE Costea, et al, Exploiting FOXM1-orchestrated molecular network for early squamous cell carcinoma diagnosis and prognosis, International Journal of Cancer, 2013, 132, 2095–2106.

Queen Mary University of London
Barts and The London

THE DISCOVERY OF A NOVEL AND PREDICTIVE BIOMARKER IN SKIN MELANOMA



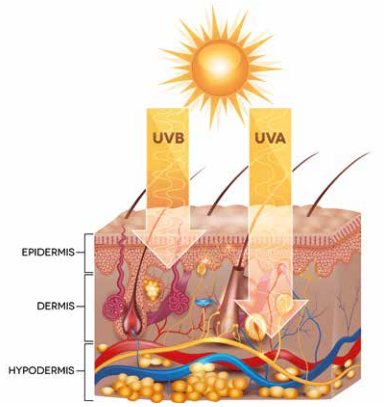
Ultraviolet radiation (UVR) from sunlight has been identified as a leading risk factor for the development of melanoma. Despite numerous research studies, the molecular mechanisms underlying the link between UVR and melanoma remain still poorly understood. **Dr Chengyu Liang**, from The Wistar Institute in Philadelphia and her collaborators from the University of Southern California have identified the function of the UV irradiation resistance associated gene (UVRAG). Their studies show that inactivation of UVRAG affects the ability of the cell to repair UVR-induced damage mechanisms. The researchers also provide compelling in vivo validation of a novel prognostic and predictive biomarker in melanoma.

Understanding the Causes of Genetic Instability in Melanoma

Each year, more than 75,000 new cases of melanoma are diagnosed in the USA, and the resultant death toll exceeds that of 10,000 individuals. Enhanced exposure to ultraviolet radiation (UVR) is generally indicated as one of the main causes of this highly aggressive and frequently drug-resistant skin cancer. However, the molecular mechanisms underlying the genesis and development of UVR-induced melanoma have not yet been fully elucidated.

Dr Chengyu Liang and her team from The Wistar Institute in Philadelphia, together with collaborators from the Keck School of Research at the University of Southern California, have identified the UVR resistance associated gene – UVRAG – as being responsible for the quick and effective repair of ultraviolet (UV) damaged skin cells. UVRAG is also generally recognised as an autophagy promoter. Autophagy is a process through which waste materials in the cytoplasm are digested within vesicle structures and subsequently recycled. A faulty autophagy mechanism is linked to an increase in uncontrolled cell proliferation, a phenomenon associated with malignancy in cancer.

Dr Liang's team has also shown that in response to intense sunlight, UVRAG has a central role in promoting the formation of organelles called melanosomes, which support melanin synthesis within them, through a mechanism that is independent of autophagy. Melanin is the light-absorbing pigment responsible for the photo-protective process commonly known as 'tanning'. The production of this pigment offers the first line of response against harmful UVR. Dr Liang and her team have conducted pioneering studies, providing much-needed insight into the mechanisms through which decreased levels of UVRAG affect the tanning response in skin cells.



Dr Liang and her team aim to verify that reduced capacity of UV-induced photolesion repair and adaptive skin pigmentation represents the main cause of genetic instability of melanoma cells, and is responsible for melanoma predisposition. To investigate the role of UVRAG they utilise state-of-the-art genetic live-cell imaging and physiological assays in cells with targeted mutations in UV resistance genes.

UVRAG: Multiple Roles Beyond Autophagy

Autophagy is a tightly regulated process responsible for the digestion and recycling of cytoplasmic components. These are engulfed in double-membrane vesicles known as autophagosomes. Autophagy maintains the quality control of cellular components and defects in autophagy are associated with numerous pathological conditions, including cancer. More than 32 genes control autophagy, but interestingly UVRAG is responsible for a cascade of reactions that culminate with the enzymatically-controlled trafficking of autophagy vesicles.

A large body of evidence has emerged showing that, in addition to its pro-autophagic role, UVRAG is a multi-tasking gene with multiple autophagy-independent roles, including maintaining chromosomal stability, repairing DNA lesions, and regulating apoptosis (the mechanism of programmed cell death). Its involvement in such a multitude of cellular processes has led to the general consensus that UVRAG is essential in tumour suppression. Although autophagy has been linked to UV protection, the inhibition of autophagy does not affect the UV-protective effect of UVRAG. This seems to suggest that the autophagic side of UVRAG is not fully responsible for its protective action against melanoma. Dr Liang and her team aim to fully explain the molecular mechanisms by which UVRAG deficiency results in an accumulation of mutations in skin cells, leading to their malignancy.

Photolesion Repair Mechanisms in Skin Cells

Exposure of the skin to excessive UVR can cause extensive damage to the DNA. If left unrepaired, this damage leads to the accumulation of mutations which can become permanent and be responsible for the induction of cancer. Cells normally protect themselves from UV-induced DNA damage by activating the nucleotide excision repair (NER) pathway. During NER, the short, single-stranded sections of DNA containing the lesion are removed, while the undamaged strand is used as a template by the enzyme DNA polymerase to synthesise a repaired, complementary short section of DNA.

The work of Dr Liang and her team sheds light on the functions of nuclear UVRAG by showing that it directly suppresses UV-linked mutagenesis. This role is particularly significant in preventing the development of skin tumours by regulating the UV-induced DNA damage repair mechanisms.

In a 2016 publication, Dr Liang and her collaborators reported that melanoma patients with lower levels of UVRAG tend to have higher amounts of UV-associated mutations in their DNA. They showed that the knockdown (or 'silencing') of UVRAG from human and mouse melanoma cells rendered them more vulnerable to accumulating a higher load of UV-induced mutations. The team demonstrated that in response to UV exposure, UVRAG accumulates in cells around the sites of photolesions and interacts with a complex of proteins, known as UV-damaged DNA binding proteins 1 and 2 (DDB1 and DDB2). Via the interaction with the DDB proteins, UVRAG activates the NER-associated protein complex

CLR4, which remodels the chromatin around the damaged DNA site, allowing other NER factors to access and repair the DNA lesions.

Importantly, a mutation in UVRAG that prevents it from binding DDB1 results in the inhibition of the NER repair mechanism. Dr Liang argues that the inactivation of UVRAG observed in some melanoma types leaves skin cells unprotected from high levels of UV radiation, causing the accumulation of large numbers of cancer-causing mutations. As such, UVRAG may function as a regulatory factor for contrasting the UV-associated genetic instability.

Although UVRAG was originally identified as an autophagy modulator, Dr Liang and her team showed that the autophagy aspect of UVRAG was not directly related to its role in the repair of the UV-induced DNA damage. This was indicated by the observation that the same mutation that prevents NER by blocking the binding of UVRAG to DDB1 does not affect the ability of UVRAG to regulate autophagy.

UVRAG Regulates Skin Cell Pigmentation

Skin pigmentation is the main cellular mechanism of protection against UV radiation. Pigment-producing cells called melanocytes respond to the action of the melanocyte-stimulating hormone (MSH) by producing melanin in lysosome-related organelles known as melanosomes. The melanosomes are transported out of melanocytes and onto the sun-exposed side of neighbouring skin cells, providing first-line protection against the penetration of UV radiation. Melanin, the pigment contained within melanosomes, protects cells by absorbing UV radiation.

Dr Liang proposes that inactivation of UVRAG could be the cause of the mislocalisation of melanosomes, altered skin pigmentation and the development of melanoma. In a study published in 2018, she reported with colleagues that UVRAG is directly involved in the formation and development of melanosomes via a mechanism that occurs independently of autophagy.

To examine the function of UVRAG in melanocytes, the team specifically 'knocked out' (inactivated) the UVRAG gene in melanoma cells. Compared with control cells expressing wild-type UVRAG, they observed significant whitening of UVRAG knockout cells.

Experiments in zebrafish, conducted as part of the same study, showed that the melanogenic activity of UVRAG observed in cell cultures is also conserved in vivo. The researchers treated zebrafish embryos with a short nucleic acid polymer to bind to the UVRAG transcript and inhibit its expression. Relative to control fish embryos, the treated zebrafish showed a significant reduction in the number of pigmented melanocytes. Deficiency of UVRAG resulted in the incorrect sorting of the melanogenic molecular machinery, which affected the pigmentation of skin cells even in the presence of MSH. Furthermore, the study showed that recovery of UVRAG in melanocytes rescued pigmentation.

Melanosome biogenesis and maturation is regulated by specific transport machinery. The early stages of cellular cargo delivery to melanosomes have been shown to require the intervention of a complex that goes by the name of biogenesis of lysosome-related organelles complex 1 (BLOC1). Dr Liang and her team showed that UVRAG directly interacts with BLOC1, contributing to its stability and mediating its cargo-sorting activity to the melanosomes. Their study demonstrated that the absence of UVRAG results in the dispersion of BLOC-1 distribution and activity, affecting melanogenesis in vitro and causing defective melanocyte development in zebrafish in vivo.

An Exciting Future

UVRAG is a promising novel prognostic and predictive biomarker in melanoma. People who present with low levels or with mutated forms of UVRAG could be at higher risk of developing melanoma. Future studies by Dr Liang and her team will be needed to help elucidate in more detail the mechanisms by which UVRAG protects against UVR-induced damage, and delineate why they fail to work in melanoma. This knowledge will allow scientists to identify targets for the development of drugs that can revert these broken mechanisms to normal functioning, an approach that may ultimately save countless lives as we continue the fight against cancer.

Meet the researcher

Dr Chengyu Liang, MD, PhD
The Wistar Institute
Philadelphia, PA
USA

Dr Chengyu Liang obtained her MD in 1995 from the Qingdao University School of Medicine and her PhD in genetics in 2004 from the State University of New York (SUNY) at Stony Brook. After completing her postdoctoral studies at Harvard Medical School in 2008 she joined the Keck School of Research, University of Southern California, where she was Associate Professor of Molecular Microbiology and Immunology until 2020. She recently joined The Wistar Institute, Philadelphia, as Professor of Molecular and Cellular Oncogenesis. Dr Liang's research is funded by the National Institutes of Health and is focused on understanding the basic mechanisms that regulate fundamental cellular processes such as autophagy, cell death, DNA damage repair, and membrane trafficking in the context of cancer and infectious disease.

CONTACT

E: cliang@wistar.org
W: https://wistar.org/
@TheWistar

KEY COLLABORATORS

Dr Meenhard Herlyn (Director, The Wistar Institute Melanoma Research Center)

FUNDING

National Institute of Environmental Health Sciences (NIEHS) ES029092
National Cancer Institute Grant (NCI) CA 140964

FURTHER READING

YF Yang, G Jang, X Yang, et al., Central role of autophagic UVRAG in melanogenesis and the suntan response, Proceedings of the National Academy of Sciences, 2018, 115, E7728–37.

YF Yang, SS He, Q Wang, et al., Autophagic UVRAG promotes UV-induced photolesion repair by activation of the $CRL4^{DDB2}$ E3 ligase, 2016, Molecular Cell, 62, 507–19.

Z Zhao, S Oh, D Ni, et al., A dual role for UVRAG in maintaining chromosomal stability independent of autophagy, Developmental Cell, 2012, 22, 1001–16.

FINDING THE MOLECULAR FINGERPRINT OF PSYCHOLOGICAL RESILIENCE IN BREAST CANCER PATIENTS

Professor Carl Borrebaeck and **Dr Ulrika Axelsson** are Director and Deputy Director, respectively, of the CREATE Health Translational Cancer Centre, Lund University, Sweden; a venue with an outstanding record of world-class cancer research. They are leading research into the fascinating topic of whether cancer patients' psychological resilience after their cancer diagnosis may be linked to biomolecular processes, suggesting a mind-body link between the ability to cope psychologically and its impact on cancer prognosis.

Why Do Some People Cope Better Than Others?

Receiving a diagnosis of cancer is a traumatic and life-changing event, and in itself may lead to significant and negative psychological impacts. We know that stress and depression can generally worsen the progression of illness and disease, and patients struggling with psychological problems tend to have a poorer life-expectancy and quality of life. However, it is observable that some patients handle these events with better resilience than others.

Cancer research is increasingly recognising that tumours do not exist in isolation but are part of, and responsive to, the wider body 'ecosystem'. For example, there is growing evidence that the effects of the mind and body are intrinsically joined, so that psychological stresses can have strong detrimental effects on cancer outcomes (prognosis). It remains unclear, however, why some individuals have greater psychological resilience, which is defined as the dynamic process in which individuals adjust, cope and adapt in the face of challenge and adversity.

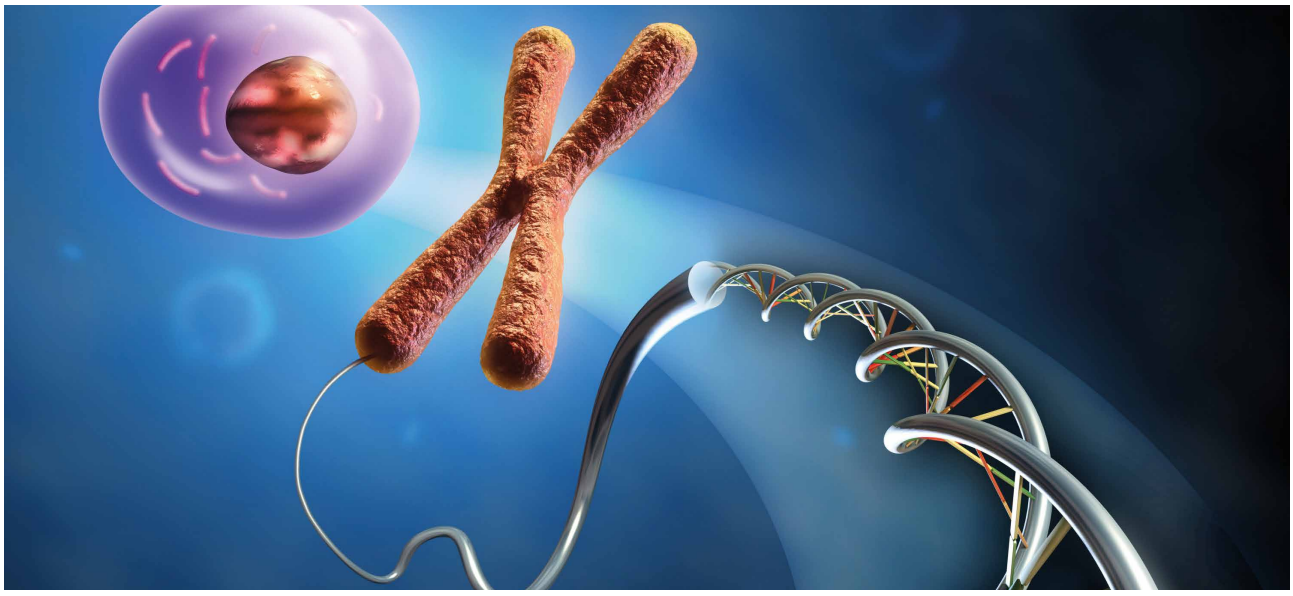
Researchers at the CREATE clinic, Sweden, are hoping to identify biochemical markers that link to and predict low psychological resilience, ideally through a simple blood test. Their long-term goal is to open up new cancer treatments through complementary medical and psychological approaches.

The CREATE project

Professor Carl Borrebaeck and Dr Ulrika Axelsson are Director and Deputy Director, respectively, of the CREATE Health Translational Cancer Centre, Lund University, Sweden. They lead a multidisciplinary research team currently running clinical studies to investigate the relationship between the psychological resilience of cancer patients and biomarker signatures. Working with breast cancer patients,

the CREATE Health project aims to use advanced 'omics' to untangle the intertwined mind-body links between psychological resilience and cancer prognosis.

The vision that resilience had an imprint in the somatic background of humans emerged in the team already 2012 and then grew into the largest clinical study conducted to date with a focus on the body and mind axis, fuelled by advanced omics technologies. Later, a scoping review by Dr Axelsson and her colleagues verified significant gaps in the knowledge regarding the connections between mind and body in the context of cancer. They also noted that there was no established protocol



From DNA to Cell

Illustration of Breast Cancer

for how to analyse these potential links, although stress and depression had been shown to affect the likelihood of tumour cells metastasising (the spreading of secondary tumours). Similarly, only a very small number of biomolecular markers had been investigated for their role in psychological resilience.

The present study, denoted as 'SCAN-B Resilience' encompasses over 1,000 patients today and is part of the broader Sweden Cancerome Analysis Network–Breast (SCAN-B) study, which aims to develop new clinical tests for breast cancer. At time of diagnosis, breast cancer patients are asked to complete the Connor-Davidson Resilience scale (CD-RISC), a standardised method to measure psychological resilience, as well as questionnaires to measure their quality of life, lifestyle and socioeconomic status and blood samples were taken for the biomolecular analyses. Patients are followed up at one year with the same questionnaires.

The Search for Biomarkers for Low/ High Psychological Resilience

The CREATE Health project is undertaking the first large-scale study to identify the epigenetic 'fingerprint' of high and low psychological resilience in a group of cancer patients. Epigenetics is the study of changes in living organisms caused by the modification of the way their genes are expressed (as opposed to changes in the genetic code through mutation or damage).

New research fields are rapidly emerging that utilise epigenetic biomarkers as a process to shed light on potential diagnostic methods and therapeutic interventions. In their simplest form, biomarkers are, ideally, easily measurable characteristics that act as an indicator or predictor of a biological process or state. They can be classified into four types: diagnostic biomarkers to determine a specific health disorder; prognostic biomarkers to chart the likely course of a disease; predictive biomarkers to indicate the likely response to a particular drug, and predisposition biomarkers that indicate the risk of developing a disease.

Omics is a catch-all term applied to new technologies that have led to the discovery of many new biomarkers. Omics include a number of investigative research fields including epigenomics, proteomics, transcriptomics and metabolomics that are characterised by high-throughput laboratory techniques that make it possible to gather, in a single experiment, enormous quantities of data about a specific type of molecule, a full set of cellular proteins or a complete set of DNA modifications, for example. While still a relatively new technology, it is moving clinical research in the direction of highly personalised, precision medicine.

Evidence of Mind-Body Interaction

Each person's response to knowing they have cancer and coming to terms with facing the challenges ahead differs hugely. However, it is evident that some people are better than others at psychologically coping, and at the same time have better outcomes, not explained by the type and severity of the cancer or their treatment.

The critical aspects of 'not coping well' or low resilience, can manifest as helplessness, powerlessness and fatalism, which are both symptoms and causes of often debilitating psychological trauma, depression and anxiety. The heterogeneous nature of mental illness, however, makes it difficult for researchers to identify the fundamental physical origins (the

Lund University, Sweden.

aetiology) of the condition(s). Broadly, we know for example, that the brain undergoes pathological changes and a reduction in neuroplasticity, the brain's ability to reorganise itself, often after injury, to create new neural connections. These processes are driven by structural, DNA transcriptional and epigenetic disruptions in several parts of the brain.

An example of the mind-body biomolecular effect is the increase in norepinephrine (a neuroendocrine hormone) during stress. Norepinephrine, in turn, elevates metalloproteinase-9 (MMP-9) levels. Importantly, both depression and stress are related to MMP-9 secretion by tumour-associated macrophages (TAM) in patients with ovarian cancer. TAM cells thus respond to stress and also act to encourage tumour growth by facilitating a proinflammatory tumour environment, creating a two-way, negative cycle of physical and psychological factors.

Professor Borrebaeck and Dr Axelsson are utilising advanced genomics and proteomics research technologies to better understand the biomolecular control of genes related to the level of resilience, in order to identify biomarkers or gene 'signatures' that will reliably signal useful and relevant information.

In this study, blood samples taken from breast cancer patients will be analysed for DNA methylation and micro RNA (miRNA) signatures. DNA methylation is a biological process in which methyl (CH3) groups are added to the DNA molecule, modifying the action of that DNA segment and expression of the genes it contains. miRNA are a class of small non-coding RNAs that control gene expression through a number of different gene translation mechanisms. Emerging research has shown that both miRNA and DNA methylation are likely to play an important role in the manifestation of psychological (and many other) disorders. By analysing patterns and occurrences of DNA methylation and circulating miRNA, omic techniques can identify potential biomarker signatures that may shed light on the complex genetic pathways behind the development of psychological responses and the biomolecular determinants of low or high resilience.

Wider research has identified that DNA methylation is potentially linked to depression through action on several genes, but the findings are preliminary. Similarly, miRNA has emerged as a key regulator of higher brain function and neuroplasticity. Importantly there are also indications that the differential co-expression of a group of miRNAs plays a direct role in human disease pathogenesis, but can also assist researchers to study the nature of the disordered biomolecular pathways involved.

Future Project Aims

At this stage of the CREATE Health research, the team is aiming to validate the value and reliability of using DNA methylation and miRNA as biomarkers for resilience. Uniquely, the project will correlate breast cancer patients' psychological status with the epigenetic results and track their relationship on quality of life and disease burden.

By focussing on the body and mind interactions of these patients, it is hypothesised that biomolecular signatures will match with high or low resilience indicators. Once biomarkers are proven as reliable indicators, simple tests will hopefully be developed that would enable the clinician to identify at an early stage whether a patient with cancer is also particularly susceptible to low-resilience. This information can alert clinicians that the patient faces a greater risk of a poorer cancer outcome and decreased quality of life. Appropriate intervention strategies can then be put in place, which can ameliorate the patient's psychological difficulties.

Establishing a greater understanding of the biomolecular processes behind psychological resilience and the complex progression of tumours and other diseases opens many avenues for future clinical developments. The identification of biomolecular signatures associated with high or low psychological resilience could potentially have a major impact on the patient, as it potentially provides the opportunity for personalised diagnosis and treatment regimens, to address challenging psychological barriers to health improvement.

For clinicians, the tests will identify high-risk patients that were perhaps previously not easily identifiable. This should significantly improve clinical outcomes for cancers by simply addressing the confounding psychological factors to success. However, a detailed understanding of the epigenetics involved will also encourage the development of new, highly targeted medical treatments such as DNA methyltransferase inhibitors, as well as new psychosocial interventions.

Meet the researchers

Professor Carl AK Borrebaeck
Department of Immunotechnology
Lund University
Sweden

Professor Carl Borrebaeck achieved degrees in Chemistry/Maths (BSc) and Chemical engineering (MSci) before achieving his doctorate in Molecular immunology from Lund University (LU), Sweden in 1979. Following postdoctoral studies at the University of California, he returned to LU as an Associate Professor of Immunotechnology in 1981. Professor Borrebaeck received his professorship in 1990 and in 2005 also became Director of CREATE, Health Translational Cancer Centre at LU. Between 2009–2014 he was Vice President of LU. In addition, Professor Borrebaeck is a successful serial entrepreneur, having co-founded multiple biotech companies including Immunovia AB, Senzagen AB, BioInvent International AB, Alligator BioScience AB, and, most recently, PainDrainer AB. Amongst his many awards, Professor Borrebaeck received The Biotech Builders Award for outstanding entrepreneurship in 2017, the Academy of Engineering Science Gold Medal for outstanding research in 2012 and the Akzo Nobel Science Award in 2009.

CONTACT

E: carl.borrebaeck@immun.lth.se
W: https://portal.research.lu.se/portal/en/persons/carl-borrebaeck(68077eca-2a0b-4da1-9080-9f8bf8b352a6).html

Dr Ulrika Axelsson
Department of Immunotechnology
Lund University
Sweden

Dr Ulrika Axelsson achieved degrees in Chemical engineering (MSci) at the Faculty of Engineering (LTH) at Lund University (LU) before she graduated from LU with a PhD in immunotechnology in 2009 and worked as a Research Scientist in at LU between 2009–2010. Since 2010, Dr Axelsson has simultaneously managed and coordinated a number of key cancer initiatives: first as Research Coordinator, then Deputy Director of CREATE, Health Translational Cancer Centre at LU (2010-date), the LU Cancer centre (Project Leader, 2012–2013), and Research Coordinator at the BioBanking and Molecular Resources Infrastructure of Sweden (2011–2014). Dr Axelsson is also currently the Business Development Manager for PainDrainer AB, Lund, Sweden.

CONTACT

E: ulrika.axelsson@immun.lth.se
W: http://www.immun.lth.se

KEY COLLABORATORS

Lisa Rydén, MD, PhD, Co-Investigator, Department of Clinical Sciences, Lund University
Ingalill Rahm Hallberg, PhD, Co-Investigator, Department of Health Sciences, Lund University
Per Johnsson, PhD, Co-Investigator, Department of Psychology, Lund university
Corinna Richter, PhD, Study Coordination, Department of Immunotechnology/CREATE Health, Lund University

FUNDING

Gunnar Nilsson Foundation
VINNOVA
Philanthropic Donation

CREATE, Health Translational Cancer Centre, Lund University

Established in 2005, CREATE Health is a unique strategic centre for Translational Cancer research mainly located at Medicon Village in Lund, Sweden. Offering an equipped and integrated 'omics' hub, CREATE Health brings together multidisciplinary Lund University Hospital researchers and clinicians from the Faculties of Medicine, Natural Science and Engineering to solve complex clinical problems. CREATE Health is primarily focussed on the selection of optimal, individually-based, cancer treatments emerging from the development of novel diagnostics and therapeutics based on identified markers and molecular signatures.

The SCAN-B resilience study and the MAD (Make a difference) for Cancer Programme are both hosted by CREATE. MAD is a unique, collaborative and multi-focused project bringing together holistic research on early diagnosis, patient stratification and targeted therapies addressing all aspects of cancer biology, with the aim of rapid implementation.

ARTIFICIAL NEURAL NETWORKS: UTILISING MACHINE LEARNING FOR EQUITABLE BREAST CANCER DIAGNOSIS

Breast cancer is the most commonly occurring cancer in women, and it is pivotal that it is diagnosed correctly and promptly. Artificial Intelligence (AI) is now widely used in diagnosis to produce more accurate results. **Dr. Roy Jafari** from the University of Redlands is training a type of AI called an Artificial Neural Network to make more equitable diagnosis decisions for patients. He hopes that focussing on decision-making will reduce stress on patients and create a better care experience.

Identifying and Treating Breast Cancer

Breast cancer is the second most common cancer in the world and the most commonly occurring cancer in women. It can present as several types and in different parts of the breast, and a patient is usually diagnosed with either the non-invasive or invasive form. Non-invasive breast cancer is characterized by the tumor being confined to the ducts, without spread to the breast tissue. More commonly, invasive breast cancer is diagnosed, where cancer cells have spread to the surrounding tissue from the ducts. Other, more unusual forms are invasive lobular breast cancer, inflammatory breast cancer, and Paget's disease of the breast.

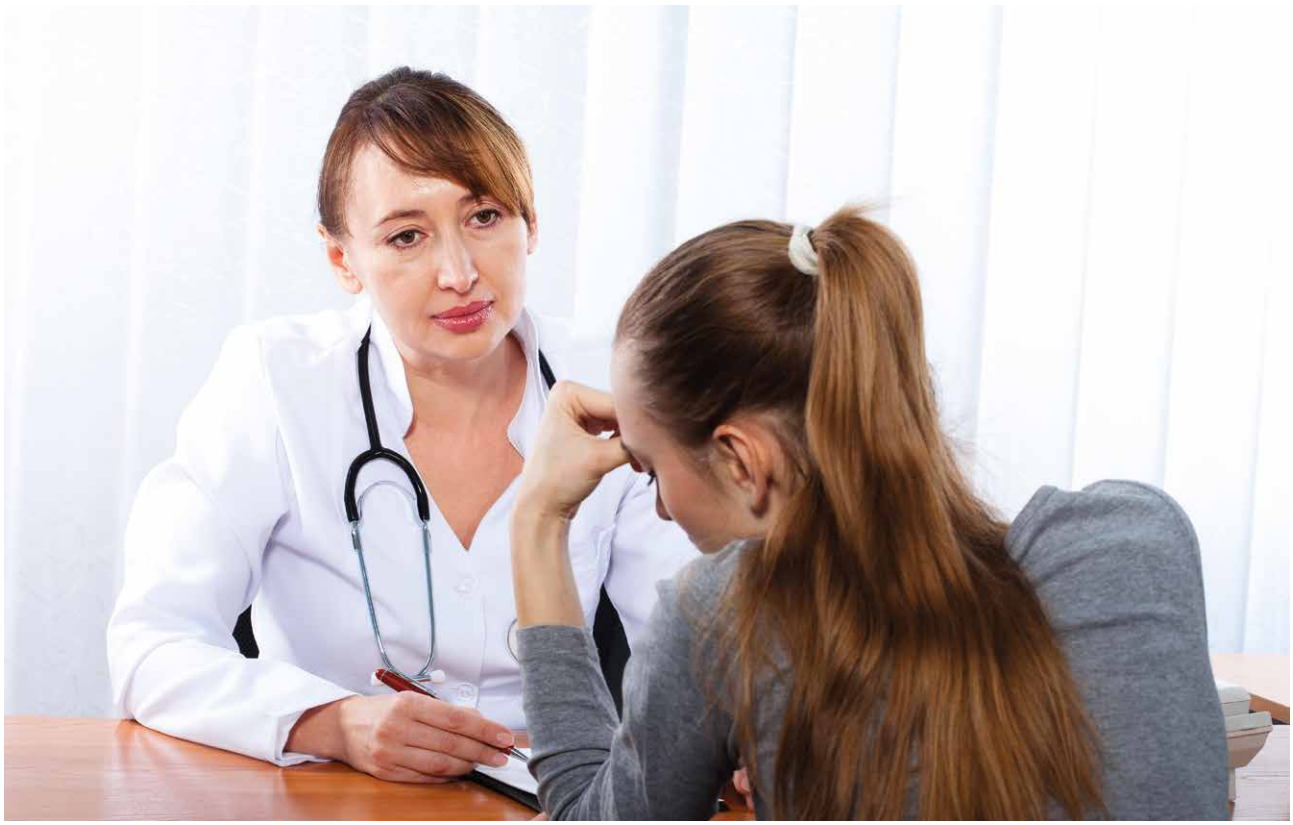
Symptoms of the disease may include a lump, tissue thickening, a rash, and other changes to the appearance of the breast. Finding any of these symptoms can prompt a person to visit their doctor for tests, which leads to a diagnosis. However, many countries including the USA, also provide screening services to women over the age of 50 or those perceived to be at high risk. This service helps to find people who are unaware that they either have or may be at risk of developing breast cancer because they are showing no symptoms.

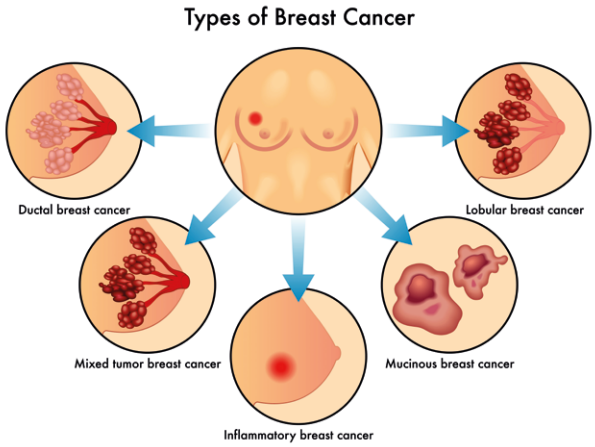
The first test carried out is usually a mammogram, which involves taking x-rays to identify and quantify abnormalities within the tissue. If any are found, the mammogram may be followed by an ultrasound and a biopsy, which is a sample of cells taken from the suspicious area. The cells are then sent to a laboratory to be examined by a histopathologist, who can establish whether or not cancer is present in the tissue.

Following a positive diagnosis, one or a combination of interventions including surgery, radiotherapy, chemotherapy, targeted therapy, and hormone therapy are used to combat cancer. Thanks to decades of dedicated research, the survival rate of breast cancer after 10 years is now 76%.

The Role of Technology in Breast Cancer Diagnosis

One interesting tool that can be used to diagnose breast cancer is a machine-learning subset of Artificial Intelligence (AI), known as an Artificial Neural Network (ANN). These fascinating computer systems are inspired by human brains, which contain millions of interconnected neurons that fire to allow function and learning. An ANN is made up of layers of units (nodes) called artificial neurons. Each artificial neuron is connected and sends signals to all the neurons in the next layer, which are ordered starting with the input layer, then the hidden layers, and finally the output layer.





The networks can be trained, and they learn to recognize and correct errors using supervised and unsupervised learning. Supervised learning involves inputting labeled data into the first layer and providing the 'answer' to the data so that when similar data is next put in, the algorithm will produce accurate results by itself. It has learned what you taught it. Unsupervised learning is the process by which the algorithm extracts its own patterns from unlabelled data.

Diagnosis of breast cancer can be achieved through inputting data of a person's test results and life circumstances into an ANN. The accuracy of this method has proven to be very high but there remains a margin of error. A false negative is the term for a diagnosis of no cancer when, in fact, cancer is present. Conversely, a false positive occurs when someone is diagnosed with cancer when they are actually cancer-free.

Most ANN protocols run on the assumption that receiving a false negative result has the potential to be significantly more damaging to a person than a false positive. The logic here is that that patient would rather take further measures to investigate their suspected cancer and eventually rule it out than cancer go undetected and worsen over time. Dr. Roy Jafari from the University of Redlands wants to challenge this expectation and investigate how the assumptions programmed into an ANN can improve its decision making.

Should We Prioritise Life Span or Life Quality?

This is an interesting question – are the consequences of a patient missing out on treatment due to a false negative worse than the psychological consequences of a false positive. The answer may seem obvious at first; surely it is better to be tested as thoroughly as possible and retested as necessary to ensure that a patient receives treatment if they need it. However, this premise, which is also coded into the ANN, is not necessarily correct. Some studies have shown that the fear and anxiety that wrong or premature cancer diagnosis can create, may have real psychological effects, not to mention the physical pain that unnecessary mammograms cause.

Dr. Jafari emphasizes that the consequences of a false positive will differ from person to person. For instance, a 45-year-old woman, with immediate family care and high emotional resilience would be encouraged to undergo further tests if her results showed she had a 10% risk of cancer. This woman would have a good support system and would be able to bounce back quicker from any emotional stress. On the other

hand, a 75-year-old woman with no immediate family care and low emotional resilience would perhaps not benefit from further testing if her cancer risk was only 10%. She may experience serious stress that could impact her mental and physical health, in addition to not having access to adequate support.

Of course, if either risk was 95%, both individuals would be tested for breast cancer further because the threat to life is significantly higher. Dr. Jafari argues that all of these factors and more (such as quality of health insurance) should be taken into account when deciding if continued tests would actually dimmish a patient's quality of life when their risk is very low anyway. Currently, most ANN approaches use the assumption that all patients would want further testing, even if their risk of breast cancer is as low as 10% – but this is not necessarily the case.

Following on from this, Dr. Jafari believes that improving ANN functioning should focus more on decision-making, rather than accuracy so that patients receive the correct care, personal to them. As he explains, 'In my research, I investigate and develop algorithmic approaches to decision-making that are both data-driven and equitable.'

Self-Organizing Error-Driven Learning

To study how altering an ANN's priorities towards better decision-making could influence outcomes, Dr. Jafari and his team developed a new method of teaching for the networks called Life-Sensitive Self-Organizing Error-Driven (LS-SOED). This method takes a patient's uniqueness into account by recognizing that if the data analytic results are inconclusive, a false positive or a false negative result will have varying consequences depending on their circumstances.

An ANN learns from its errors, so if the calculations to rectify these are driven by accuracy, the networks will focus on improving accuracy, not improving decision making. The LS-SOED method aims to put the ANN's power into making better decisions for patients, using both supervised and unsupervised learning. Data from patients is organized into a base, where their personal similarities and differences are mapped and recognized by the system (this is the self-organizing part of the title). This base allows LS-SOED to use decision error-driven learning along with clearer decision goals so that the ANN decision making improves.

A combination of underlying methods assists the new programming. Self-Organizing Map recognizes patterns and maps them, Multi-Layered Perceptron uses this map to predict patient types and Fluid Genetic Algorithm deals with inconclusive data.

Findings from the New Method

The new LS-SOED method applied to an ANN provided some interesting results. In line with their original thinking, Dr. Jafari and his team showed that a highly accurate ANN does not always give the best diagnosis decisions. They compared two sets of data analyzed using LS-SOED with three other advanced data analytic techniques. The first group of 253 patients diagnosed using decisions made by the LS-SOED method were predicted to save 30 years of human life between them. The second group of 57 were predicted to save 8 years, meaning collectively 38 years would be saved between 340 people.

Dr. Jafari notes that implementing these decision-making improvements comes with a great computational cost and is very complex. But this is only the beginning, the complexity of these systems will pave the way for more research into the promising possibilities for AI and machine learning.

Meet the researcher

Dr. Roy Jafari
School of Business
University of Redlands
Redlands, CA
USA

Dr. Roy Jafari completed his Bachelor of Science in Industrial Engineering at Tafresh University in Iran and then went on to obtain a Master of Science in Industrial Engineering from the University of Tehran. After moving to the USA to continue his studies, he graduated with a Ph.D. from Mississippi State University in 2018. He served as Assistant Professor of Industrial and Manufacturing at California Polytechnic State University from September 2018 to July 2020, and now he holds the role of Assistant Professor of Business Analytics at the University of Redlands. In his academic positions, he researches diagnosis decisions and data science. His current work investigates how machines can use data analytics to make smarter and more equitable decisions for real-world applications such as breast cancer diagnosis.

CONTACT

E: roy_jafari@redlands.edu
W: https://roy-jafari.com
https://www.linkedin.com/in/roy-jafari-marandi-84077932/
https://www.researchgate.net/profile/Roy_Jafari
https://twitter.com/jafariroy

FURTHER READING

R Jafari-Marandi, Supervised or Unsupervised Learning? Investigating the role of pattern recognition assumptions in the success of binary predictive prescriptions, Neurocomputing, 2021, 434, 165–193, https://www.sciencedirect.com/science/article/abs/pii/S0925231220319639?via%3Dihub

H Long, JM Brooks, M Harvie, A Maxwell, DP French, How do women experience a false-positive test result from breast screening? A systematic review and thematic synthesis of qualitative studies, British Journal of Cancer, 2019, 121, 351–358.

R Jafari-Marandi, S Davarzani, M Soltanpour Gharibdousti, BK Smith, An optimum ANN-based breast cancer diagnosis: Bridging gaps between ANN learning and decision-making goals, Applied Soft Computing, 2018, 72, 108–120.

M Bond, R Garside, C Hyde, A crisis of visibility: The psychological consequences of false-positive screening mammograms, an interview study, British Journal of Health Psychology, 2015, 20, 792–806.

AGAINST BREAST CANCER

Shockingly, there are around 55,200 new breast cancer cases in the UK every year. As such, breast cancer is the most common invasive cancer in women and a leading cause of cancer death. In this exclusive interview, we speak with **Richard Bahu**, Chair of Trustees at the UK research charity Against Breast Cancer to hear about their vital work.

How did Against Breast Cancer come to fruition?

The total focus of Against Breast Cancer (ABC) is to stop secondary breast cancer from claiming lives. ABC was founded by Dr Anthony Leathem and his wife Patricia. At the time, Anthony was a pathologist at the Middlesex Hospital (later University College London) and his wife Patricia was a breast cancer theatre nurse at Oxford's Churchill Hospital. They were deeply upset by the number of post-mortems he carried out each day on young women with breast cancer. They had to act by personally fundraising to support his research into breast cancer survival. This included Patricia planting their entire allotment with sweet peas to sell, and Anthony in his white coat outside Oxford Street tube station with a collection tin! Anthony's research paper describing a difference between aggressive and non-aggressive breast cancer cells was the springboard for setting up ABC in 1993.

How does Against Breast Cancer support research? What types of research do you fund?

Our research strategy is set out in our Roadmap with three key areas: prevention, detection and new therapies. Prevention includes understanding how diet and lifestyle factors affect the risk of cancer recurrence. Detection aims to design better tools for the earlier detection of secondary cancer through biomarker discovery. New therapies aim to exploit the body's immune system to create more effective treatments.

Our research strategy has created a significant critical mass of world-class researchers at Southampton University. We complement this with seed funding of researchers who have an exciting research idea but insufficient background research to apply for major funding. Often, these seed-funded projects enable them to successfully apply for major follow-on programmes. We are also committed to encouraging and developing young scientists in breast cancer research through a major fellowship programme at Oriel College, Oxford University.

How is the general public involved in your work?

We wouldn't be where we are today without the involvement and support of the public. We have amazing supporters who help raise funds for our research projects. There are many ways people get involved, from taking part in local and national events, corporate giving, donating bras and clothes, volunteering their time and skills, and providing touching case studies. We are also grateful to those who leave a legacy in their wills. Some major companies and organisations choose ABC as their charity of the year and we work with them to give their staff a positive experience of our charity work. We also have research advocates who are people with experience with breast cancer and who provide valuable input into the types of projects that we fund.

One amazing and extremely moving example is that of Danielle. She was the focal point for a fundraising campaign in which a lock of her hair taken before her treatment was turned into diamond – the world's most precious stone. At the end of the campaign, the diamond was incorporated into a necklace by a leading designer and donated to her young daughter.

We are, of course, in the midst of the COVID-19 pandemic. What specific difficulties has this brought to the work of Against Breast Cancer?

Clearly, this has had a dramatic effect not just on our fundraising but also on our staff and supporters. Being a smaller charity, we can react quickly to these challenges. Our team has risen to the challenge by being creative in moving activities online and into a virtual world. We have retained all our staff whilst other charities have sadly let many people go. This is a reflection of the lean management of the charity by an outstanding management team. We have also benefited from prudent management of our funds which has enabled us to cope robustly with the financial pressures.

There has been an impact on our research and with our full support, some of our researchers have joined the effort to combat the virus. However, we have been very pleased with how our researchers have sought to move their projects forward wherever possible. Again, we have continued to support all our funded projects and do not envisage cutting back any of them.

What would you say are Against Breast Cancer's biggest achievements to date?

Reaching our 25th anniversary in 2018 was a big milestone for the charity. We are particularly proud of achieving our Biobank which is a collection of samples and lifestyle data collected from over 3,000 women over a period of 5 years post-cancer diagnosis. This is such a valuable research resource and one of the largest of its kind in the UK. To date, our research funding has now exceeded £7m and we have laboratories located at one of the world's leading centres researching secondary breast cancer. ABC's committed funding up to 2028 sees a portfolio of £4.5m with further funding envisaged for seed grants.

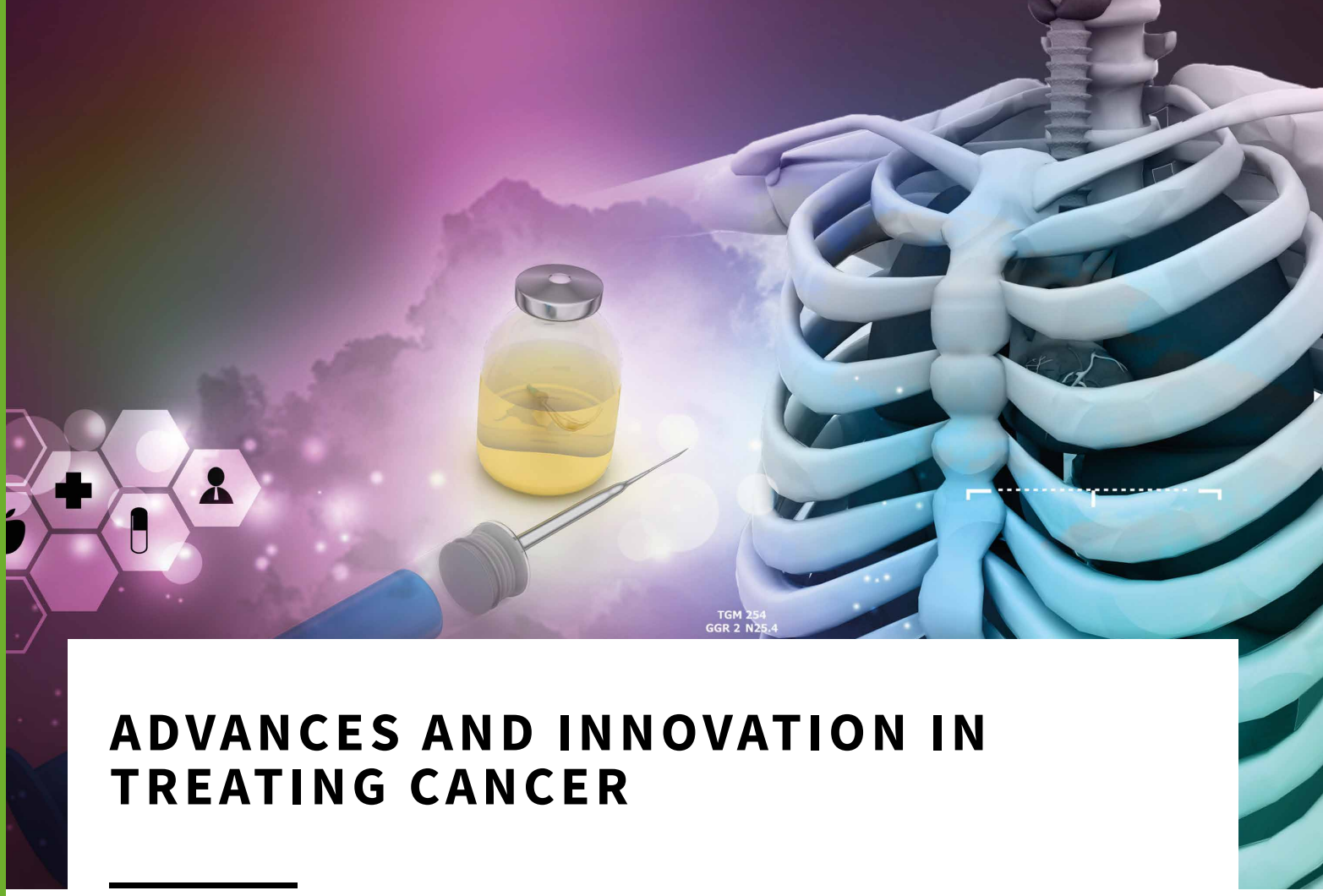
Looking now to the future, what are the main challenges and goals for Against Breast Cancer over the next 5–10 years?

ABC is now on a clear path to have a major impact on secondary breast cancer deaths over the next 5–10 years. The biggest challenge is managing the expectations of our supporters as they obviously want quick results whereas the complexity of the research requires a steady and sustained long-term effort to which we are committed. This means we have to provide clear and truthful communication about our research as often people see articles in the press and on social media which talk about breakthroughs but which often are still some way from being implemented in clinical practice.

W: https://www.againstbreastcancer.org.uk/
@againstbc

AGAINST® breast cancer

TREATING CANCER



ADVANCES AND INNOVATION IN TREATING CANCER

Finding a 'cure' for cancer is often referred to as the holy grail of medical research. But the complexity of cancer, attributable to the variation in the causes and risk factors, as well in the ways in which different types grow and spread, makes it incredibly unlikely we will ever have a single cure. A further complication arises in that even following complete remission, in which there are no longer any detectable signs or symptoms of cancer, it can remain in the body and strike again – sometimes many years later. This means that while cancer may be treated, complete cure is much more difficult to guarantee, and we need a broad range of therapies if we are to cover the whole spectrum of cancer. In this final section, we meet the researchers dedicated to exactly that – the development of novel and innovative therapeutics, that taken together, bring us closer to effectively treating cancer in all its forms and stages.

We open this final section by meeting Dr Scott Gerber at the University of Rochester Medical Center, who focusses his research on the particularly aggressive form of cancer known as pancreatic ductal adenocarcinoma (PDA). Unfortunately, this major cause of cancer mortality is often detected only in the late stage of the disease and fails to respond to approaches such as chemotherapy or radiotherapy. We read of Dr Gerber's pre-clinical work developing a novel combined therapy to improve the therapeutic response of tumours even at late-stage identification and thus increase the survival of PDA patients.

Dr Yi Sheng at York University, Canada, is also focussed on early-stage laboratory research with the aim of improving treatment for cancer. Ubiquitin is a small protein that is attached to other proteins in the cell to signal specific biological processes. Alteration of the ubiquitin system is critical in many diseases, including the majority of cancers. We read how Dr Sheng is characterising the ubiquitinylation process of key proteins to identify important new therapeutic targets influencing the progression of cancers, as well as other conditions.

Dr J. Kenneth Hoober and Dr Laura L. Eggink at Susavion Biosciences, USA, have identified some peptides that can inhibit the growth of cancer cells in several animal models of cancer. Importantly, cancer cells not only evade the immune system, they also actively suppress it. We read how the peptides identified by Susavion can generate a strong anti-tumour response and are effective across a range of diseases – either as a monotherapy or in combination with other treatments.

Remaining on the theme of preclinical laboratory work, we turn to Dr Rock J. Mancini from Washington State University who is working to overcome

the role of multidrug resistance in the failure of chemotherapy as a cancer treatment. We read of Dr Mancini's work exploiting the proteins that are over-expressed in drug-resistant cancers to convert inactive prodrug substrates into active drugs that initiate an immune response specifically targeted at cancer cells.

In order to better understand individual response to cancer treatment, Dr Joshua B. Rubin at Washington University, USA, has been exploring the reasons for the established differences in the prevalence and survival of male and female cancer patients. Dr Rubin and his collaborators are the first researchers to identify sex-specific differences in malignant tumour transformation. We read how these important findings will help to optimise sex-specific approaches to cancer treatment and contribute to the improvement of the outcomes and survival of cancer patients.

Dr John Paul Y.C. Shen of the University of Texas MD Anderson Cancer Center is also working to improve the effectiveness of cancer treatments. We read of his work creating comprehensive molecular 'maps' of cancer cells and their interactions in his mission to better understand the cancer genome. By understanding cancer at the molecular level and transferring this knowledge to the clinic, we are becoming closer to being able to provide personalised treatment for cancer in the clinic.

The translation of laboratory research into effective clinical treatment is also the quest of Dr Sean E. Lawler at Brigham and Women's Hospital, Harvard Medical School. With a focus on brain tumours and other central nervous system diseases, Dr Lawler is working to deliver therapies to the brain by overcoming the obstacle of the blood-brain barrier. This approach is particularly important for the treatment of the lethal brain tumour known as glioblastoma.

We then turn to Dr Patrick C. Still at California State University, Dominguez Hills, who takes a different approach to developing effective interventions for cancer. Dr Still works with an enthusiastic team of undergraduate researchers to identify and screen anti-cancer compounds derived from plant materials. While working to meet the need to identify novel treatments for cancer, involvement in his studies incorporating the structure elucidation and biological testing of compounds from plants provides valuable undergraduate research experiences for students.

Dr Hiromi Wada at the Japanese Society of Inflammation and Metabolism in Cancer and colleagues including Dr Reo Hamaguchi, are taking the novel approach of investigating the effects of an alkaline diet (e.g., fruit, vegetables, legumes, nuts and seeds) on the tumour microenvironment, and its potential to enhance the efficacy of anti-cancer treatments. We read how

alkalisation (achieved through diet or oral supplementation) may lead to improved outcomes, even in patients with late stage or metastatic (i.e., spreading) tumours which are traditionally very difficult to treat or control.

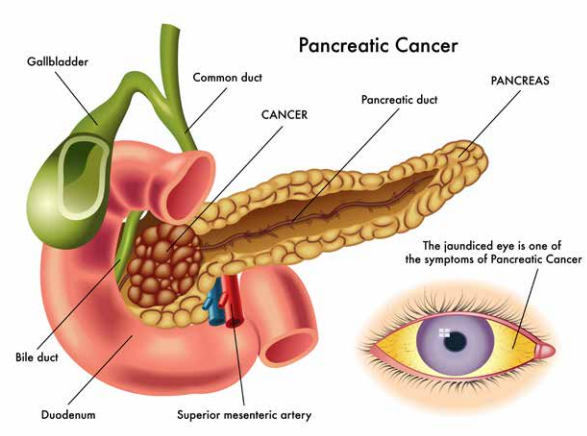
We then turn to an alternative approach provided by Dr Mark Herzberg at the University of Minnesota. During the course of his extensive research into the antimicrobial and antibacterial properties of small proteins known as calgranulins, Dr Herzberg serendipitously uncovered the potential use of the calprotectin complex as a therapeutic agent for certain types of human cancer. We read how this may be particularly effective in limiting the local invasion and spread of tumours in head and neck cancers.

Our final featured researcher, Dr Kenneth Pienta at the Johns Hopkins School of Medicine, has made the exciting discovery that that in every type of cancer, a special type of rare cancer cell – a polyaneuploid cancer cell (PACC) – exists and hides within the greater cancer cell population. As such, Dr Pienta and his team propose that polyaneuploid cancer cells are the critical treatment target in cancer. We read of his inspired 'call to arms' for scientists and researchers across diverse disciplines to unite knowledge and efforts in the bid to develop successful treatments for all types of cancer.

We conclude this section with an exclusive interview with Worldwide Cancer Research's Director of Research, Dr Lynn Turner. We read how 'the other big C' – the COVID-19 pandemic – has affected critical research into cancer, and what challenges our clinicians, researchers and patients now face as a result.

A NOVEL COMBINATION THERAPY FOR PANCREATIC DUCTAL ADENOCARCINOMA

Pancreatic ductal adenocarcinoma (PDA) is an aggressive type of cancer. It is relatively common and is one of the leading causes of cancer mortality. Unfortunately, it is often detected only in the late stage of the disease and fails to respond to pre-surgical approaches, such as chemotherapy or radiotherapy, that are needed to shrink the tumour mass before surgical removal. **Dr Scott Gerber** at the University of Rochester Medical Center, USA, is working with colleagues to develop a novel combined therapy to overcome this issue and increase the survival of PDA patients.



Why is Survival of Pancreatic Ductal Adenocarcinoma Patients So Poor?

The prognosis for pancreatic ductal adenocarcinoma (PDA) patients is very poor. The only cure for this cancer is the surgical removal of the tumour but by the time that patients are diagnosed, the tumour tends to be very advanced. Consequently, only a minority of patients – between 10 and 20% – are eligible for surgery or respond to initial treatment, also called neoadjuvant therapy, to shrink their tumour such that the patient is appropriate for surgery.

There are currently several potential options for shrinking the mass of tumours. However, in PDA, chemotherapy or combined chemotherapy and radiotherapy regimes have proven to be largely ineffective in reducing the size of the advanced tumours.

An Alternative to Conventional Radiotherapy

Recently, stereotactic body radiation therapy (SBRT), a method which uses radiation to target the tumour in an extremely precise fashion, is now showing some promise as a neoadjuvant to shrink the tumour before surgical removal.

Typically, conventional radiotherapy consists of small doses of radiation delivered over 4–6 weeks. This method is somewhat ineffective in treating PDA and often results in damage to normal tissue. However, the precision targeting of SBRT allows for higher doses of radiation to be administered over days, rather than weeks, and results in much higher levels of tumour cell destruction, while the damage to local, healthy tissues is minimised.

It is now widely accepted that the immune system mediates many of the anti-tumour effects of radiotherapy. Importantly, it has been shown that SBRT does a better job stimulating the immune system to fight cancer when compared to conventional radiotherapy. This is an important aspect of the treatment as it enables a stronger immune attack on the tumour cells, which have been exposed by the SBRT treatment. In addition, SBRT induces processes within the tumour that encourage the release of chemoattractants, which are chemical messengers that encourage vital immune cells to infiltrate the remaining tumour.

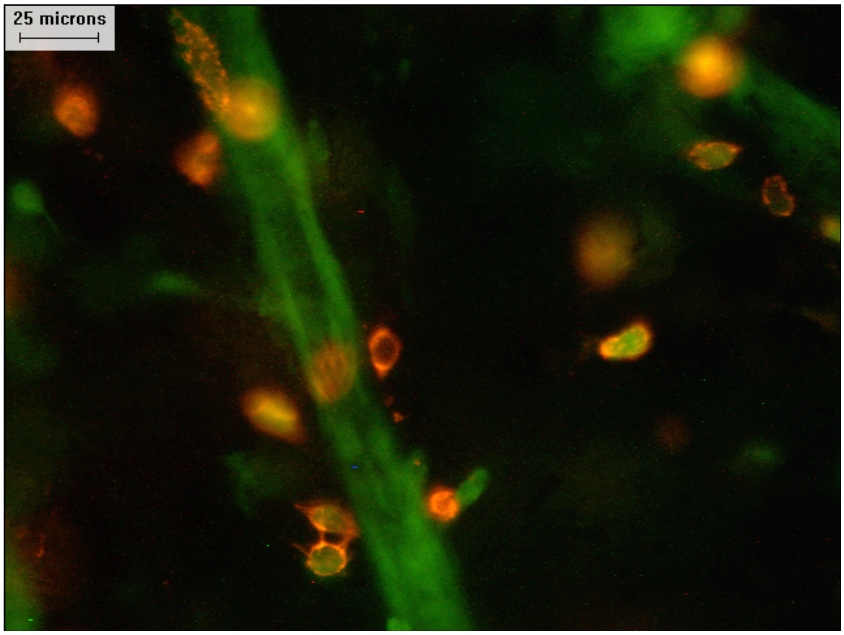


Image of anti-tumour CD8+ T cells (orange) infiltrating the tumour microenvironment (black) with tumour blood vasculature (green) Credit: Scott Gerber.

Stereotactic Body Radiation Therapy to Treat PDA

Dr Scott Gerber at the University of Rochester Medical Center, USA, and his team recently completed a clinical trial examining how well SBRT activated the immune system in PDA. On comparing untreated to SBRT-treated tumours, they found that CD8 T-cells, an immune cell type important to initiating tumour cell death, had infiltrated into the middle of only the SBRT-treated tumours. However, it is important to note that the treatment did not alter the numbers of CD68+ immune system cells in the tumour, which are key to the tumour's survival as these cells suppress the immune system response. Overall, SBRT did initiate an anti-tumour immune response, but could not sustain this response long enough to eliminate the tumours. Therefore, the group looked toward immunotherapy to enhance SBRT and bolster the immune attack on the tumour.

The Problems with Immunotherapy in PDA

Immunotherapy for cancer treatment, in general, is a relatively novel approach, and to date, there have been only limited benefits, especially for patients with PDA. There are several different modes of immune therapies that may be used in PDA tumour treatment. However, depending on the precise strategy used, most are ineffective, can be relatively toxic to the patient and, in other cases, can lead to the tumour becoming resistant to treatments. This is largely due to a unique tumour microenvironment where cancerous PDA cells hijack certain immune cells and cause them to promote tumour progression rather than inhibit it.

This phenomenon also makes it difficult for immunotherapies, which rely on stimulating immune cells against the tumour, to work well in this particular malignancy. In PDA, many of the anti-tumour mechanisms are inactive resulting in the survival of the tumour. To overcome these hurdles, Dr Gerber's team made use of a potent anti-tumour cytokine, called Interleukin-12, to ramp up the immune system.

Targeted Delivery of Interleukin 12

Interleukin 12 (IL-12) is a pleiotropic cytokine, a small, protein chemical messenger that can affect many different cell types. In early tests, IL-12 appeared to have significant potential as an anti-tumour cytokine that operates by promoting activation of T-cells. However, early trials generated poor results and showed high levels of toxicity to patients when it was administered systematically. More recently, researchers have focused on delivering doses of IL-12 directly into the tumour, anticipating that targeted delivery will provide the benefits of IL-12 therapy whilst minimising the toxicity issues unveiled in the early testing.

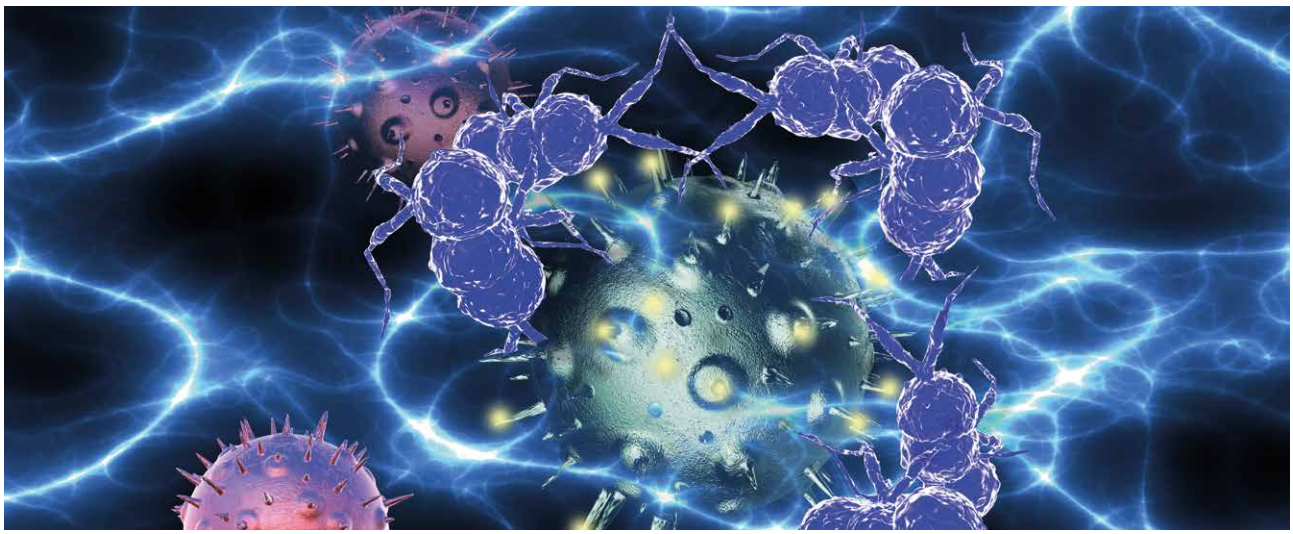
Dr Gerber and his colleagues have adopted the novel approach of encapsulating immune-modulatory IL-12 in microspheres, which are small particles. The microspheres serve a dual function. First, they protect the IL-12 from destruction by enzymes in the body, and second, they allow a slow, sustained release of IL-12 into the tumour.

The Development of a Combination Therapy

Dr Gerber and his colleagues tested their critical prediction that the immune system effects derived by SBRT can be further enhanced by providing IL-12 directly to the tumour. In their tests, IL-12 loaded microspheres were injected directly into tumours in an animal model of pancreatic cancer, in which the IL-12 was then slowly released over a period of up to two weeks.

The team dosed the animal models with a quantity and schedule of radiation similar to the clinical SBRT schedule, using a small animal radiation research platform. In their test animals, the team demonstrated a substantial destruction in tumour mass following SBRT-like treatment.

Using animals in which tumours had been transplanted into the pancreas, the team found that following the combination therapy (SBRT + IL-12 microspheres) the tumours were undetectable at 20 days post-implantation, and this remained the case as far out as 120 days. This



combined approach was superior to treatment with either IL-12 microspheres or SBRT alone. Additionally, assessment of the post-treatment tumours at day 11 of the study showed that the combination treatment resulted in the tumour being overwhelmed by immune cell infiltration.

The team tested the individual and combination therapies on a range of animal models, and overall, they found combination treatment was vastly superior to individual therapy modes. In one case, SBRT treatment resulted in 20% of test subjects having increased survival at 120 days of the study. However, with the combination therapy, 100% of the studied animals survived to this point. This is an unprecedented finding, in that the combination therapy fully eradicated the hard-to-treat PDA tumour. Of great significance is the fact that this combined therapy not only eradicated the tumour mass from a transplanted model which is known to be sensitive to radiotherapy but showed the same effect in a model which is known to be insensitive to radiotherapy.

The Immune Response to Combination Therapy

It is generally accepted that many of the functions of Il-12 are mediated through stimulation of other cytokines, and specifically, IFN-δ (interferon-gamma). IFN-δ is an important cytokine for immune responses, and a key function of this cytokine is its ability to induce a range of actions, which are vital in anti-tumour processes.

When Dr Gerber and his team analysed the cytokine profiles of tumours treated with combination therapy, they found that the expression of both Il-12 and IFN-δ was increased compared to individual treatments. These increases occurred on the day after treatment and were sustained for 24 and 48 hours, respectively. The team demonstrated the requirement for IFN-δ to induce tumour death by testing a model which was unable to produce this important cytokine, and they found that tumours in these mice did were not eradicated in the same way as in the mice which were capable of producing IFN-δ.

On assessing the cell types found within the treated tumours, the team noted that the tumours exposed to the combination treatment showed significantly higher levels of CD45+ cells, which are IFN-δ positive, and CD4 and CD8 T-cells, which can target the tumour directly or change the tumour microenvironment.

As IFN-δ is a cytokine which promotes an inflammatory response from other immune system cells, Dr Gerber and colleagues investigated the effect of the increased levels of IFN-δ on the other immune cell types within the tumour. It is known that immunosuppressive cells are increased in response to radiotherapy in PDAs, and the team predicted that the combination approach could overcome this suppression. They found that tumour-associated myeloid cells, which can be increased by SBRT, could be modified by IL-12-microsphere treatments. For example, the team demonstrated that tumour-associated myeloid cells which are immune suppressive following radiotherapy are reprogrammed following exposure to Il-12 and actually then assist in the destruction of the tumour.

Building on Pre-clinical Success

The pre-clinical work conducted by Dr Gerber and colleagues shows great promise in providing treatment for PDA, for which currently, the vast majority of patients do not survive past five-years. As Dr Gerber notes, 'One of the key findings to our work was that the combination therapy was not only effective against the primary tumour, but was also able to destroy established metastases. This is important, as metastatic disease is what eventually kills most cancer patients.'

Looking to the future, Dr Gerber further shares, 'We feel this combination therapy could give pancreatic cancer patients much needed "hope" in their fight against the disease.' Now, the team are setting up clinical trials in humans to assess the effects of this potentially revolutionary treatment and to ascertain the benefits to locally-advanced and metastatic PDA patients.

Meet the researcher

Scott Andrew Gerber, PhD
Associate Professor
University of Rochester Medical Center
Rochester, NY
USA

Dr Scott Gerber is an Associate Professor in the Department of Surgery at the University of Rochester Medical Center with over two decades of experience. After completing his PhD in Immunology at the University of Rochester, New York, in 2005, Dr Gerber undertook a three-year postdoctoral position at Yale University, before returning to University of Rochester. The majority of Dr Gerber's research has been focused on cancer therapies, and his major interest is in overcoming tumour-induced immune suppression and enhancing the efficacy of radiotherapy treatment. His team has recently identified a highly promising combination of therapies, using a targeted radiotherapy treatment and intra-tumour injection with immune-modulating microspheres that, if successful in human trials, will result in a vast improvement in treating or extending survival, which is currently dismally low, of pancreatic cancer patients.

CONTACT

E: scott_gerber@urmc.rochester.edu
W: https://www.urmc.rochester.edu/labs/gerber.aspx
W: https://www.urmc.rochester.edu/labs/tumor-immunology.aspx

KEY COLLABORATORS

Dr David C. Linehan, MD
Dr Edith M. Lord, PhD
Dr Nejat K. Egilmez, PhD
Dr Bradley N. Mills, PhD

FUNDING

National Institutes of Health (R01CA230277)

FURTHER READING

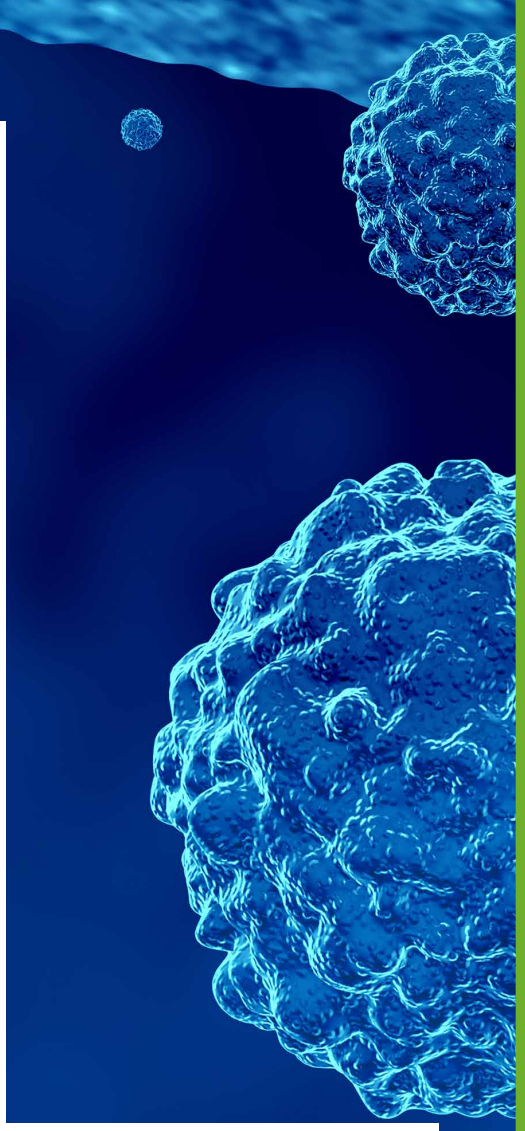
J Ye, BN Mills, T Zhao, et al, Assessing the Magnitude of Immunogenic Cell Death Following Chemotherapy and Irradiation Reveals a New Strategy to Treat Pancreatic Cancer, Cancer Immunology Research, 2020, 8, 94–107.

HJ Han, JD Murphy, S Qin, et al, Microspheres Encapsulating Immunotherapy Agents Target the Tumor-Draining Lymph Node in Pancreatic Ductal Adenocarcinoma, Immunological Investigations, 2020 (online ahead of print). Doi:10.1080/08820139.2020.1765795.

BN Mills, KA Connolly, J Ye, et al, Stereotactic Body Radiation and Interleukin-12 Combination Therapy Eradicates Pancreatic Tumors by Repolarizing the Immune Microenvironment, Cell Reports, 2019, 29, 406–421.

UNIVERSITY of ROCHESTER MEDICAL CENTER

THE UBIQUITIN AND PROTEASOME SYSTEM IN TUMOUR MANAGEMENT AND DRUG DISCOVERY



Ubiquitin is a polypeptide that is tagged on to various proteins to signal a range of biological processes. The alteration of the ubiquitin system plays a pivotal role in the pathogenesis of diseases including autoimmune disorders and cancer. The process of ubiquitinylation involves a cascade of enzymes, E1, the activating enzyme, E2 (conjugating enzymes) and E3 (ligases). Characterisation of the ubiquitinylation process of key proteins that impact stem cells, immune cells and cancers is vital to identify therapeutic targets influencing the progression of autoimmune conditions and cancers. The ubiquitin system is compromised in the majority of cancers and is the focus of research by **Dr Yi Sheng** of York University, Canada.

Ubiquitin and Ubiquitin Conjugation

Ubiquitin is a small protein that is attached to other proteins in the cell to signal specific biological processes. This is a reversible process that is used to alter the function of the proteins in various ways. The process of attaching ubiquitin to another protein is termed ubiquitin conjugation, a highly regulated, sequential, multi-step process.

Single ubiquitin molecules can be attached to proteins, a process known as monoubiquitinylation, which is associated with various biological processes. However, ubiquitin itself can further form polyubiquitin structures, effectively chains of ubiquitin attached to an initial target protein. The length and specific linkages used between the ubiquitin molecules indicate vastly different biological outcomes for the targeted protein.

Maintaining the balance in this dynamic, complex system requires not only the ability of enzymes to bind ubiquitin to target proteins but also remove ubiquitin. Removal of ubiquitin is facilitated by ubiquitin-specific proteases which catalyse the separation of ubiquitin from the protein.

P53 and Ubiquitylation Control of Tumourigenesis

P53 is a protein that is known to be a tumour suppressor. It plays a pivotal role in safeguarding the integrity of the genome and preventing tumourigenesis, the development and progression of cancer cells. P53 achieves this by preventing the proliferation of damaged cells, which occurs through one of two key mechanisms. More specifically, by stopping the cell cycle (termed cell cycle arrest) or by inducing programmed cell death (also known as apoptosis). In normal cells, the level of p53 is closely regulated. Regulation is managed by a group of enzymes called

E3 ligases. These enzymes drive the ubiquitylation of p53 by attaching the small protein, ubiquitin, to p53. This process signals that the tagged p53 should be degraded.

Human Malignant Cells and p53-E3 ligases

MDM2, an important human E3-ubiquitin-protein ligase, is overexpressed in a number of human malignancies, and as such, it is an attractive target for novel cancer therapies. From a potential therapy perspective, inhibition of the E3 ligase MDM2 in tumours should result in an increase of p53 and subsequently

increased levels of p53-activated cell death in cancers that are overexpressing MDM2.

In the past decade, the number of E3 ligases identified by researchers has increased substantially to include Pirh2, COPI, TOPORS and HUWE1 (also known as Mule or ARF-BP1). This broad range of E3 ligases results in greater complexity of the p53-ubiquitylation pathway and offers an increased number of potential drug targets in p53-dependent cancers.

MDM2 is found to be overexpressed in more than 10% of human cancers, including 40–60% of human osteogenic sarcomas (bone cancers) and around 30% of soft tissue sarcomas. A sarcoma is a malignant tumour which arises from connective-type tissues including muscle, tendons and blood vessels. MDM2 is also frequently overexpressed in the malignancies of blood cells, which causes the inhibition of p53.

Molecular and Functional Comparison of MDM2 and other E3 ligases

Dr Yi Sheng of York University, Canada, and her colleagues have studied a number of E3 ligases associated with p53 degradation, with the most commonly known MDM2 protein to identify differences as well as similarities in the structures and functions that may be exploited to inform novel targets for cancer drug therapies.

First, comparing MDM2 with Pirh2, which is also prevalent in a number of human cancers, Dr Sheng found that, similarly to MDM2, Pirh2 reduces p53 levels via ubiquitinylation. However, Pirh2 ubiquitylation occurs in a manner which is fully independent of MDM2. This means that the inhibition of p53 by Pirh2 may play an important role in tumourigenesis.

Dr Sheng and her team identified important structural differences between MDM2 and Pirh2. In addition, they conducted tests to determine their respective ubiquitylation activities, both autoubiquitylation – self-tagging with ubiquitin – and p53 ubiquitinylation.

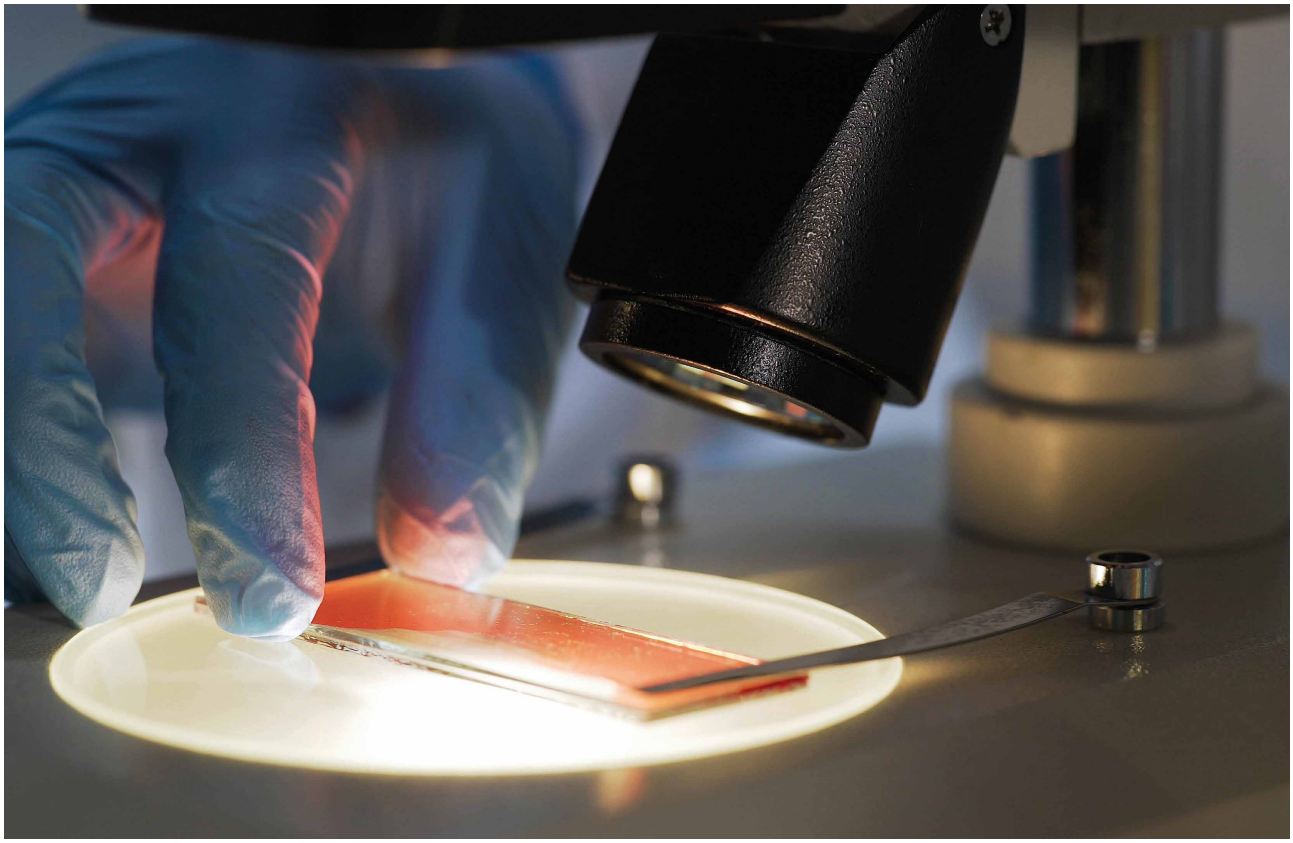
Dr Sheng and her team found that MDM2 performs as a more effective ubiquitylation enzyme for both auto- and p53-ubiquitylation than Pirh2. During their testing, the team discovered that these two E3 ligases, MDM2 and Pirh2, target different sites on the p53 ubiquitin tagging. While the significance of this is currently unclear, it may offer future drug targeting options.

Following the work on MDM2 and Pirh2, Dr Sheng and her team conducted structural and activity comparisons of the E3 ligases MDM2 and MDMX. They successfully identified specific regions of the ring structures of these molecules which are essential for ubiquitinylation. This information provides a potential route for designing MDM2 inhibitors which directly influence its E3 ligase activity and prevent the pivotal p53 ubiquitinylation in tumours.

Research has revealed that the p53 regulators MDM2 and MDMX operate in different ways, each serving to provide a specific and unique role in regulating the cell death (MDM2) and cell cycle arrest (MDMX) actions of p53.

Regulation of the Wnt Pathway

Another pathway which is regulated by ubiquitinylation is the Wnt pathway, which is key in both embryonic development and maintenance of



homoeostasis in adult tissues. Two of the most important pathways managed by the Wnt pathway are cell proliferation and stem cell self-renewal. Genetic manipulation or failed modulation of the Wnt pathway contributes to cancer development in a number of tissues.

ß-catenin is a protein which regulates and coordinates gene transcription, the first step in gene expression. Specifically, it is the intracellular signal transducer in the Wnt signalling pathway. Mutation or overexpression of ß-catenin is associated with many cancers. In turn, ß-catenin is regulated and destroyed by the APC protein complex.

In collaboration with the Tak Mak Lab, Dr Sheng and her team's research has demonstrated that in conditions of hyperactive Wnt signalling, an E3 ubiquitin ligase (Mule) targets ß-catenin for degradation, which in turn stops the activation of Wnt signalling. The work demonstrated that a combined loss of both Mule and APC promotes the conversion of some stem cells into cancer stem cells, initiating cancer development.

This knowledge offers a number of intervention points for the manipulation of 'Mule', ß-catenin and the Wnt pathway functions to impact the initiation and development of tumours.

Future Research

The ubiquitin proteasome system (UPS) governs the vast majority of cellular protein degradation. The UPS function is compromised in most cancer cells. As described above, MDM2, an E3 ligase, is a negative regulator of p53, a potent tumour suppressor protein. MDM2 promotes ubiquitylation and degradation of p53.

In blood cell tumours, MDM2 overexpression is frequently detected where p53 is commonly inactivated through negative regulation. Dr Sheng and her team's current research focuses on identifying and developing lead compounds that inhibit the E3 ligase activity of the tumour-promoting protein MDM2.

Dr Sheng and her team have used computational methodologies to screen a quarter of a million naturally occurring candidate compounds to determine whether they are capable of binding the specific region of MDM2 (the RING domain) that is responsible for the E3 ligase activity.

The team have identified candidate compounds as an inhibitor of MDM2 E3 ligase activity, and subsequently activates p53 leading to programmed cell death in human bone cancers. The team's on-going and future work is based on understanding the effect of this molecule in terms of toxicity and efficacy when tested against a panel of haematopoietic cell lines that have been isolated from blood cancer patients.

The outcomes from this planned research will contribute to the body of knowledge surrounding the molecular mechanism of the cancer loop incorporating p53-MDM2-MDMX, and will also provide critical information to the scientific community on the benefits of targeting MDM2 E3 ligase activity as a therapeutic strategy.

Meet the researcher

Dr Yi Sheng
Associate Professor
Department of Biology
Faculty of Science
York University
Toronto
Canada

Dr Yi Sheng is an Associate Professor of Biology in the Faculty of Science at York University, Toronto, Canada. She completed her Bachelor's and Master's degrees at Zhongshan (Sun Yatsen) University in 1996 and thereafter undertook doctoral studies at the University of Toronto, which she completed in 2003. After completing a Postdoctoral Fellowship with the University Health Network, she undertook an Assistant Professorship at York University and in 2013 was promoted to Associate Professor at the same institution. Dr Sheng's work is focused on defining the mechanisms of the ubiquitin system and its effect on cancer pathways, and includes the development of novel therapeutics for potential cancer therapeutics.

CONTACT

T: (416) 7362100 ext 33521
Fax: (416) 7365898
E: yisheng@yorku.ca
W: http://www.yorku.ca/yisheng/

KEY COLLABORATORS

Cheryl Arrowsmith (Princess Margaret Cancer Center, Toronto, Canada)
Tak Mak (Princess Margaret Cancer Center, Toronto, Canada)
Brian Raught (Princess Margaret Cancer Center, Toronto, Canada)
Vivian Saridakis (York University, Canada)

YORK UNIVERSITÉ UNIVERSITY

FURTHER READING

O Egorova, HHC Lau, K McGraphery, Yi Sheng, Mdm2 and MdmX RING Domains Play Distinct Roles in the Regulation of p53 Responses: A Comparative Study of Mdm2 and MdmX RING Domains in U2OS Cells, International Journal of Molecular Sciences, 2020, 21, 1309.

C Dominguez-Brauer, R Khatun, AJ Elia, et al., E3 ubiquitin ligase Mule targets ß- catenin under conditions of hyperactive Wnt signaling, Proceedings of the National Academy of Sciences of the United States of America, 2017, 114, E1148–E1157.

C Dominguez-Brauer, R Khatun, AJ Elia, et al., E3 ubiquitin ligase Mule targets ß-catenin under conditions of hyperactive Wnt signaling, Proceedings of the National Academy of Sciences of the United States of America, 2017, E1148–E1157.

K Wheaton, F Sarkari, B Stanly Johns, et al., UbE2E1/UBCH6 Is a Critical in Vivo E2 for the PRC1-catalyzed Ubiquitination of H2A at Lys-119, Journal of Biological Chemistry, 2017, 292(7), 2893–2902.

O Egorova, Y Sheng, A site-directed mutagenesis study of the MdmX RING domain, Biochemical and Biophysical Research Communications, 2014, 447, 696–701.

F Sarkari, K Wheaton, A La Delfa, et al, Ubiquitin-specific protease 7 is a regulator of ubiquitin-conjugating enzyme UbE2E1, Journal of Biological Chemistry, 2013, 288(23), 16975–16985.

J Shloush, JE Vlassov, I Engson, et al, Structural and Functional Comparison of the RING Domains of Two p53 E3 Ligases, Mdm2 and Pirh2, Journal of Biological Chemistry, 2011, 286(6), 4796–4808.

Y Sheng, JH Hong, R Doherty, et al, A Human Ubiquitin Conjugating Enzyme (E2)-HECT E3 Ligase Structure-function Screen, Molecular & Cellular Proteomics, 2012, 11(8), 329–341.

GLYCOMIMETIC PEPTIDES AS IMMUNE SYSTEM ACTIVATORS IN THE TREATMENT OF CANCER AND VIRAL INFECTIONS

Immune system cells express a number of receptors that bind to sugar ligands. This binding initiates the activation of T-cell lymphocytes and natural killer cells. **Dr J. Kenneth Hoober**, **Dr Laura L. Eggink** and the team at Susavion have designed peptides that bind to different receptors on immune cells. The peptides effectively extend the lives of mice with glioblastoma and ovarian cancer, and prevent the replication of viruses in the presence of non-neutralising antibodies. The mechanism of action of their peptides could inspire the development of effective treatments for Covid-19 and other viral infections.

Designing Novel Peptides to Modulate the Immune System

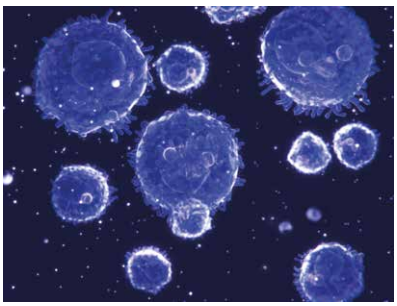
Cell to cell recognition occurs through the interactions of chemically complementary molecules on the surfaces of opposite cells. Cell to cell recognition is pivotal in a plethora of physiological processes. For example, it allows embryonic cells to arrange themselves into developing tissues, and immune cells to 'sense' the presence of pathogens and signal their destruction. The recognition is a biochemical event that happens when the receptor on one cell binds to a ligand molecule on another cell. Ligands often belong to a class of biological molecules known as glycoproteins, which are proteins to which sugars are attached.

Dr J. Kenneth Hoober, Dr Laura L. Eggink and the team at Susavion Biosciences developed peptides that mimic the action of glycoproteins and have the ability to modulate the immune system. More specifically, they designed peptides that are recognised with high affinity by specific receptors on immune cells.

An important upshot of their work is that they have identified some peptides that can inhibit the growth of cancer cells in several animal models of cancer. Cancer cells not only evade the immune system, they also actively suppress it. Susavion's peptides mimic carbohydrate ligands and reactivate the immune system, generating a strong anti-tumour response. The peptides are effective across a range of diseases as a monotherapy or in combination with other treatments. They exert their action at very low concentrations and their efficacy is achieved without any significant toxicity.

Blocking the Replication of HIV

The team at Susavion Biosciences had previously identified several peptide sequences that mimic the terminal sugars of complex glycans. Peptide



svH1C, one of the molecules developed by the team, was designed to mimic the action of sialic acid. Sialic acids are derivatives of a sugar with nine carbons, known as neuraminic acid and are expressed on the surface of cells. Glycoproteins that are rich in sialic acids bind to a large family of receptors known as siglecs (sialic acid-binding immunoglobulin-like lectins), which have a key role in the maturation of immune cells.

Given the importance of siglecs as regulators of the immune system, the researchers at Susavion Bioscience were intrigued by the possibility that

the design of peptides that mimic sialic acids could lead to the synthesis of ligands capable of binding siglecs with enough affinity to achieve modulation of the immune system.

The team showed that svH1C is not constrained by a rigid structure, and thus interacts with binding sites from a flexible conformation. The peptide binds with high avidity to several recombinant human siglec receptors. Furthermore, subcutaneous, alternate-day injections of svH1C into mice induced several-fold increases in populations of several types of immune cells in the peritoneum. These results confirmed that svH1C mimics sialic acids and interacts with cell-surface receptors with enough potency to induce an immune response even at low concentrations.

More importantly, the team also reported that svH1C strongly inhibited infection by HIV-1 of peripheral blood mononuclear cells (mostly T cells) in culture and completely inhibited infection in the presence of non-neutralising antibodies taken from the serum of HIV-positive patients. The researchers point out that the complete inhibition cannot be caused by the serum, as in experiments where the serum was used on its own against HIV, the maximum amount of inhibition reached was 30%. They suggested instead that the binding of low concentrations of svH1C to one or more of the siglec receptors stimulated antibody-mediated destruction of the virus by phagocytosis, the process by which immune cells engulf and digest bacterial or viral particles.

A Cancer Immunotherapy Strategy

Dendritic and other cells involved in the immune system's first line of defence internalise, process and present antigens to lymphocytes, initiating the proliferation of B- and T-lymphocytes. Dendritic cells are equipped with a receptor known as CLEC10A (Ca^{2+}-dependent lectin domain family 10 member A, CD301), which acts as a pathogen-recognition receptor that binds structures containing the sugar *N*-acetylgalactosamine (GalNAc). CLEC10A has been proposed as a target for immunotherapy of cancer, a therapeutic strategy that aims to increase the immune system's ability to recognise and destroy cancer cells.

Most human tumours display an immunosuppressive microenvironment that reduces the ability of antigen-presenting cells to recruit other cells in the immune cascade that control tumour growth, such as T-lymphocytes and natural killer cells. Many cancer cells manage to escape the action of the immune system by losing the cell-surface proteins that would normally act as tags that trigger an immune response. CLEC10A plays an important role in the maturation of dendritic cells and initiation of an immune response, by a cascade that results in T-cell activation. This prompted the researchers at Susavion to verify the hypothesis that a peptide binding to the GalNAc-binding site of CLEC10A could serve as an activator of immunity against cancer.

Two Promising Tools in the Fight Against Cancer

The Susavion team developed two peptides, named as svL4 and sv6D, both resembling the structure of *N*-acetylgalactosamine. The team confirmed through their studies that both peptides bound the CLEC10A receptor and promoted the recruitment, proliferation and activation of several types of immune cells. The strong immune response was localised in the peritoneal cavity and this led the team to test whether the peptides would be effective in treating cancers of peritoneal organs.

They developed binding experiments to evaluate the affinity of the two peptides to the receptors and observed that they were both able to bind even when present at very low concentrations. Of the two peptides, sv6D gave the most promising results, significantly extending the life of mice with implanted ovarian cancer cells. Peptide sv6D was as effective as the chemotherapeutic drug paclitaxel. Additionally, treatment with sv6D after paclitaxel further extended survival without developing additional toxicity.

On the other hand, svL4 strongly inhibited the growth of implanted glioma tumour cells in the brain of mice and enhanced survival after a brief, low dose radiation treatment. Similarly to that observed with sv6D, no toxicity has been detected with svL4. The peptide appears well tolerated and effective at very low concentrations.

A Molecular Switch?

Dose-response experiments with svL4 and sv6D revealed important aspects of the physiological roles of the receptor CLEC10A. Interestingly, low doses of the peptides produced an activation of the CLEC10A-mediated immune response. Increasing the dose of the peptides caused a peak in activation, followed by a steep inhibition of the immune response at higher doses of the peptides. These data suggest that the CLEC10A receptor might act as a molecular switch that can either initiate or block the immune cascade in response to the concentration of ligand.

CLEC10A could be targeted pharmacologically by different doses of modulator peptides, depending on the desired therapeutic outcome. In the context of immunosuppression, low concentration of the peptides would activate the immune system, while in the context of inflammation a higher concentration of Susavion's peptides might be desirable.

A Bright Future for Susavion Biosciences

Susavion Biosciences, Inc. is pioneering the development of effective drugs based on peptides that work by activating the immune system without causing toxicity. The peptides in question mimic the sugar chains that bind to receptors expressed by immune cells. The scientists at Susavion propose that their peptides could be used therapeutically on their own or in combination with other treatments. Susavion's peptides activate dendritic cells and macrophages, which are key in initiating the concerted action of all the other immune system cells. They resemble the action of *N*-acetylgalactosamine on the lectin receptor CLEC10A expressed by dendritic cells. Susavion's approach could be adopted to address the biological problem of tumour-induced immune evasion.

The peptide sv6D has excellent potential to become a breakthrough product for ovarian cancer, which is among the most challenging cancers to treat. Current therapies for ovarian cancer are associated with high levels of toxicity. The low toxicity of sv6D makes it a promising compound, which will improve the survival and the quality of life of ovarian cancer patients. Susavion also developed the peptide svH1C as a highly specific mimetic of sialic acid. The peptide binds with high avidity to siglec receptors. svH1C completely inhibits HIV-1 replication in cultures of monocytes with serum from HIV-positive patients.

In future applications, the team at Susavion hopes to broaden the applicability of their approach. The selective modulation of CLEC10A receptors and the activation of tumour-specific cytotoxic T cells can treat glioma, melanoma and other cancers. svL4 and sv6D modulate the response in a dose-dependent manner. Interestingly, higher concentrations of the peptides are inhibitory towards the immune response, rather than activating it. This means that the use of svL4 and sv6D can be adapted to other inflammatory diseases. Importantly, the inhibition of HIV infection by svH1C, in the presence of non-neutralising antibodies, offers a therapeutic avenue to explore in the context of viral infections where a pathogen, or vaccine, should not induce a neutralising set of antibodies. Such a strategy would include – but is not limited to – the development of treatments to help meet the global challenge posed by Covid-19.

Meet the researchers

Dr J. Kenneth Hoober, PhD
Chief Scientific Officer
Susavion Biosciences, Inc.
Tempe, AZ
USA

Dr J. Kenneth Hoober obtained his PhD in Biochemistry in 1965 at the University of Michigan. Before becoming Chief Scientific Officer at Susavion Biosciences, he was Chair in Life Sciences at Arizona State University. Dr Hoober has co-authored over 100 publications.

CONTACT

E: jkhoober@susavion.com
W: www.susavion.com

Dr Laura L. Eggink, PhD
President
Susavion Biosciences, Inc.
Tempe, AZ
USA

Dr Laura L. Eggink is a former Assistant Professor of Biomedicine and Biotechnology at Arizona State University, USA. She is an expert in cell signalling and peptide mimetics and is co-inventor with Dr Hoober on 14 patents.

CONTACT

E: Laura.Eggink@susavion.com
W: www.susavion.com

KEY COLLABORATORS

Katherine F. Roby, PhD, Research Professor, University of Kansas Medical Center
Carl V. Hanson, PhD, Director, Viral and Rickettsial Disease Laboratory, California Department of Public Health
Mark C. Preul, MD, Director, Neurological Research Laboratory, Barrow Neurological Institute

Susavion BIOSCIENCES, INC.

FURTHER READING

LL Eggink, KF Roby, R Cote, JK Hoober, An innovative immunotherapeutic strategy for ovarian cancer: CLEC10A and glycomimetic peptides, Journal for ImmunoTherapy of Cancer, 2018, 6, 28.

LL Eggink, GA Spyroulias, NG Jones, CV Hanson, JK Hoober, A Peptide Mimetic of 5-Acetylneuraminic Acid-Galactose Binds with High Avidity to Siglecs and NKG2D, 2015, PLoS ONE, 10, e0130532.

JK Hoober, ASGR1 and Its Enigmatic Relative, CLEC10A, International Journal of Molecular Sciences, 2020, 21, 4818.

LL Eggink, M Salas, CV Hanson, JK Hoober, Peptide Sugar Mimetics Prevent HIV Type 1 Replication in Peripheral Blood Mononuclear Cells in the Presence of HIV-Positive Antiserum, AIDS Research and Human Retroviruses, 2010, 28, 149–160.

EXPLOITING DRUG METABOLISM TO ACTIVATE IMMUNITY AGAINST CANCER

Multidrug resistance is one of the main culprits underlying the failure of chemotherapy as a cancer treatment. Whilst many therapies are initially effective, a considerable proportion of patients eventually incur a poor prognosis and recurrence of malignant spread due to developing drug resistance at a later stage. **Dr Rock J. Mancini**, from Washington State University, has devised an approach that exploits proteins over-expressed in drug-resistant cancers to convert inactive prodrug substrates into active drugs that initiate an immune response targeted at cancer cells.

Fighting Multidrug Resistance in Cancer

In recent years, there have been significant advances in the treatment of several types of cancer with chemotherapy and immunotherapy alike. Many cancers are either resistant to specific chemotherapeutic drugs or acquire drug resistance at a later stage, despite initially responding favourably to therapy. However, immunotherapy has also emerged as a new class of cancer treatment with efficacy that is fundamentally orthogonal to acquired chemotherapeutic drug resistance.

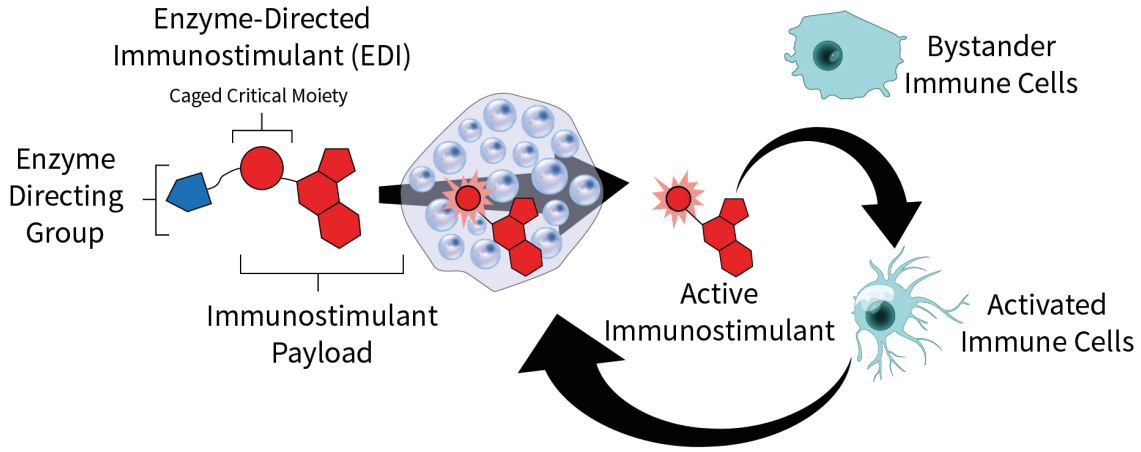
There are several mechanisms through which cancer cells develop drug resistance. One commonality across many drug-resistant cancers is that they use molecular transporters that derive energy from intracellular adenosine triphosphate to actively pump cancer drugs out of the cells; a series of biochemical reactions resulting in the mechanisms of drug resistance known collectively as 'drug efflux'.

To address this, Dr Rock J. Mancini, Assistant Professor of Organic Chemistry at Washington State University, is pioneering methods that exploit drug efflux to link the immune system, at a chemical level, to drug-resistant cancers. Dr Mancini is working to create an array of smart immunostimulant drugs that are finely modulated by stimuli including enzyme activity, temperature, and disease-specific biomarkers that are present in or on cancer cells.

Dr Mancini's team of researchers have devised an ingenious strategy that exploits drug efflux to raise an immune response against cancer cells, an approach that could provide clinical advantage to patients with multidrug-resistant cancers. The approach uses smart immunostimulants that are metabolised by cancer cells, which then secrete immunostimulant drugs via drug efflux, effectively alerting the immune system to the cancer. By coupling cancer cell metabolism and drug efflux to activate the immune system, the team hopes to affect the ability of cancer cells to escape their fate. The hope is that, by selectively activating immune cells in close proximity to cancer cells, this technology will enable a new way in which drug-resistant cancers can be eradicated, and tumour growth and spread can be halted.

Molecular structure of a smart immunostimulant developed by the Mancini Laboratory.

Overview of Bystander-Assisted ImmunoTherapy (BAIT)



A Trojan Horse to Trigger Immunity Against Cancer Cells

Over the past decades, medicinal chemists have contributed to the rational design and production of synthetic immunostimulants, compounds that interact with specific receptors on immune cells, triggering a proinflammatory response. However, immunostimulants are susceptible to diffusion, off-target activity, and other drug resistance mechanisms, including the drug efflux pathway described above.

Dr Mancini's group intentionally uses immunostimulants as drug efflux substrates to hijack the drug resistance mechanism to produce molecules that activate immune cells. Specifically, his research group is recognised as the first to develop enzyme-directed immunostimulants (immunostimulants activated by enzymes present in cancer cell metabolism) and demonstrate that they undergo drug efflux from multidrug-resistant cancers.

Cocking the Molecular Guns

One approach to addressing drug resistance is directed enzyme prodrug therapy (DEPT), which attempts to outcompete drug efflux by increasing the local concentration of therapeutic drugs in the area surrounding tumour cells. In DEPT, enzymes within a solid tumour convert an enzyme-directed prodrug into an active anti-tumour chemotherapeutic. With this approach, there is the potential for diffusion of the active drug away from the target cell, where it can interact with other nearby cells via a phenomenon known as the 'bystander effect'. The bystander effect can be beneficial, resulting in the permanent damage of nearby cancer cells, or unwanted, resulting in overall toxicity from the damage of nearby non-cancerous cells.

Importantly, drug efflux enhances the bystander effect. Dr Mancini and his team created a way to harness the bystander effect for potential therapeutic advantage by replacing the chemotherapeutics typically used in DEPT with immunostimulants. This modification makes the bystander effect a desirable outcome, causing multidrug-resistant cancer cells to activate bystander immune cells.

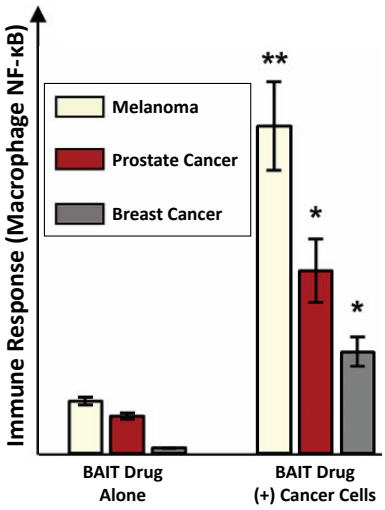
Dr Mancini and his team named their approach 'bystander-assisted immunotherapy' (BAIT). In BAIT, an inert prodrug is first metabolised by enzymes within multidrug-resistant cancer cells to produce an active immunostimulant metabolite. Following this conversion, the immunostimulant is transported to the extracellular space, via drug efflux. Once in the extracellular space, the immunostimulant activates bystander immune cells, resulting in an immune response initiated at the site of the cancer.

The advantage of engaging immune cells to target tumours is that, once they are trained to recognise cancer cells, they have the ability to destroy them with high specificity, leaving healthy cells nearby intact. Given that enzyme-directed immunostimulants are generated within the tumour microenvironment, they are perfectly placed to target tumour cells with high efficacy and low toxicity.

Cancer Takes the Bait

In two different papers, published in 2016 and 2018, Dr Mancini and his team reported the creation of the first enzyme-directed proimmunostimulants in the form of imidazoquinoline-pyranosides, substrates for hydrolase enzymes, such as the alpha-mannosidases expressed by cancer cell metabolism. Effectively acting like a piece of cheese in a mousetrap, the BAIT prodrug is harmless and inert until it is eaten (metabolised) by cancer cells, ultimately initiating an anti-cancer immune response.

In the system developed by Dr Mancini's laboratory, alpha-mannosidase converts the prodrug substrate into the active immunostimulant imiquimod. The studies were conducted *in vitro* on cultured prostate cancer cells, and the enzyme-mediated production of imiquimod resulted in the activation of macrophages and dendritic cells, providing robust evidence that the



Data taken from Ryan et al., Comparing the immunogenicity of glycosidase-directed resiquimod prodrugs mediated by cancer cell metabolism, Acta Pharmacologica Sinica, 2020, doi: 10.1038/s41401-020-0432-4

mechanisms of drug efflux in cancer cells can be exploited via BAIT. Like most cancer immunotherapies, BAIT offers the advantage of specifically targeting one undesired type of cell, unlike other chemotherapeutic agents that cause high levels of toxicity due to their lack of specificity.

Dr Mancini and his team used cell cultures to demonstrate that enzyme-directed immunostimulants only target those cultures that overexpressed the corresponding proteins associated with multidrug-resistant cancer cells. This indicates that this method triggers a cancer-specific immune response without causing inflammatory toxicity, and could be used alongside blockers of immunosuppression to improve efficacy. Preliminary *in vivo* mouse studies demonstrate that enzyme-directed proimmunostimulants are well-tolerated and do not cause systemic inflammatory toxicity in otherwise healthy mice. The team is currently testing the efficacy on a model of drug-resistant breast cancer in mice, providing evidence that immunostimulants produced by the enzyme-directed BAIT method are more effective when compared to directly administered parent immunostimulant.

Comparing the Immunostimulant Effect Across Different Cancer Cell Lines

In optimizing the BAIT approach, Mancini and his group compared the immunogenicity of several enzyme-directed prodrugs that all have the imidazoquinoline immunostimulant resiquimod embedded in their structure. Like imiquimod, resiquimod is a ligand for immunogenic toll-like receptors. This compound was chosen for its potency at nanomolar concentration, rather than simplicity, as was the case in earlier iterations. The cancer types studied were skin cancer, prostate cancer, and breast cancer, because they are among the most frequently diagnosed cancers in the USA.

The group published their latest results in 2020 which confirm that immunogenicity across different cancer cell lines is due to the enzymatic conversion of substrate prodrug into the immunostimulant resiquimod, liberated following drug efflux. The team compared cancer cell metabolism of the prodrug to adding the enzymes responsible for metabolism separately, both of which resulted in increased production of active immunostimulant and the activation of immune cells. It was established that melanoma cells *in vitro* showed the greatest extent of immunogenicity, followed by prostate cancer cells and breast cancer cells.

Not surprisingly, the group established that different cell lines displayed different levels of enzyme expression and activity. Those differences in glycosidase activity and drug efflux across different cancer cell lines will provide insight into which factors affect the cancer-mediated activation of immune cells, serving as a powerful diagnostic tool to predict the efficacy of BAIT in specific cancers.

Optimizing BAIT Drug Design and Other Future Developments

The team is currently optimising a lead enzyme-directed immunostimulant for further studies *in vivo*. Dr Mancini and his group also hope to confirm the observation that cancer cell drug efflux, a trait linked to drug resistance, is the most important factor that drives efficacy of the prodrugs. To test this hypothesis, his lab has spent the past months growing cells that are resistant to chemotherapy, a process that involves adding chemotherapeutic drugs to the cell culture and selecting the resistant cancer cells that survive. Overall, the findings suggest that BAIT is best suited for cancer cells that overexpress proteins associated with drug efflux, in particular multidrug-resistant cancers that do not respond to chemotherapy. However, to understand the process at a molecular level, more studies will be needed to characterise all of the possible routes of efflux, as the immunostimulant might be a substrate for many different transport proteins involved in drug efflux.

Given that the efficacy of traditional chemotherapeutics is attenuated by mechanisms of drug resistance, Dr Mancini's research efforts will continue to provide tools to interrogate the immune system, shedding light on how immune cells can be activated and initiate a targeted response against cancer. In the long term, the team has the ambition of contributing significantly to the field of medicinal chemistry by designing the next generation of immunotherapeutics and cancer vaccines.

Meet the researcher

Dr Rock J. Mancini
Department of Chemistry
Washington State University
Pullman, WA
USA

Dr Rock J. Mancini is Assistant Professor of Organic Chemistry at Washington State University. Dr Mancini obtained his PhD in Organic Chemistry from the University of California, Los Angeles, in 2012. He subsequently trained as a synthetic immunologist from 2012 to 2015 while pursuing his postdoctoral studies at the University of California, Irvine. Since starting his tenure track position in 2015, his research interests have evolved to incorporate organic chemistry, chemical biology, and polymer chemistry. The research focus of his laboratory is the development of methods to both control and interrogate the immune system, aimed at creating the next generation of immunotherapeutics and vaccines. Dr Mancini mentors several junior researchers in his team and encourages them to reach their full potential as scientists by acquiring a deep understanding of basic and applied research processes, from hypothesis generation to its proof of concept and ultimate dissemination to the scientific community.

CONTACT

E: rmancini@wsu.edu
W: https://chem.wsu.edu/faculty/mancini-rock/
@ManciniLab_WSU

WASHINGTON STATE UNIVERSITY

KEY COLLABORATORS

Dr Amy E. Nielsen, Clinical Assistant Professor of Chemistry, Washington State University
Dr Maryam Davaritouchaee (Postdoctoral Scholar, Mancini Group)
Austin Ryan and Anthony Burt (Graduate Student Researchers, Mancini Group)

FUNDING

The research reported in this publication was supported by the National Cancer Institute of the National Institutes of Health under Award Number R01CA234115. The content is solely the responsibility of the researchers and does not necessarily represent the official views of the National Institutes of Health.

FURTHER READING

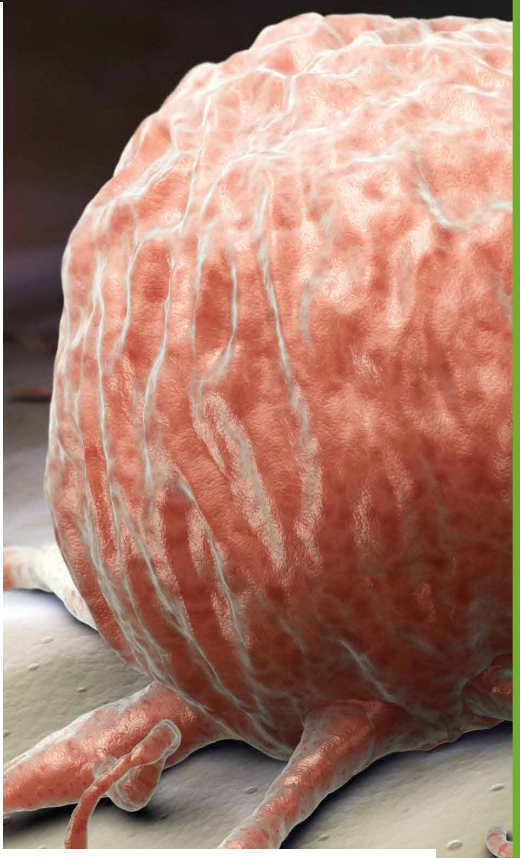
AT Ryan, AJ Pulukuri, M Davaritouchaee, et al, Comparing the Cancer Cell Metabolism and Immunogenicity of Glycosidase-Directed Resiquimod Prodrugs Mediated by Cancer Cell Metabolism, Acta Pharmacologica Sinica, 2020, 41, 9915–1004.

AJ Burt, JD Hantho, AE Nielsen, RJ Mancini, An Enzyme-Directed Imidazoquinoline Activated by Drug Resistance, Biochemistry, 2018, 57, 2184–2188.

JD Hantho, TA Strayer, AE Nielsen, RJ Mancini, An Enzyme-Directed Imidazoquinoline for Cancer Immunotherapy, ChemMedChem, 2016, 11, 2496–2500.

UNDERSTANDING SEX DIFFERENCES IN CANCER PROMISES BETTER TREATMENT AND SURVIVAL

Differences in the prevalence and survival of male and female cancer patients have long been acknowledged but not well understood. **Dr Josh Rubin** (Departments of Paediatrics and Neuroscience at Washington University) and his collaborators have been the first to identify sex-specific differences in malignant transformation. This evidence will help to optimise sex-specific approaches to cancer treatment and contribute to the improvement of the outcomes and survival of cancer patients.



Sex Differences in Brain Tumours

'Cancer exhibits a significant sex difference in rates and survival. Males get more cancer and die more often from their cancer than females do' explains Dr Josh Rubin from the Departments of Paediatrics and Neuroscience at Washington University School of Medicine, USA. Many of the disparities between male and female disease can be explained by differences in sex hormones. One example is the higher occurrence of autoimmune disease in females of reproductive age and the increased incidence of cardiovascular disease in postmenopausal females.

However, not all sex disparities can be explained by the activity of circulating sex hormones. For example, certain brain tumour types occurring in very young and peripubertal children are twice as likely to develop in boys than in girls. In cases such as these, circulating sex hormones can neither explain the vulnerability of males nor the protection of females. These observations led the research group headed by Dr Rubin to investigate the alternative mechanisms by which sex may be influencing brain tumourigenesis in humans.

Higher Prevalence of Glioblastoma in Males

In adults, glioblastoma (GBM) is the most common type of brain cancer. Multiple recent reports from collaborator, Dr Jill Barnholtz-Sloan, have established that GBM is much more prevalent in men and the 5-year survival rate is significantly lower in men compared with females with GBM. In a 2014 paper, Dr Rubin and colleagues reported their investigation into whether there is an effect of sex on cell-intrinsic sexual dimorphism in tumour progenitor cells.

At the time, GBMs were divided into four subtypes (classical, mesenchymal, neural and proneural), based on their gene expression profile. Looking at two data sets for GBM, the team found that all subtypes other than classical GBMs were more prevalent in males with the greatest difference – two-fold – for the mesenchymal subtype. This suggested to the team that the effect of sex on GBM was dependant on the molecular

subtype of the disease. Dr Rubin and his group went on to demonstrate, for the first time, that there are sex-specific, cell-intrinsic differences in malignant transformation.

To accomplish this, Dr Rubin and Dr Tao Sun focused on sex differences in the cellular response to loss of two tumour suppressor genes commonly mutated in GBM, Neurofibromin (NF1) and p53. The loss of both NF1 and p53 in astrocytes, the cell lineage from which glioblastoma rises, led to malignant transformation of male, but not female, astrocytes,

suggesting that sex is a factor in glioma formation upon loss of NF1 and p53 function.

The first step was to isolate male and female astrocytes with complete loss of *Nf1* (*Nf1*$^{-/-}$) and to assess whether they differed in their growth rates. While the loss of *Nf1* alone was not associated with any sex difference in growth rates, the additional loss of p53 function resulted in a greater increase in the growth of male astrocytes as compared with female astrocytes. Upon further activation of the epidermal growth factor receptor (EGFR) – a protein involved in cell growth and differentiation (and frequently activated in GBM) male cells, but not female cells were fully transformed.

In order to assess whether this would result in sex differences in *in vivo* tumourigenesis, Dr Rubin and colleagues implanted the male and female cells into the brains of male and female immunocompromised mice. They found that 100% of recipient mice implanted with the male astrocytes developed tumours and died of the disease. In contrast, only 36% of recipient mice implanted with the female astrocytes developed the disease and died. A closer look at the tumours revealed that tumourigenesis was determined by the sex of the implanted cells and not by the sex of the recipient mouse.

To better understand this result, the team examined the regulation of the retinoblastoma protein (RB) pathway which is a negative regulator of proliferation. RB loss or inactivation is one of the most common features of human cancers. Experiments revealed that male astrocytes exhibited much greater time dependant phosphorylation of RB (which inactivates the inhibitory functions of RB) compared with female astrocytes, strongly indicating that RB regulation is sexually dimorphic in these astrocytes and leads to greater proliferation in male cells. When p53 and RB pathways were inactivated in male and female *Nf1*$^{-/-}$ astrocytes, male and female astrocytes are equally transformed. Dr Rubin and colleagues concluded that sexual dimorphism in the regulation of RB function is likely to be contributing to sex differences in cancer.

Sexual Dimorphism and Decreased Survival in Males

Next, Dr Rubin and Dr Joseph Ippolito wanted to investigate the role of metabolism in the sex disparity seen in brain cancers. Metabolism is a key factor in tumour survival and tumourigenicity, and one of the hallmarks of cancer metabolism is enhanced glucose uptake and its conversion to lactate, even in the presence of oxygen. This process results in the rapid generation of adenosine triphosphate through glycolysis, and also contributes to the pathways required for proliferation.

The data revealed that the overexpression of glycolytic genes resulted in decreased survival in males. Moreover, patients within this high-glycolytic group showed significant differences in the presence of key genomic alterations, including isocitrate dehydrogenase (*IDH*) mutation compared with the low-glycolytic group. Overall, the study showed that glycolytic stratification defined poor prognosis in males. The research unexpectedly revealed that females with high-glycolytic gene expression and

wild type *IDH* mutation survived longer than all other wild type patients. The findings from these studies suggest a synergistic relationship between sex, tumour metabolism and genomic alterations in glioma.

Cell Cycle Inhibitors p16 and p21

Dr Rubin and his team, having previously found that sex differences in the regulation of RB correlated with differences in clonogenic cell function, cell proliferation and *in vivo* tumourigenesis, then wanted to investigate how cyclin dependant kinase (CDK) inhibitors contribute to sex differences in RB regulation, tumourigenesis and response to DNA damage. CDK inhibitors function as cell cycle inhibitors and the team looked at p16, p21 and p27 in mice GBM astrocytes under conditions that promote RB-dependant growth arrest.

They first examined the expression of the CDK inhibitors. Female cells had higher levels of p21 protein expression compared to male GBM astrocytes and female tumours expressed significantly higher levels of p21. These data confirmed to the team that there are significant sex differences in the expression of these key negative regulators of cell growth.

To investigate whether these differences in *p21* expression might underlie female protection from transformation, Dr Rubin and Dr Najla Kfoury looked at whether there were differences in the expression of these regulators in response to changes in growth factor availability and DNA damage, two stressors that activate CDK inhibitors under normal circumstances. Serum (growth factor) was withdrawn for 48 hours from GBM astrocytes and the researchers found that *p16* mRNA and protein levels were significantly higher in female but not in the male astrocytes. When DNA damage was initiated by etoposide there was a substantial increase in p21 mRNA and protein in both female and male GBM astrocytes, but the increase was far greater and more significant in female astrocytes. They went on to demonstrate that when they deleted p16 and p21 from female GBM astrocytes, they responded like male astrocytes to these conditions, indicating that expression of CDK inhibitors was required for the relative female protection from transformation.

Sex Differences in GBM Treatment Response and Survival

More recently, Dr Rubin and Dr Kristin Swanson used a method based on magnetic resonance imaging to calculate tumour growth velocity in patients as a measure of response to treatment. The study found that females had a better response to standard treatment than males with GBM. The data suggest that females with GBM may benefit more from standard treatment than males with GBM and the difference in response may be due to tumour growth velocity which in turn may contribute to survival. Next, Dr Rubin and Dr Will Yang looked at potential differences in GBM by examining transcriptome (collection of all RNA transcript) data from The Cancer Genome Atlas for GBM. These experiments identified sex-specific molecular subtypes of GBM and revealed that expression of cell cycle regulators correlates with survival in male GBM and integrin signalling correlates with survival in females, further supporting the importance of sex differences in GBM.

Implications for the Future of GBM Diagnosis and Treatment

The research by Dr Rubin and colleagues highlights the significance of understanding the molecular basis for sexual disparity in GBM. This understanding will lead to the development of diagnostics and treatments that take sexual disparity into consideration. Dr Rubin explains 'The importance of what we have found is how it will inform efforts to develop sex-specific approaches to cancer therapy'. Targeting sex-specific components of the disease undoubtedly holds the potential to improve outcomes for both male and female cancer patients.

Meet the researcher

Dr Joshua B. Rubin
Washington University in St. Louis
School of Medicine
Neuroscience
St. Louis, MO
USA

Dr Joshua B. Rubin is currently a Professor of Paediatrics and Neuroscience at Washington University of Medicine in St. Louis, USA. Dr Rubin obtained his MD and PhD from the Albert Einstein College of Medicine in New York City in 1994. Following his doctoral research, Dr Rubin completed a residency in Paediatrics at Boston Children's Hospital followed by a fellowship in Paediatric Haematology and Oncology at the Dana Farber Cancer Institute and Harvard Medical School to train, where he concentrated on paediatric neuro-oncology. Dr Rubin's research team focuses on the mechanisms of tumourigenesis and resistance to therapy with the aim of improving the outcomes of patients with malignant brain tumours. Amongst numerous positions on advisory boards and panels, Dr Rubin has been an author on over 100 publications. As an esteemed international basic and clinical researcher, Dr Rubin has been awarded substantial and prestigious funding to support his work.

CONTACT

E: rubin_j@wustl.edu

KEY COLLABORATORS

Rosy Luo (Washington University School of Medicine)
Robi Mitra (Washington University School of Medicine)
Will Yang (Washington University School of Medicine)
Joseph Ippolito (Washington University School of Medicine)
Sonika Dahiya (Washington University School of Medicine)
Kristin Swanson (Mayo Clinic, Scottsdale)
Jill Barnholtz-Sloan (Case Western Reserve University)
Justin Lathia (Cleveland Clinic)
Michael Berens (TGen)
James Connor (Pennsylvania State University)

Washington University in St. Louis

CURRENT FUNDING

National Institutes of Health
Children's Discovery Institute
Prayers from Maria Foundation
Barnes Jewish Foundation
American Brain Tumor Foundation

FURTHER READING

JB Rubin, JS Lagas, L Broestl, J Spomagel, N Rockwell, G Rhee, SF Rosen, S Chen, RS Klein, P Imoukhuede, J Luo, Sex differences in cancer mechanisms, Biology of Sex Differences, 2020, 11, 17.

M Dong, G Ciofi, J Wang, KA Waite, QT Ostrom, C Kruchko, JD Lathia, JB Rubin, ME Berens, J Connor, JS Barnholtz-Sloan, Sex differences in cancer incidence and survival: A pan-cancer analysis, Cancer Epidemiology, Biomarkers and Prevention, 2020, 29, 1389.

W Yang, NM Warrington, SJ Taylor, P Whitmore, E Carrasco, KW Singleton, N Wu, JD Lathia, ME Berens, AH Kim, JS Barnholtz-Sloan, KR Swanson, J Luo, JB Rubin, Sex differences in GBM revealed by analysis of patient imaging, transcriptome, and survival data, Science Translational Medicine, 2019, 473, eaao5253.

N Kfoury, T Sun, K Yu, N Rockwell, KL Tinkum, Z Qi, NM Warrington, P McDonald, A Roy, SJ Weir, CA Mohila, B Deneen, JB Rubin, Cooperative p16 and p21 action protects female astrocytes from transformation, Acta Neuropathologica Communications, 2018, 6, 12.

JE Ippolito, AK Yim, J Luo, P Chinnaiyan, JB Rubin, Sexual dimorphism in glioma glycolysis underlies sex differences in survival, JCI Insight, 2017, 2, e92142.

T Sun, NM Warrington, J Luo, MD Brooks, S Dahiya, SC Snyder, R Sengupta, JB Rubin, Sexually dimorphic RB inactivation underlies mesenchymal glioblastoma prevalence in males, The Journal of Clinical Investigation, 2014, 124, 4123–33.

EXPLOITING THE CANCER GENOME FOR PERSONALISED THERAPIES

The effectiveness of cancer treatments could be hugely improved by a greater understanding of the cancer genome. This is the focus of the work of **Dr John Paul Y.C. Shen**, MD, of the University of Texas MD Anderson Cancer Center, who is creating comprehensive molecular 'maps' of cancer cells and their interactions. Understanding cancer at a molecular level is the first step towards Dr Shen's very real hope of bringing personalised cancer treatments into the clinic.

From Laboratory to Clinic

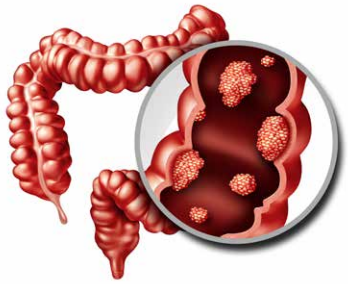
Cancer is the result of an accumulation of mutations that lead to uncontrolled cell growth, and so it makes sense that a better understanding of these genes could lead to an improvement in cancer treatments. A cell's complete set of genes is known as its genome, and for Dr John Paul Y.C. Shen, MD, a fuller understanding of the cancer genome is paving the way for delivering more effective chemotherapies in practice. Dr Shen is a physician-scientist at the University of Texas MD Anderson Cancer Centre, where he is an Assistant Professor in the Department of Gastrointestinal Medical Oncology and heads a research laboratory of experimental and computational scientists developing targeted cancer treatments.

Dr Shen aims to understand cancer on a molecular level and then transfer this knowledge to the clinic, facilitating the prediction of which chemotherapy will work for each patient. As a physician, Dr Shen treats patients with malignancies of the gastrointestinal tract including colon (colorectal) and appendix (appendiceal) cancers. His research aims to combat two major limitations that are faced in this field: a general lack of targeted therapies, and a lack of predictive biomarkers indicating which therapy a patient is likely to respond to. And it is working! So far, work in Dr Shen's laboratory has contributed to three clinical trials of novel drugs which have the potential to directly benefit the lives of colorectal and appendiceal cancer patients.

A Synthetic-Lethal Gene Pair

The major pitfall of traditional, also called cytotoxic chemotherapies is that in addition to cancer cells, they attack other rapidly growing cells in the body, such as those found in hair roots. Consequently, hair loss, nausea and vomiting, and decreased blood counts are among the many unpleasant side effects faced by cancer patients while undergoing treatment. This problem can potentially be overcome through the use of drugs which selectively target cancer cells while sparing normal cells, in a process known as 'synthetic lethality'.

Synthetic lethality depends on the phenomenon that two independent



gene mutations can cause an unexpected characteristic to arise in a cell, that is unrelated to either original gene. When this occurs, it is indicative of both original genes being part of the same biological process or pathway. Thus, damaging the two genes may result in a dramatic change, such as cell death, while damaging only one will not. The two genes are then termed a 'synthetic-lethal' gene pair.

The cancer drug Olaparib exploits this concept. Developed in 2014, it is effective against ovarian, fallopian tube, and peritoneal cancers, which share a common BCRA gene mutation. Olaparib targets the other gene in BCRA's synthetic-lethal gene pair, resulting in the death of cancer cells while normal cells remain healthy.

Discovering other such lethal gene pairs presents a significant challenge, due to the complexity and variability of cancer cells' genetic interactions. In 2017, Dr Shen was part of a team that addressed this problem in a pioneering manner.

A Novel Gene Editing Approach

While a postdoctoral fellow in the University of California's Department of Medicine in his then-mentor's lab, Dr Shen helped to develop a novel method of searching for synthetic-lethal gene pairs. The technique, which was a novel application of the CRISPR/Cas9 gene editing process, resulted in over 120 potential new targets for cancer drugs.

CRISPR is a bacterial derived mechanism used in the laboratory to knock out target genes. It does this in two stages: first, DNA recognition processes lead to the location of a pre-determined target gene in the genome, and, second, a protein called Cas9 cuts the DNA, inducing a mutation which inactivates said gene. The organism's genes are therefore edited in a way that allows scientists to investigate the functions of particular genes of interest.

In this study, Dr Shen and his team used CRISPR to inactivate 73 genes in lab-cultured kidney cells, lung cancer cells, and cervical cancer cells, for testing a total of 150,000 unique gene interaction combinations. They then looked at the effects on cell growth, and ultimately uncovered 120 new synthetic-lethal interactions. This was the first time that such a low-cost and high-throughput approach had been carried out to make this discovery, and it presented opportunities both to gain a greater understanding of cancer development, and to develop new therapies.

Promises and Pitfalls of Synthetic Lethal Interactions

The marvellous opportunities presented by CRISPR screening for synthetic-lethal gene pairs are aided by decreased cost and increased throughput compared to previous methods. Synthetic lethal interactions fall into the growing field of precision oncology, as they overcome the difficulties faced by more traditional chemotherapies of damaging healthy cells. In the future, novel drug development must consider the source of cancer since many of the synthetic-lethal gene pairs were only fatal in one of the three cell types. Additionally, the team's findings must be validated in further cell types and mice models; this nevertheless presents a promising start to the development of more targeted cancer therapies.

Perhaps the greatest barrier faced by Dr Shen's work in developing synthetic-lethal therapies is the diversity of cancer at a molecular level. Even two seemingly identical tumours of the same type in different patients have vastly different genetic makeups; this is also true of different cells within the same single tumour. This explains why chemotherapy can never be 'one-size-fits-all', and why molecular profiling of patients' cancers is beneficial when planning treatment options. Along these lines, proposed novel cancer therapies must be heavily context specific.

Considering the complexities of the relationships involved, Dr Shen's team pointed out that in the development of new synthetic lethal drugs, it will be just as useful to know when a drug will not work, as it will be to know when one will. Patients will be genetically profiled to reveal any predispositions to certain drugs in a system which is already in

existence. During colorectal tumour diagnoses, patients are currently screened for mutations which correlate with a lack of response to anti-EGFR antibody treatments. Screening processes of the same kind could easily be applied to synthetic-lethal drug interactions.

The Genetic Landscape of Appendiceal Cancers

The cancer genome is incredibly complex and incompletely understood, and therefore, treatment guidelines are typically based on data from clinical trials. This presents an issue with rarer cancer types, including appendiceal cancer, where very little clinical trial data are available. Patients presenting with appendiceal cancers tend to receive treatment in the form of colorectal cancer chemotherapy regimens, despite the multitude of differences between the two types. Dr Shen aimed to remedy this through his 2018 molecular profiling of 703 different appendiceal cancer samples, which enabled the discovery of novel biomarkers for these elusive tumours.

Each cancer was sequenced and analysed for individual mutations, while looking for trends in the entire cohort that could help to guide treatment. The team found that appendiceal cancers had molecular profiles that were distinct from colorectal cancers, suggesting that their treatments should vary. In addition, two genes were identified as biomarkers for appendiceal cancer (TP53 and GNAS), which is significant due to their ease of detection. This comprehensive portrait of the genomic landscape of appendiceal cancer highlights the importance of the molecular profiling of cancers in order to discover new biomarkers and advance existing treatments. More directly, it will help with the development of future clinical studies in appendiceal cancer, to allow a more specific treatment approach going forward.

Paving the Way for Precision Medicine in the Treatment of Appendiceal Cancer

For Dr Shen, his work in the fight against cancer is just getting started. Despite research demonstrating the differences in tumour molecular profiles between the two, the practice of treating appendiceal cancer with colorectal cancer chemotherapies is likely to continue unless specific cancer treatments for the former are developed. Dr Shen's proposal illustrates his ambition to carry out a series of experiments that will provide a foundation for future drug developments that are specific to appendiceal cancers.

Dr Shen hopes to construct, characterise, and test preclinical models of appendiceal cancer to identify new drug targets in appendix cancer. He is also working to establish a database of appendix cancer mutation and transcription data, where patients could submit data on their own tumour in an anonymous fashion. By 'crowd-sourcing' the collection of appendix cancer data, he hopes to collect enough data to understand how appendix tumours differ from one another. Considering the ability of cross-interactions between seemingly unrelated genes to cause synthetic lethality, it is clear that cancer should be considered a network-based disease. However, traditional biological pathways are heavily rewired in cancer cells, and different tumour types contain unique genetic networks which are context specific.

This environmental specificity adds another layer of complexity, part of why Dr Shen is trying to generate more data from mouse models of appendix cancer where the tumours grow in the peritoneal cavity, which surrounds the intestines, similar to the usual spread of appendix cancer in humans. He is also working with collaborators to use automated intelligence (AI), also called machine learning, to help make predictions from this complex dataset. The end goal of identifying more effective appendiceal cancer therapies and/or predictive biomarkers will take years of dedicated effort, but meaningful progress is expected in the near future.

Meet the researcher

Dr John Paul Y.C. Shen, MD
Department of Gastrointestinal Medical Oncology
University of Texas
Houston, TX
USA

Dr John Paul Y.C. Shen is a physician-scientist who received his MD from Washington University in Saint Louis in 2008, before undertaking clinical training at the University of California, San Diego in internal medicine, haematology, and oncology. He then completed a postdoctoral fellowship in cancer genomics under the mentorship of Professor Trey Ideker in 2018, alongside his career as a physician. Today, he works as an Assistant Professor in the Department of Gastrointestinal Medical Oncology and a Cancer Research and Prevention Institute of Texas (CPRIT) Scholar in Cancer Research at the University of Texas MD Anderson Cancer Centre, where he heads a laboratory of scientists studying the genomics of colorectal and appendiceal cancers. His long-term research goal is to better understand the cancer genome for the delivery of more effective chemotherapies. Among many other accolades and awards, in 2016 he was selected as a NextGen Star by the American Association for Cancer Research.

CONTACT

E: jshen8@mdanderson.org
W: https://www.mdanderson.org/research/departments-labs-institutes/labs/john-paul-shen-laboratory.html
jpshen_md

THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center

KEY COLLABORATORS

Scott Kopetz (MDA)
Michael Overman (MDA)
Kanwal Raghav (MDA)
Keith Fournier (MDA)
Wenyi Wang (MDA)
Jianzhu Ma (Purdue)
Silvio Gutkind (UCSD)
Dionicio Siegel (UCSD)
Xiling Shen (Duke)

FUNDING

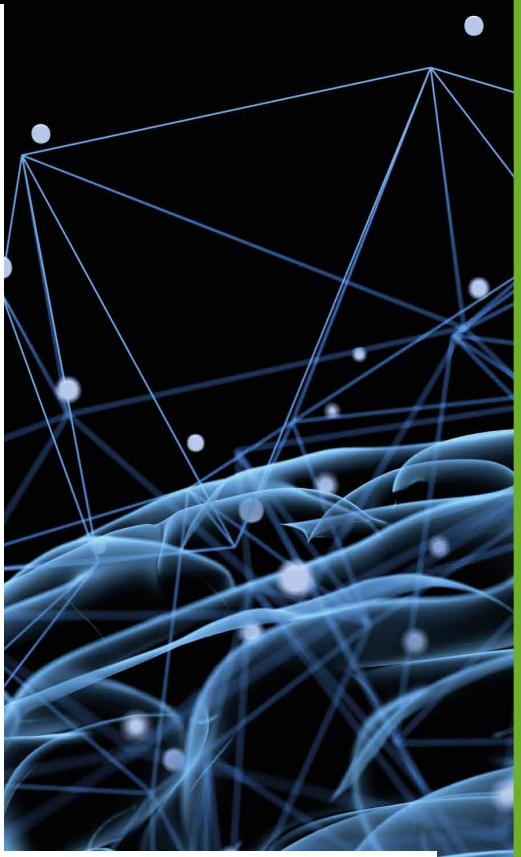
Cancer Research and Prevention Institute of Texas (CPRIT)
National Cancer Institute (K22 CA 234406-01)

FURTHER READING

C Ang, J Shen, C Hardy-Abeloos, et al, Genomic Landscape of Appendiceal Neoplasms, JCO Precision Oncology, 2018, 2, 1–18.
J Shen, T Ideker, Synthetic Lethal Networks for Precision Oncology: Promises and Pitfalls, Journal of Molecular Biology, 2018, 430, 18, 2900–2912.

J Shen, D Zhao, R Sasik, J Leubeck, et al, Combinatorial CRISPR–Cas9 screens for de novo mapping of genetic interactions, Nature Methods, 2017, 14, 573–576.

OVERCOMING THE BARRIERS TO BRAIN TUMOUR THERAPY



Brain tumours and other central nervous system diseases can be exceptionally difficult to treat. This is often due to the blood-brain barrier which can pose a significant obstacle when trying to get drugs to their intended site of action. **Dr Sean Lawler** and his team from Brigham and Women's Hospital, Harvard Medical School are aiming to bridge the gap between laboratory research and clinical treatment in their quest to find new ways to effectively deliver therapies to the brain and treat challenging diseases, especially the lethal brain tumour glioblastoma.

The Blood-brain Barrier: A Help and a Hindrance

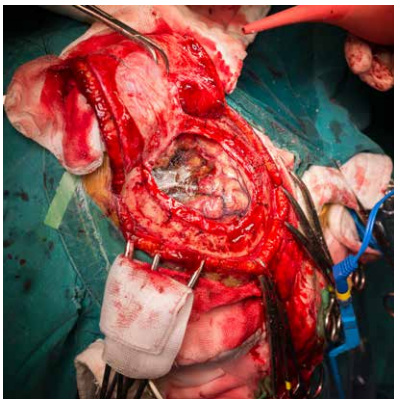
Diseases of the central nervous system (CNS) and brain can be severely debilitating or deadly, and most are difficult to treat effectively. Cancers like glioblastoma present with bleak survival expectations of an average of only 15 months, and are among the most challenging tumours to treat. This is in part due to the presence of the blood-brain barrier (BBB), a shield-like border of specialised cells which surrounds all the blood vessels in the brain. The BBB allows oxygen and essential nutrients to pass from the blood to the cells of the brain but is highly selective in allowing other molecules, including many therapeutic drugs to cross.

The BBB is critical in maintaining tight control over the CNS, where the entry of pathogens, toxins, and even immune cells can be dangerous. But despite its essential role, when we want to treat diseases of the brain the BBB can hinder our efforts to deliver drugs to where they need to be by blocking their entry and actively removing them from the CNS. This means scientists have had to develop novel ways to get through, either by smuggling molecules past the BBB checkpoint in disguise or bypassing the BBB entirely and injecting drugs straight to the target.

Brain tumours come with further difficulties, arising from their ability to hide from the immune system, preventing our natural defences from taking them down. Dr Sean Lawler and his team from Brigham and Women's Hospital, Harvard Medical School, are working to develop new ways to tackle brain tumours. They are creating models of the BBB to test new compounds and hoping to translate novel therapies from the laboratory to clinical trials.

Bridging the Gap

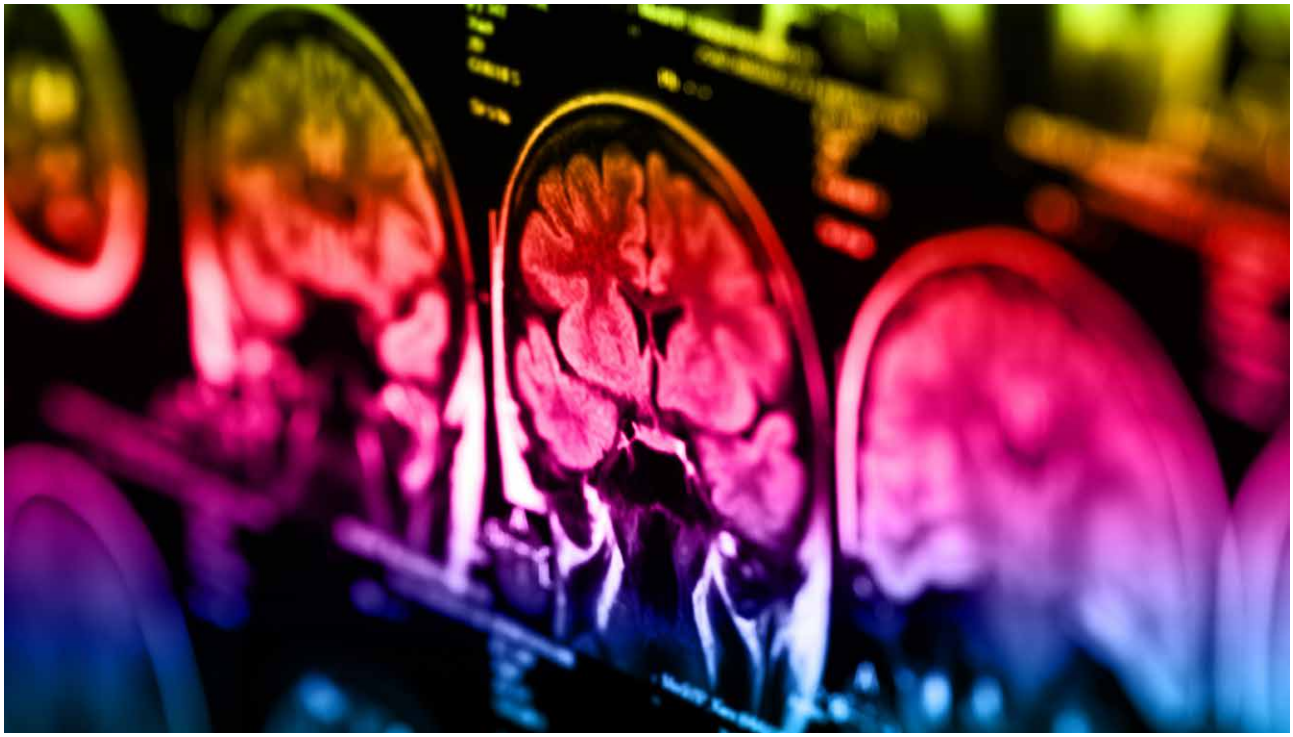
'Our goal is to be able to effectively deliver therapies to the brain,' says Dr Lawler about his work. Biomedical science is at a pivotal moment, where our ever-improving understanding of diseases is coinciding with advancements in our technological abilities, allowing exciting new avenues to open up. Dr Lawler's work focuses on translational medicine, which is



the practice of getting new therapies from the laboratory to the clinic and then using observation of patient responses to further improve and develop the concept. Dr Lawler's team is highly collaborative, using skills of physicists, chemists, biologists and mathematicians to try to solve the riddle of effective drug delivery to the brain.

Developing a useful model of the BBB is central to progressing drug discovery before costly human trials can take place. Unfortunately, due to the extremely specialised nature of these cells, it is almost impossible to culture them *in vitro* and retain the

‘Our goal is to be able to effectively deliver therapies to the brain.’



unique characteristics they possess in the brain. Key proteins which modulate the passage of molecules and the tight junctions between cells are lost when the cells are grown in the laboratory, which means that testing drugs on them is not representative of what would happen *in vivo*.

Dr Lawler and his team have been able to create a spheroid model of the BBB which keeps many of its functions outside of the body. Co-culturing the various cells which make up the BBB causes them to self-assemble into a 3D structure by allowing them to interact with each other as they would *in vivo*. Dr Lawler’s model successfully reproduced functions of the natural BBB, such as the proteins and junctions, and offers a convenient way to study transport from the bloodstream to the brain. This can aid the screening of drugs, to see what kind of molecules are allowed to cross, and critically, to find novel therapeutics for brain diseases.

Using Vectors to Cross the Blood-brain Barrier

Many attempts to get drugs through the BBB have focused on temporarily disrupting the barrier, either chemically or mechanically. Unfortunately, these techniques come with many limitations, including the side effect of letting toxins enter the brain, causing neurotoxicity.

A different way to overcome this challenge is to use molecular delivery vectors, called ‘BBB shuttles’. The vector acts as a vehicle to carry the drug through the cellular blockade. Often, this is achieved by attaching proteins to the drug which interact with specific receptors on the surface of the BBB cells. Many different strategies have been trialled to find the most efficient vector and recent work has shown that a process known as peptide macrocyclisation can significantly improve the uptake of the vector-drug combinations across the BBB.

Normally, proteins used in vectors are liable to be degraded quickly, reducing the amount available. Dr Lawler and his team have shown that a chemical process, known as nucleophilic aromatic substitution, can turn proteins into ring-like structures (macrocycles) which prevents them from being degraded as well as augmenting their uptake into cells and enhancing their ability to cross the BBB.

In a set of experiments, Dr Lawler used his spheroid BBB model to demonstrate that macrocycles can efficiently penetrate the brain and showed that in mice macrocycle uptake was significantly increased compared to other proteins. This research holds a huge amount of promise for the future of delivering therapeutics to the CNS, and elucidation of the exact method of transport will help scientists learn more about the BBB and how to get past it.

Boosting the Immune System

Treating brain diseases like glioblastoma doesn’t solely rely on getting chemotherapy drugs into the brain. Dr Lawler and other translational research scientists have also been looking at ways in which the immune system can be targeted to help fight tumours from within.

Since tumours are highly capable of evading the immune system, attempts have been made to ‘reactivate’ the

immune response to cancer. Cancer cells can prevent the crucial interactions between immune cells necessary for T-cell activation, and a class of drugs known as immune-checkpoint inhibitors attempt to override this. Unfortunately, specific aspects of glioblastoma tumours mean that these inhibitors are less effective than in other cancers, and few patients respond as robustly as we would hope. Evidently, there is a need for better immune-related cancer therapeutics.

Dr Lawler has been studying gene-mediated cytotoxic immunotherapy (GMCI) for glioblastoma. This complex approach involves the injection of a non-replicating virus into the tumour where the virus expresses a specific gene. Upon administration of a known drug, called ganciclovir, the gene converts this into a toxic product, allowing extremely targeted chemotherapy right in the tumour itself.

On top of this, GMCI is capable of activating a strong immune response which remains at the site of the tumour and can prevent further growth. Dr Lawler wanted to find out if combining GMCI with immune-checkpoint inhibitors would enhance the activity of both treatments. In a phase 1 clinical trial using mice, he and his team demonstrated that this combination increased the number of long-term surviving animals compared to each drug acting alone. His data suggested that the treatments act synergistically, with each enhancing the activity of the other.

Going forward, Dr Lawler hopes to find out whether this combined therapy can overcome the initial resistance of glioblastoma to treatment, which is often seen in newly diagnosed cases. For now, the results provide an encouraging platform for further investigation into combined immunotherapies.

Forward Thinking

It remains clear that glioblastoma is an especially challenging cancer to treat, and progress has been incremental at best. One of the more promising areas of research which Dr Lawler is undertaking involves immune sensing molecules such as STING, or Stimulator of Interferon Genes. These immune sensors are naturally activated when a pathogen invades a cell, resulting in potent activation and recruitment of immune cells.

Researchers hope to find compounds which can boost these immune sensing pathways and response, potentially combining them with immune-checkpoint inhibitors, but as of now, the STING pathway in glioblastoma is almost entirely unknown. There are little to no data detailing expression levels of the proteins in the pathway in glioblastoma, or how this may vary between patients, and this is a major hurdle when developing therapeutics to target this pathway.

Dr Lawler is convinced of the importance of STING and other immune sensing pathways as targets for cancer treatment, and his future research aims for the full characterisation and identification of new pharmacologic agents which can target and activate them in the right way. It seems likely that targeting the STING pathway could overcome the immune suppression induced by the tumour and trigger inflammation, something which is key for immune-checkpoint inhibitors to work. Overall, the STING pathway is a hugely important candidate for finding new anti-tumour agents and enhancing the activity of already known ones. Dr Lawler and his team are currently working to develop new methods that could allow sustained drug release within the tumour to allow the proper immune response to develop.

The future of glioblastoma research will see great steps forward as our understanding of immune mechanisms in the brain and in cancer improve. Further to this, as technology advances our ability to model complicated biological structures such as the BBB will support research and drug development and hopefully speed up the time it takes for drug candidates to make it from the laboratory to the clinic. The work of Dr Lawler is just one foray into these new capabilities and incorporating all the knowledge we gain from patient observation along the way should help lead the charge toward developing new and effective therapies for glioblastoma.

Meet the researcher

Dr Sean Edward Lawler
Associate Professor of Neurosurgery
Department of Neurosurgery
Brigham and Women's Hospital
Harvard Medical School
Boston, MA
USA

Dr Sean Lawler is a translational brain tumour scientist from Brigham and Women's Hospital, Harvard Medical School. He earned his doctorate from Birkbeck College, University of London in 1992 and worked on the fundamentals of signal transduction in San Francisco, Paris, Dundee and at Massachusetts General Hospital until 2004 when he joined the faculty at The Ohio State University. A stint in his hometown at the University of Leeds was followed by him joining the faculty of Harvard Medical School in 2013 where he now holds the position of Associate Professor in the Department of Neurosurgery at Brigham and Women's Hospital. His work has focused on brain tumours with an emphasis on glioblastoma, and the use of immunotherapeutics to treat cancer. He is an Editorial Board Member of *Neurooncology*, a member of the American Society of Clinical Oncology, Society for Neurooncology and American Association for Cancer Research, and in 2017 he received the prestigious BWH Innovator Award.

CONTACT

E: slawler@bwh.harvard.edu
W: researchfaculty.brighamandwomens.org/BRIProfile.aspx?id=6541

KEY COLLABORATORS

E. Antonio Chiocca MD, PhD, Chairman of Neurosurgery, Brigham and Women's Hospital, Boston
Brad Pentelute PhD, Professor of Chemistry, Massachusetts Institute of Technology, Boston
Charles S. Cobbs MD, Director, Ben and Catherine Ivy Center for Advanced Brain Tumor Research, Swedish Neurosciences Institute, Seattle
Charles H. Cook MD, Associate Professor of Neurosurgery, Beth Israel Deaconess Medical Center, Boston

FUNDING

Brigham and Women's Hospital Innovator Award
B*CURED foundation
US National Cancer Institute (R01CA237063, R01CA184283)

FURTHER READING

SE Lawler, MC Speranza, C Passaro, et al., Preclinical investigation of combined gene-mediated cytotoxic immunotherapy and immune checkpoint blockade in glioblastoma, Neuro-Oncology, 2018, 20, 2, 225–235.

CM Fadzen, JM Wolfe, CF Cho, et al., Perfluoroarene-Based Peptide Macrocycles to Enhance Penetration Across the Blood-Brain Barrier, Journal of the American Chemical Society, 2017, 139, 44, 15628–15631.

CF Cho, JM Wolfe, CM Fadzen, et al., Blood-brain-barrier spheroids as an *in vitro* screening platform for brain-penetrating agents, Nature Communications, 2017, 8, 15623.

HARVARD MEDICAL SCHOOL

USING PLANTS AS A SOURCE OF ANTI-CANCER COMPOUNDS FOR UNDERGRADUATE RESEARCH EXPERIENCES

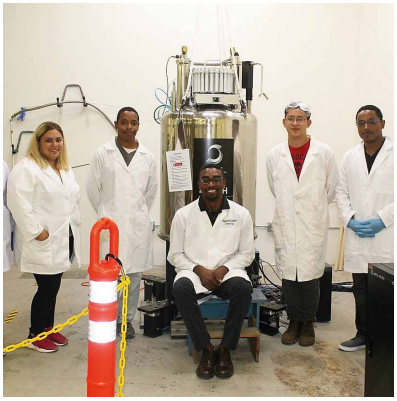
Cancer, in all its forms, is one of the major causes of death across the world and we are in urgent need of more effective interventions for this global killer. Drugs used to treat diseases like cancer can be either synthetic in origin, semi-synthetic derivatives of natural products, or unmodified natural products. **Dr Patrick Still** and his team at California State University, Dominguez Hills (CSUDH), conduct research to identify and biologically screen anti-cancer compounds derived specifically from plant materials. Studies involving structure elucidation and biological testing of compounds from plants have provided undergraduate research experiences for students across chemical and biological sciences majors on the CSUDH campus.

Natural Products Analysis for Workforce Readiness

Cancer accounts for about one in four deaths in the USA and is second only to heart disease in terms of lives lost. Healthier lifestyles, more routine screening and the development of increasingly advanced treatments have helped reduce the number of cancer-related deaths but only by about 2% each year. Unsurprisingly, efforts to further reduce the mortality rate remain a priority for researchers.

Dr Patrick Still's laboratory at California State University, Dominguez Hills, focuses on discovering natural products from plants that show activity against cancer cells. As the name implies, natural products are molecules which are produced in plants as well as animals, bacteria, marine organisms and fungi. Dr Still explains, 'Plants have been a source of drugs to treat human disease for centuries. With the rise of Organic Chemistry as a formal discipline in the early 1800s, many of the active compounds found in plant extracts were chemically elucidated.'

Undergraduate research in natural products chemistry while enrolled in chemical or biological sciences majors provides students with workforce readiness skill-sets that potential employers find desirable. Once the extraction from the plant material is completed, independent study students are guided through operating in-house biological assays on semi-purified chromatographic subfractions. In a process known as bioactivity-guided fractionation, only the subfractions showing activity against cancer cells are further purified. The iterative process of purification and testing against cancer cells is repeated until a purified compound is obtained. Testing is carried out using various types of



Dr Patrick Still and his team. Credit Patrick Still.

cancer cells including breast, brain and pancreatic cancer cells grown for the purposes of testing (in vitro), or within an organism (*in vivo*). The former is normally carried out first, while the latter is conducted through collaborations with laboratories that specialise in animal studies.

‘Finding anti-cancer compounds provides workforce readiness skill-sets for undergraduate research experiences.’

Credit Patrick Still.

Using a range of chemical techniques, potential lead compounds can be isolated and then analysed. Dr Still and his team use an analytical technique known as nuclear magnetic resonance (NMR) spectroscopy to ‘elucidate the chemical structure of biologically active compounds we isolate from nature.’ Broadly speaking, NMR allows scientists to identify the positions of the hydrogen and carbon atoms in a molecule, and how the atoms connect together. Knowing the detailed structure helps the team identify if the molecule is novel, in other words, whether it has been reported previously in the chemical literature. Undergraduates, in sum, solidify skill-sets in compound purification, cell culture and spectroscopy during the bioactivity-guided isolation process.

Anti-cancer Agents That Also Affect the Central Nervous System

Receptors are parts of the cell which are responsible for exchanging messages and triggering biochemical events. They achieve this by receiving molecules or ions with the required structure, in a similar way to which a key can fit into a lock. A molecule that triggers a biochemical event by binding a receptor is an agonist. Compounds which block or reduce the exchange of signal transmission are known as antagonists.

One of Dr Still’s most important achievements to date is the isolation of a cytotoxic nicotinic receptor antagonist known as ‘Microgrewiapine A’, a piperidine-class alkaloid. The

Credit Patrick Still.

compound was purified from the shrub *Microcos paniculata* collected in Vietnam and it was discovered that only micromolar concentrations were needed to inhibit the proliferation of cancerous colon cells. Microgrewiapine A was found to have antagonistic activity with 60 and 70% inhibition against the human α4ß2 and α3ß4 nicotinic acetylcholine receptors (nAChR), respectively. This was the first example of a dual cytotoxic and nAChR piperidine antagonist to have been isolated from the genus Microcos.

It was determined that the concentration of Microgrewiapine A needed to inhibit healthy colon cells was about five times higher than the levels needed to inhibit the same proportion of cancerous colon cells. This shows that Microgrewiapine A can effectively target cancerous cells over healthy cells, which, in general, is a critical requirement for all anti-cancer treatments.

Suppressing the Spread of Melanoma

One of the limitations of cancer treatments is how these therapies also affect the white blood cells that fight infection. For example, chemotherapy not only reduces the size of tumours but in some cases also lowers the number of white blood cells. Depletion of the immune response is an undesirable effect of chemotherapy since stimulation of innate immunity is associated with fighting off a variety of cancers.

Dr Still was involved in a collaborative study investigating ellagic acid and ellagic acid peracetate (a chemically modified form), studied in the context of melanoma. Ellagic acid occurs naturally in many dietary fruits with reports of anti-tumour activity. While there are published in vitro studies for both compounds, there were no *in vivo* results of ellagic acid peracetate available at that time.

In contrast to the purification of a compound from the crude plant extract, this collaborative study prepared ellagic acid and ellagic acid peracetate by synthetic means and published these synthetic procedures and *in vivo* work in 2012. The results were encouraging, showing that while ellagic acid did not significantly reduce tumour size, mice treated with ellagic acid peracetate showed a 70% decrease in the original weight of the tumour.

The team also characterised the immunomodulatory role of both compounds and found that ellagic acid peracetate raised the count of white blood cells. Meanwhile, ellagic acid did not result in any increase to white blood cell count. Importantly, natural killer cell levels were unaffected after the administration of ellagic acid peracetate. These combined results show that ellagic acid peracetate not only targets melanoma but also increases the natural immunity levels of the host.

Using Repositories for Bioactive Compound Discovery

Dr Still and his team's future research goals are now to harness the materials stored at the National Cancer Institute Natural Products Repository in the USA. The repository holds a large database of approximately 80,000 plant-based specimens, many of which could prove to have promising biological activities against cancers like melanoma. This exciting work may allow Dr Still's team to provide an even better understanding of how best we might be able to eventually combat cancer utilising the power of nature. These future research projects involving extracts screened in-house will continue to provide projects for undergraduates to discover new biological activities and hone knowledge of organic structure analysis using methods that industrial employers find desirable in a new hire.

Meet the researcher

Dr Patrick C. Still
Department of Chemistry and Biochemistry
California State University, Dominguez Hills
Carson, CA
USA

Dr Patrick C. Still received his PhD in Medicinal Chemistry from The Ohio State University, Columbus, in 2013. Following this, Dr Still carried out postdoctoral research for two years at the University of California, Santa Cruz, before joining the faculty at California State University, Dominguez Hills (CSUDH) in 2015. Dr Still's research group focuses on extracting natural products from plant material and identifying bioactive molecules using in-house and collaborative cell-based screening. A major part of Dr Still's research is the structural elucidation of bioactive molecules using nuclear magnetic resonance (NMR). Dr Still lead the establishment of the NMR Facility at CSUDH by coordinating the purchase of the $400,000 instrument for the university and is also responsible for new user training and maintenance.

CONTACT

E: pstill@csudh.edu
W: https://www.naturalproductsgroupcsudh.com/

KEY COLLABORATORS

Dr A. Douglas Kinghorn, The Ohio State University, Department of Medicinal Chemistry & Pharmacognosy
Dr Yulin Ren, The Ohio State University, Department of Medicinal Chemistry & Pharmacognosy
Dr Li Pan, Research Scientist Taste Innovation, Kerry
Dr Dennis McKay, Pharmacology, The Ohio State University, Department of Pharmacology

FUNDING

National Institutes of Health (NIH)/National Institute of General Medical Sciences (NIGMS)
'Discovery of Natural Products from Botanical Sources' (Grant: 5SC2GM12272 PI: Still)

WM Keck Foundation
'Spectroscopy Advancement Laboratories for Diverse Students' STEM Success (SALDS^3): PI: Still

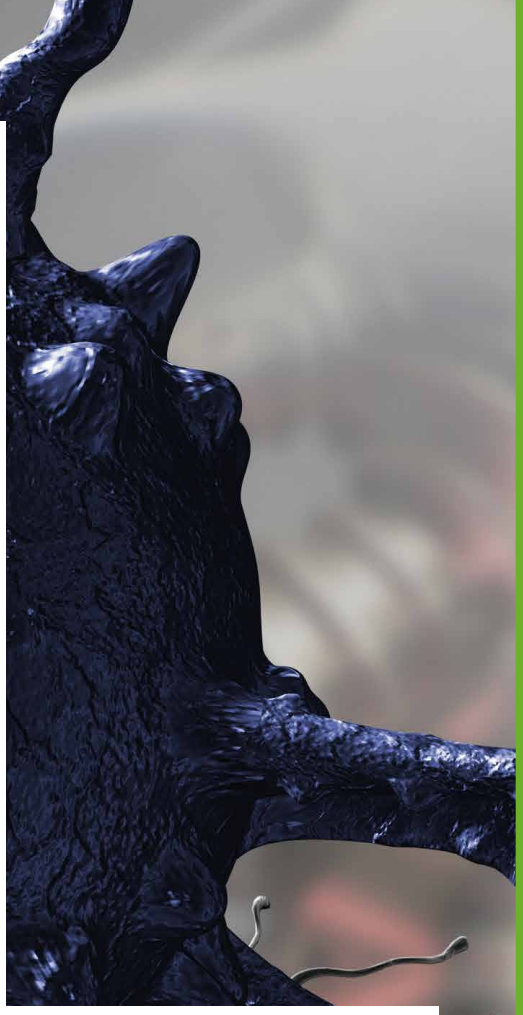
FURTHER READING

M Waseem, J Q.-L. Williams, A Thangavel, PC Still, P Ymele-Leki, A structural analog of ralfuranones and flavipesins promotes biofilm formation by *Vibrio cholerae*, PLoS ONE, 2019, 14, e0215273.

LB Pérez, PC Still, CB Naman, Y Ren, L Pan, H Chai, EJC de Blanco, TN Ninh, BV Thanh, SM Swanson, DD Soejarto, AD Kinghorn, Investigation of Vietnamese Plants for Potential Anticancer Agents, Phytochemistry Reviews, 2014, 13, 727.

CSUDH
CALIFORNIA STATE UNIVERSITY, DOMINGUEZ HILLS

CAN AN ALKALINE DIET IMPROVE CANCER OUTCOMES?



There is a strong body of evidence from animal and human studies showing that the acidic external microenvironment (local environment) of cells associated with tumours plays a significant role in the progression and migration of cancers. Indeed, in a rat model, systemic buffering which reduces acidic pH levels also reduces both cancer progression and drug resistance. **Dr Hiromi Wada** at the Japanese Society of Inflammation and Metabolism in Cancer and his colleagues are investigating the effect of an alkaline diet on the tumour microenvironment, and its potential to enhance the efficacy of anti-cancer treatments.

The Impact of Diet

It has been proposed that an alkaline diet may bring benefits to the conventional treatment of cancer, based on its ability to modify the pH of the tumour microenvironment. Alkaline foods include fruit, vegetables, legumes, nuts, and seeds. However, there is currently a lack of robust clinical evidence demonstrating a relationship between diet and cancer development and progression.

More specifically, prospective cohort studies in which groups of individuals have been observed over periods of time have failed to demonstrate an association between the dietary consumption of fruit and vegetables and the prevention of cancer. However, observational case control studies have suggested some interesting associations. These discrepancies in findings may be due to methodological differences and confounding variables among studies, such as the insufficient follow up of patients.

An Alkaline Diet and Advanced Non-small Cell Lung Cancer (NSCLC)

To better understand the relationship between an alkaline diet and cancer, Dr Hiromi Wada at the Japanese Society of Inflammation and Metabolism in Cancer and colleagues conducted a retrospective assessment of eleven patients diagnosed with either advanced stage or recurrent NSCLC with an epidermal growth factor receptor (EGFR) mutation – that is, a DNA mutation in the genes that code for this protein which is important in cell division and survival.

The patients had been treated with tyrosine kinase inhibitor (TKI), a protein which targets the mutated EGF-receptor and had also been directed to consume a diet rich in alkaline foods.

The compliance of patients with an alkaline diet was confirmed by urine pH analysis. The team found, in some cases, the TKI treatment was reduced to almost half of the standard treatment dosage (Figure 1). When compared to similar studies, the resulting progression-free survival (PFS; defined as the length of time during and after treatment that a patient still has the disease but without worsening) increased from 13 months to 19 months. Furthermore, overall survival increased from 22.8 months to 28.5 months.

While Dr Wada and his colleagues acknowledge that there was no direct comparator group in this study, the findings when considered with the existing literature support their important proposal that combining an alkaline diet with a lower dose of EGFR-TKI treatment may reduce the toxic side effects of the treatment while extending the PFS of the patients.

Alkalisation Therapy and Stage IV Metastatic or Recurrent Pancreatic Cancer

Pancreatic cancer is highly aggressive and associated with extremely poor outcomes even when treated with combined chemotherapy regimens. In another clinical trial, Dr Wada and his colleagues supplemented chemotherapy treatment of advanced

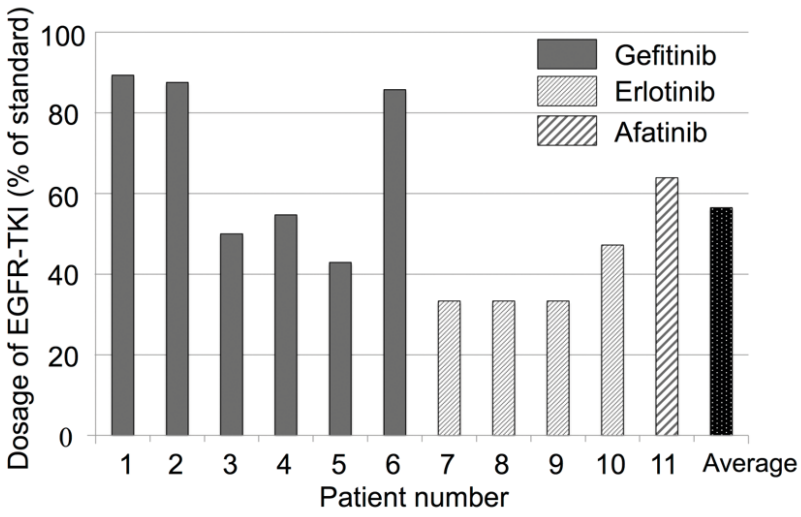


Figure 1. Dosage of EGFR-TKI (from Anticancer Research, 2017, 37, 5141–45).

pancreatic cancers by prescribing an alkaline diet, comprising of a minimum of 400 grams per day of fruits and vegetables along with a total exclusion of meat and dairy, and daily consumption of an oral sodium bicarbonate solution. The study outcomes, measured in 28 participating patients, were urine pH and mortality.

Following alkalisation therapy, mean urine pH was significantly higher than at the initiation of the trial, and the research team found that patients with higher urine pH (i.e., being more alkaline) had a higher overall survival (OS) rate than those with lower (more acidic) urine pH. These patients had an OS rate of 16.1 months compared to only 4.7 months for patients with more acidic urine (Figure 2A). Similarly, the patients who had a greater difference in urine pH (>1) from pre- to post-alkalisation therapy also had a greater OS. In these cases, the OS was 16.1 months compared to 4.3 (Figure 2B).

Intra- and Extra-cellular pH: Effects on Tumour Physiology and Pathology

In general, cancer cells exhibit a shift towards alkalinity in the intracellular environment. In other words, the cytoplasm within the cancer cells is alkaline (pH>7) and the corresponding extracellular environment is acidic.

Alkalisation within the cancer cells is pivotal to the initiation of malignancy and the progression of the tumour, with some researchers reporting associations with key tumour processes, including the induction of multi-drug resistance and the inhibition of programmed cell death (apoptosis).

The Sodium/Hydrogen Transporter (Isoform 1) NHE1

NHE1 is responsible for maintaining the acid/alkali balance within normal cells. With a typical ('set point') level of 6.9–7.1, this transporter is largely inactive in the steady state. However, in transformed cells, the NHE1 transporter has an altered set point and remains active in the pH 7.2–7.7 range. Consequently, this leads to an alkaline environment within the cells and an acidic environment immediately surrounding the cells.

The theory of alkalisation is based on altering the pH of the environment surrounding the tumour cells, such that increasing the pH of the acidic surrounds to be close to that of the inside of the tumour cells (pH 7.2–7.7) prevents the transport action of NHE1 and prevents the self-driven proliferation of cancer cells.

NHE1: Tumour Proliferation and Metastasis

Other researchers have confirmed the association between NHE1, tumour proliferation, and increased internal cellular pH by blocking either cell proliferation or the action of the transporter and determining the intracellular pH.

Furthermore, the alkaline internal environment which results from the action of the NHE1 transporter promotes the production of factors which, in turn, induce angiogenesis, the formation of new blood vessels that is necessary for the dissemination of metastatic tumours. Furthermore, the acidic external pH promotes the actions of a number of factors that support the proliferation and mobilisation of tumour cells.

One of the most significant aspects of the action of NHE1 is that it does not require any growth factor to stimulate its action. This means that a change in intercellular pH is all that is required to induce the conditions that lead to cell proliferation.

Alkalisation and the Effect on Cancer Immunotherapies

In addition to chemotherapies, antibody therapies are also used as therapies in some cancer treatments. A treatment for esophagogastric junction adenocarcinoma, Nivolumab, was used in conjunction with alkalisation in one elderly patient. Dr Wada and colleagues reported positive findings in this case study, where the patient consumed an alkaline diet in combination with oral sodium bicarbonate. They showed that tumour markers reverted to normal levels from extremely high values prior to treatment, and furthermore, a computerised tomography scan at 12 months post-treatment identified shrinkage of the esophagogastric junction tumour and the liver metastases.

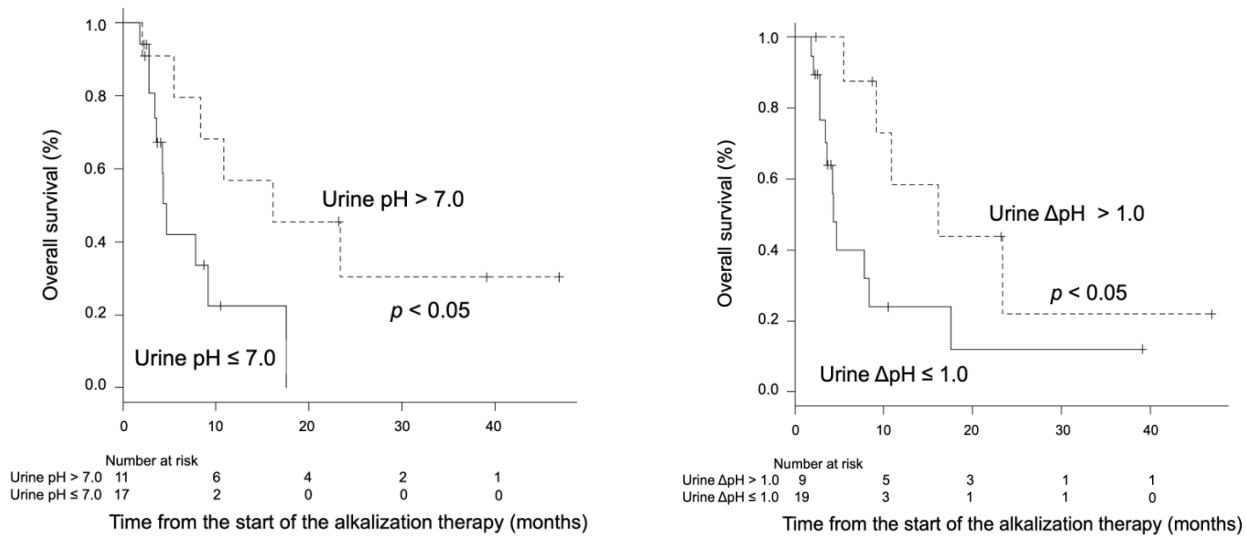


Figure 2. Association between overall survival of advanced pancreatic cancer patients and urine pH (A) or urine ΔpH (B) (from Anticancer Research, 2020, 40, 873–80').

Dr Wada and his group note that this case study, the first of its kind, highlights the potential correlation between alkalinisation of the tumour microenvironment and positive outcomes from antibody therapy in cases that have shown no benefit from multiple other therapies.

Chemotherapy Resistance and Diet

It is known that the internal (cytoplasmic) pH of most cancers is alkaline, and some researchers have shown that this is a central aspect of chemotherapy resistance. Specifically, in alkaline environments, some chemotherapy drugs are shown to be present within the tumour cells at lower concentrations in alkaline conditions than in acidic (lower pH) conditions.

This means that when NHE1 is active and the intercellular pH increases, chemotherapy treatments are substantially less effective, and the tumour cells become resistant to the actions of the drugs. As all of the cancer cells are not killed by the treatment – common in most situations – the internal pH of the remaining cells increases further and the cells become more resistant to the drugs. The drugs cannot induce apoptosis and the tumour advances.

In some tests using cultured cell lines, the resistance to key chemotherapy drugs has been shown to increase almost 2000-fold in response to an increased intercellular pH of 0.44.

Dr Wada and colleagues argue that the beneficial results they have observed in patients with aggressive, late stage cancers that frequently become resistant to chemotherapy support their concept that alkalisation of the tumour microenvironment through the manipulation of diet and consumption of oral sodium bicarbonate solution leads to a reduced pH in the internal cellular environment. Ultimately, this manipulation of the pH in the local tumour environment may increase the sensitivity of the tumour cells to chemotherapeutic agents.

The Future

Dr Wada and colleagues discuss the limitations of their work to date, necessitated by the nature of treating late-stage cancers. However, their findings associated with alkalisation (achieved through diet and, in some cases, supplementation with oral sodium bicarbonate) in patients with late stage or metastatic tumours indicate improved patient outcomes. These include OS, PFS, tumour and metastatic reduction, and improved sensitivity of the tumours to chemotherapeutic and antibody-based drugs. In one case, Dr Wada and his team even report improved outcomes with alkalisation therapy in a patient with a multi-drug resistant tumour.

In addition to their impressive clinical findings, Dr Wada and his team present a clear mechanism for the pathology and physiology of cancers in association with an acidic external and alkaline intracellular environment, thus demonstrating how the pH balance leads to cellular proliferation, angiogenesis, and formation of metastatic tumours.

Dr Wada and his team are also interested in the area of mental well being and cancer development and progression, advocating that suppressing overactivation of the hypothalamic-pituitary-axis, the body's central stress response system, prior to cancer treatment brings tangible benefits to the patient. One's emotions have a direct effect on the pituitary, which leads to the production of stress-associated hormones, disrupting the body's hormones and dampening the immune response. As such, Dr Wada also advocates a treatment approach aiming to minimise stress and enhance the benefits of cancer interventions.

Meet the researchers

Dr Hiromi Wada, MD, PhD
Professor Emeritus
Department of Thoracic Surgery
Faculty of Medicine
Kyoto University
Japan

Dr Hiromi Wada graduated from the Faculty of Medicine at Kyoto University, Japan, as a Doctor of Medicine. He then worked at the Institute for Chest Disease Research and Institute for Frontier Medical Sciences at Kyoto University before becoming a Professor in the Department of Thoracic Surgery, which is also at Kyoto University. Dr Wada is currently a Professor Emeritus at Kyoto University, Director of the Karasuma Wada Clinic, and Representative Director of the Japanese Society of Inflammation and Metabolism in Cancer.

CONTACT

E: wadah@kuhp.kyoto-u.ac.jp

Dr Hiromasa Morikawa, MD, PhD
Karasuma Wada Clinic
Kyoto
Japan

Dr Hiromasa Morikawa received his MD in 2001 from the School of Medicine at Kyoto University, Japan. After working as a thoracic surgeon, he received a PhD for his research on T-cell immunology conducted at Dr Shimon Sakaguchi's laboratory. After working as an Assistant Professor there, he moved to Karolinska Institutet in Sweden, working in computational medicine and immunology at Dr Jesper Tegner's laboratory as a Vinnmer-Marie Curie Fellow. He is currently working at Karasuma Wada Clinic in Japan and is a Board Member of the Japanese Association for Chest Surgery.

CONTACT

E: hiromasa-morikawa@umin.ac.jp
W: https://www.linkedin.com/in/hmorikawa/

Dr Reo Hamaguchi, MD
Japanese Society of Inflammation and Metabolism in Cancer
Kyoto
Japan

Dr Reo Hamaguchi graduated from Kanazawa University School of Medicine and is currently undertaking graduate studies at Tokyo University School of Medicine. He is a Certified Internist at the Japanese Society of Internal Medicine, a Specialist in Chest Medicine, and a Certified Oncologist. Dr Hamaguchi is also the Chief Physician at the Mirai Medical Clinic in Myogadani, Tokyo. Over his career, he has worked as a pulmonologist in the treatment of patients with general internal medicine diseases as well as cancer. He currently studies under the supervision of Dr Wada, and treats cancer patients through the improvement of diet and the enhancement of immunity in consideration of the inflammation and metabolism of cancers.

CONTACT

E: reo-h@nifty.com

Dr Ryoko Narui, MD
Japanese Society of Inflammation and Metabolism in Cancer
Kyoto
Japan

Dr Ryoko Narui graduated from Kobe University School of Medicine and is a Board Certified Surgeon at the Japan Surgical Society. After working as a surgeon in general surgical disease as well as gastrointestinal cancer and breast cancer, Dr Narui completed a Fellowship in Integrative Medicine at the University of Arizona, Center of Integrative Medicine, led by Dr Andrew Weil. She received a scholarship from CWAJ and completed a master's degree in Medical Humanities at King's College, London. She currently studies under the supervision of Dr Wada.

CONTACT

E: narui@thermo-cc.com

CALPROTECTIN – FROM NATURAL ANTIMICROBIAL TO ANTI-TUMOUR THERAPEUTIC?

Calgranulins are relatively small proteins, usually around 100 amino acids long. Calprotectin is a complex of two of these small proteins, S100A8 and S100A9, getting its name from its protective, antimicrobial properties. **Dr Mark Herzberg** at the University of Minnesota, USA, has extensively researched the antimicrobial action of this protein complex, and this knowledge is now leading serendipitously to the development of potential therapeutic agents for certain types of human cancer.

Structural Biology of Various Calprotectin (S100A8/A9) Complexes

In humans, these proteins are found most commonly as heterodimers, that is, a complex of one of each protein, S100A8 and S100A9. Homodimers, which contain the same protein twice in a complex, homotrimers (three of the same protein), homotetramers (four of the same protein), and monomers (individual proteins) are all theoretically possible to form but are rare in nature.

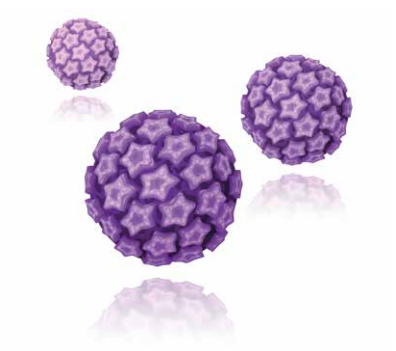
Antimicrobial Mechanisms of Calprotectin

The antimicrobial mechanism of this important protein complex is thought to rely on the binding property for copper, manganese, and zinc. These essential transition metals are required for the growth of many bacteria and fungi, and when bound to calprotectin appear to inhibit both intra- and extracellular growth of the microorganisms. Calprotectin binds some metals more easily than others making the antimicrobial activity dependent on an organism's need for one rather than another. This means that the protein may also favour binding to the metal that is most prevalent in the environment at any given time.

In addition to this antimicrobial function, calprotectin contributes to the barrier functionality of the mucosal epithelial membranes that line the body cavities, such as the mouth, and separates the internal organs of the human body from the outside to help resist bacterial invasion.

Also contributing to fighting invasive microorganisms, the individual units of calprotectin and other related proteins, known generally as calgranulins, are also linked to modulation of the immune and inflammatory cell response. Some evidence argues that calprotectin is an 'alarmin' that signals the body to elicit robust inflammation in response to an infection.

Interestingly, calprotectin has anti-inflammatory features too. The anti-inflammatory mechanism is thought to occur by scavenging and neutralising nitric oxide and reactive oxygen species, which are powerful oxidants produced by the host's own inflammatory response to infection.



In this way, calgranulins are capable of limiting self-induced damage to host tissues while reacting to an infection that breaches the mucosal surface. Given the many calprotectin producing and containing cell types, such as neutrophils, macrophages, and epithelial cells, researchers propose that calprotectin may operate in a cell-type-specific manner.

Mucosal Epithelia and Enhanced Bacterial Resistance

The mucosal membranes provide both a physical barrier and the molecular mechanisms to help evade bacterial assault. Dr Mark Herzberg at the University of Minnesota and

his colleagues have addressed the question of whether the existing innate, non-specific immune response can be enhanced.

Researchers have demonstrated that calprotectin is a pivotal factor in innate-epithelial-immunity against a broad spectrum of bacteria. Using a cell culture model of keratinocytes, a mucosal epithelial cell type, Dr Herzberg and his team have identified the critical segments in the calprotectin protein complex that are important in preventing infection by invading bacteria.

Relying in part on their knowledge of the functional structure of calprotectin, Dr Herzberg and his team applied molecular biology methods to insert protein-coding messenger RNA (mRNA) transcripts into the mucosal epithelial cells. The outcomes of these tests showed that insertion of mRNA encoding either the members of the calprotectin protein complex or these proteins in combination with another distinct antimicrobial protein, cathelicidin, enhanced the resistance of the cells to invasive human pathogens, including *Listeria* and *Salmonella*, for up to 48 hours. This means that protection against infection by these bacteria can be selectively increased for a period of time.

Epithelial Tissues and Head Neck Squamous Cell Carcinoma and Calprotectin Expression

Head and neck squamous cell carcinoma (HNSCC) accounts for approximately 90% of head and neck cancers, and arises in the mucosal membranes of the mouth, throat, and nose. In normal squamous (e.g., stacked up) epithelial cells lining the surface of these anatomic sites, calprotectin is produced at high concentrations. Unlike the squamous epithelium of the head and neck, other normal epithelial tissues only express very low or no calprotectin. In cancers arising in the breast, thyroid, gastric, liver, colorectal, ovarian, prostate, bladder, and lung, such as adenocarcinomas, high levels of the calprotectin are produced. In HNSCC, however, the expression of calprotectin decreases drastically.

This discrepancy between high and low calprotectin tumour types and the apparent 'switching off' of this anti-microbial protein complex when squamous mucosal epithelial cells become cancerous caught the interest of Dr Herzberg and his colleagues.

Head and neck cancer is the sixth most prevalent cancer globally, and the number of cases has increased substantially in the past ten years. According to the Cancer Genome Atlas project, the two subunits of the calprotectin complex, S100A8 and S100A9, are downregulated in over 90% of HNSCCs compared to normal tissues.

The level of downregulation in HNSCCs appears independent of the clinical features of the tumour, including stage and location. Studies by the Herzberg team suggest that it is possible that the decrease in calprotectin is an indicator of the initiation of cancer development, with pre-neoplastic (dysplastic) cells in otherwise healthy tissue showing progressive downregulation of S100A8 and S100A9 as they approach cancerous transformation.

Similar to cigarette-smoking and consumption of tobacco-like products, infection by high-risk human papillomavirus (HPV) is highly associated with the development of HNSCC. Data from the Herzberg group, supported in part by the Cancer Genome Atlas, suggests strongly that the decrease in calprotectin levels is even greater in HPV-positive tumours than in HPV-negative ones (tobacco-associated).

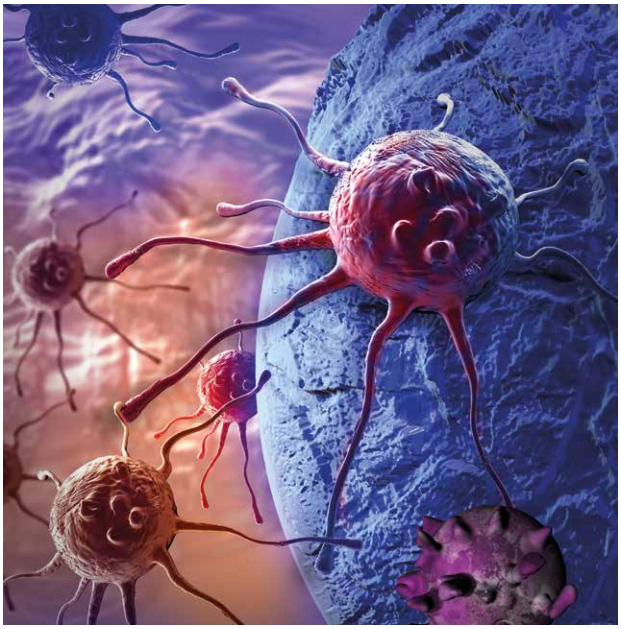
Conducting tests in a well-differentiated (looking much like normal cells) oral squamous cell carcinoma cell line, Dr Herzberg and his team found that decreasing the calprotectin levels via manipulation of mRNA expression increased the ability of the tumour cells to invade and spread, features of a more aggressive cancer. However, when the calprotectin levels were normally expressed in the same cell line, tumour initiation and local spread actually decreased. Furthermore, in a mouse model, transplanted tumour cells that are negative for calprotectin produced larger tumours than tumours produced by calprotectin-positive transplanted cells. On a molecular level, both S100A8 and S100A9 are associated with the downregulation of genes that promote tumour migration and initiation. In other words, calprotectin makes tumour cells more 'normal.'

Calprotectin and Head and Neck Cancer Progression and Patient Survival Outcomes

In human oral and oropharyngeal cancers, in cases where S100A8/A9 is more highly expressed, levels of the epithelial growth factor receptor (EGFR), a protein that sustains cell proliferation and growth, are low. In contrast, histologically aggressive tumours (high-grade) with low calprotectin levels show high EGFR levels. Also, cells expressing calprotectin show increased programmed cell death (apoptosis), which is key to the susceptibility of a tumour to shrivelling and dying in response to both chemo- and radiotherapies. Furthermore, the team has shown that levels of calprotectin and overall survival of patients with HNSCC are directly related.

Despite global advances in cancer treatment, the five-year survival rate for patients with HNSCC remains only at 50–66%. Dr Herzberg and colleagues propose that calprotectin may represent a potential therapeutic target for this disease. Restoring levels of S100A8/A9 to normal in pre-cancerous and existing tumours may prevent or decelerate tumour initiation or progression. These investigators speculate that intervening at multiple points may halt tumorigenesis and enhance susceptibility to existing treatment regimens.

Although calprotectin appears to limit local invasion and spread of a tumour, metastasis may not be affected. In non-squamous epithelial cell tumours, such as breast, prostate, colorectal and thyroid, high levels of calprotectin are associated with primary tumour development and with metastatic spread. Dr Herzberg's team was interested to test whether, in the case of HNSCC, lower levels of calprotectin would be associated



with metastases. From paired primary HNSCC and lymph node metastatic tumours, they found that the levels of calprotectin in the metastatic samples were lower than in the primary tumours, although not statistically so. These data indicate that calprotectin may not affect metastasis in HNSCC.

Calprotectin, Caspases, and Response to Cisplatin-based Treatments: Unravelling the Survival Statistics

Findings from the Cancer Genome Atlas show strong correlations between caspases, enzymes that regulate programmed cell death, and S100A8/A9 in HNSCC patients. When S100A8/A9 expression is lost in head and neck cancer cells there is a corresponding loss of caspase 3/7 activity and subsequently, the cell death pathway is attenuated. This process minimises the effect of radiation treatments since apoptosis would not be induced following treatment. Notably, research from the Herzberg group showed that increased S100A8/A9 levels in HNSCC cell lines confers sensitivity to cisplatin treatment and facilitates cell death.

The relationships between the calprotectin dimer, caspases, sensitivity to cisplatin and EGFR, are likely to explain the better survival rates observed in HNSCC patients who exhibit higher levels of calprotectin.

Looking to The Future

Dr Herzberg and his colleagues have significantly increased our understanding of the antimicrobial and anti-inflammatory actions of calprotectin. By exploring the effects of modulating calprotectin levels in HNSCC tumours, they have opened an extensive opportunity for the development of multifaceted novel therapeutics for HNSCC. These potential new therapeutics could improve the response to both chemo- and radiotherapy, as well as modulating the tumour cell-death process.

Meet the researcher

Dr Mark Herzberg
Department of Diagnostic and Biological Sciences
University of Minnesota
Minneapolis, MN
USA

Dr Mark Herzberg holds a doctorate in dental surgery as well as a research doctorate. For three decades, he has been the Director of the Minnesota Craniofacial Research Training Program, funded by the National Institutes of Health and based at the University of Minnesota. Dr Herzberg's interest in the antimicrobial protein known as calprotectin started in the 1990s, and he has provided a comprehensive account of the mechanisms and actions of this protein in protecting the mucosal tissues from invasion by microbial pathogens. More recently, Dr Herzberg has applied his knowledge and understanding of the multifaceted role of this protein to cancer research. His findings demonstrate an important role for calprotectin in head and neck squamous cell carcinoma (HNSCC), the tumours of which demonstrate a different pattern of expression of this protein compared to other epithelial cell cancers. This work is directing the development of novel, HNSCC-specific therapeutics.

CONTACT

E: mcherzb@umn.edu
W: https://www.dentistry.umn.edu/bio/oral-biology/mark-herzberg

KEY COLLABORATORS

Dr Karen F Ross
Dr Bruno Lima
Dr Prokopios Argyris
Dr Ali Khammanivong
Dr Will Boyle
Dr Massimo Costalonga
Dr Peter Bittner-Eddy
Dr Flavia Saavedra
Dr Lori Fischer
Dr Alexandre Zaia
Dr Xianqiong Zou

FUNDING

National Institutes of Health/National Institute of Dental and Craniofacial Research

FURTHER READING

PP Argyris, Z Slama, C Malz, et al, Intracellular calprotectin (S100A8/A9) controls epithelial differentiation and caspase-mediated cleavage of EGFR in head and neck squamous cell carcinoma, Oral Oncology, 2019, 95, 1–10.

PP Argyris, ZM Slama, KF Ross, et al, Calprotectin and the initiation and progression of head and neck cancer, Journal of Dental Research, 2018, 97, 674–682.

X Zou, BS Sorenson, KF Ross, MC Herzberg, Augmentation of epithelial resistance to invading bacteria by using mRNA transfections, infection and immunity, 2013, 81, 3975–3983.

BS Sorenson, A Khammanivong, BD Guenther, et al, IL-1 receptor regulates S100A8/A9-dependent keratinocyte resistance to bacterial invasion, Mucosal Immunology, 2012, 5, 66–75.

C Champaiboon, KJ Sappington, BD Guenther, et al, Calprotectin S100A9 calcium-binding loops I and II are essential for keratinocyte resistance to bacterial invasion, Journal of Biological Chemistry, 2009, 284, 7078–7090.

K Hsu, C Champaiboon, BD Guenther, et al, Anti-infective protective properties of s100 calgranulins, Anti-Inflammatory & Anti-Allergy Agents in Medicinal Chemistry, 2009, 8, 290–305.

AA Zaia, KJ Sappington, K Nisapakultorn, et al, Subversion of antimicrobial calprotectin (S100A8/S100A9 complex) in the cytoplasm of TR146 epithelial cells after invasion by *Listeria monocytogenes*, Mucosal Immunology, 2009, 2, 43–53.

UNIVERSITY OF MINNESOTA
Driven to Discover℠

ARE POLY-ANEUPLOID CANCER CELLS THE KEYSTONE CURE FOR CANCER?

‘Our audacious idea is to cure cancer.’ **Dr Kenneth Pienta** at the Johns Hopkins School of Medicine, USA, speaks with genuine passion about his ground-breaking research. With his team, he has recently discovered that in *every* type of cancer, a special type of rare cancer cell – a polyaneuploid cancer cell (PACC) – exists and hides within the greater cancer cell population. The team hypothesises that ‘PACCs are a master mediator of therapy tolerance’ and thus, the critical treatment target. Now, in a call to arms, Dr Pienta is asking researchers and scientists across diverse disciplines to unite in developing a cure for cancer.

The Challenge of Cancer

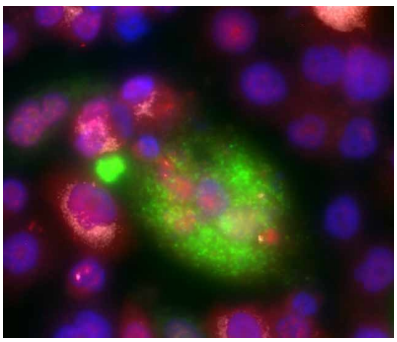
Cancer is a global health concern. There are over 100 types of cancer, which taken together, kill more than 10 million people across the world each year. Although localised cancers can be treated successfully through excision or localised radiotherapy, metastatic cancers spread throughout the body and are incurable, eventually leading to death. Once cancer cells have metastasised, they grow aggressively and are resistant to virtually all treatments. Despite enormous funds and dedicated efforts put into cancer research over the last half-century, half of all people diagnosed with cancer still die from their disease.

Traditional thinking in cancer therapy accepts that cancer is incurable once it spreads. There are billions of cells in a tumour and it only takes one cell to randomly mutate into a form with increased tolerance to survive treatment and then to clone itself. Cancer therapies have focussed on multi-drug chemotherapies to tackle the progression of new cancers as they occur, or to develop drugs that target resistance-associated mutations. However, cancer cells have proven resistant to all high-tech medical innovations to date. Researchers have not understood exactly what cells or mechanisms that cause this remarkable resilience amongst cancer cells.

Dr Kenneth Pienta at the Johns Hopkins School of Medicine, USA, is leading an amazing and bold line of discovery in cancer research. Critically, his team now believes that polyaneuploid cancer cells (PACCs) are the master mediators of this resilience and provide an adaptive way for tumours to survive almost any type of stress.

Cancer as an Ecosystem

Dr Pienta’s work is a fantastic example of thinking outside the box and began with the consideration that cancer research might learn from the study of ecological systems. His team considered the concept of keystone species in known ecosystems. Here, specific species, such



Polyaneuploid Cancer Cell. Credit Laboratory of Kenneth Pienta

as the elephants in the Serengeti or the wolves of Yellowstone Park, are known to be critical to that ecosystem such that if they are removed, the ecosystem will either dramatically change or collapse. The researchers had come to the belief that the connectivity and interdependence of animal ecosystems could be applied to cancer cell populations, encouraging an entirely different observational approach to how these cells interact and respond holistically within the whole body of which they are part. The PACCs are now being explored as keystone species of the cancer cell population.

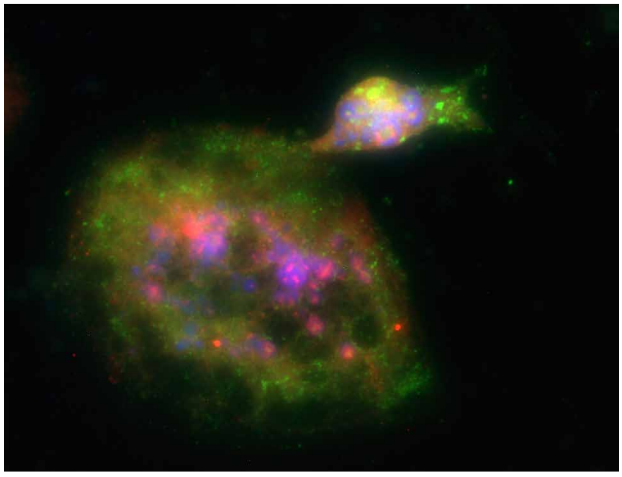
The Tale of the Grasshopper and the Locust...

Two key questions across all types of cancer research are, why do some cancer cells become immune to treatment and why do they metastasise? Returning to thinking about cancer cells as an ecosystem paradigm, discussions with cancer biologist Dr Sarah Amend and ecologist Dr Joel Brown turned to the example of short-horned grasshoppers. When food is abundant, grasshoppers are typically green and healthy solitary specimens, but in times of nutritional stress, the next generation becomes yellow locusts, growing wings and changing their behaviour to become more gregarious, swarm-forming animals that travel to seek new food sources. Once resources are again abundant, the next generation once again transmogrifies back to the grasshopper morph.

Applying this to a cancer cell ecosystem, in times of induced stress (i.e., therapy), normally stable epithelial cancer cells are known to transition into mobile cells and relocate. To observe this transition, Dr Pienta began a unique project with Professor Robert Austin at Princeton University, USA. In an incredible innovation, Professor Austin was able to create a cancer ecosystem on a (micro)chip. Described as an 'engineered cancer micro-environment,' the mini-ecosystem could be visually tracked by a camera and computer to watch the changes in each cancer cell as it occurred in real-time.

Prostate cancer cells were seeded into the micro-environment and treated with docetaxel, an anti-prostate cancer drug at a lethal dose expected to kill 99.9% of the cancer cells. Dr Pienta describes how after 14 days, the cancer cells were largely eliminated, but then, unexpectedly, some grew back. Dr Pienta noted, 'for the first time – in live time – I watched the birth of drug resistance, it was sobering and totally scary!' After successfully killing nearly all the cancer cells, the cancer cells returned against all expectations, but now they were resistant to the drug therapy.

Under higher magnification, they observed that large, highly mobile cells began to emerge as the cells died off, surviving despite the seemingly lethal dose, reminiscent of the birth of locusts. The cells were giant and polyploid (containing extra sets of chromosomes). Somehow, these abnormal cells were able to asymmetrically divide, birthing a bloom of new small clone cells that were drug resistant. The team termed the cells poly-aneuploid cancer cells (PACCs); aneuploidy meaning having an abnormal number of chromosomes. Initially, Dr Pienta and his team were sceptical, 'We sat on this [finding], we just didn't believe it – if it is this fundamentally important, how did we [the scientific community] miss this, all these years?' In fact, PACC cells had been reported in the literature for over 100 years (often referred to as polyploid or polymorphous giant cancer cells, multinucleated cancer cells, or blastocyst cancer cells) and had been observed after therapeutic intervention. However, it had long been assumed that these giant polyploid cells where simply senescent dying cells that could not divide. As a result, PACCs were largely ignored and even screened out of automated cellular recognition programmes looking for metastatic cancer cells. Interestingly, across diverse research fields, PACCs were noticed yet nobody had really put together that PACCs could be the central, common actuator of tumour formation, metastasis, and therapeutic resistance across all cancer types – the hypothesis that Dr Pienta and his team are compelled to take forward.



Polyaneuploid Cancer Cell Budding. Credit Laboratory of Kenneth Pienta.

PACCs: Hiding in Plain Sight

Returning to the cancer ecosystem, Dr Pienta believes that PACCs form a keystone species and that they must be targeted to truly eliminate all cancer. With his team, Dr Pienta has since discovered that in every type of cancer, PACCs represent a small percentage of the cancer cell population. When therapeutic stress of any kind is introduced to the ecosystem, the majority of cancer cells die, resulting in a decreased tumour size, but the surviving cells form PACCs, which then repopulate the tumour with resistant cancer cells. The team hypothesise that 'PACCs are a master mediator of therapy tolerance,' with the ability to give rise to a new generation of super-resistant cancer cells. PACCs are thus a 'species' adapting for survival and not simply a mutated cancer cell. Like the locust, they are formed in response to stress rather than simply existing in a heterogeneous cell population – and critically – may be the true source of cancer resistance and lethality.

To further understand these intriguing discoveries, Dr Pienta continues to work with a cross-disciplinary group of investigators. In preliminary data from ongoing research, it seems that PACC cells are formed in two known ways. These are either by failed cytokinesis, in which the cells try to divide, double their DNA content and nuclei but fail to divide, or the fusion of cells which merge together. It seems likely that these processes begin in a primary tumour when it is stressed by low oxygen or low nutrients, as a way to protect itself. Working with geneticist Dr Laura Buttitta at the University of Michigan, the PACC cells were found to enter a 'hibernating' state (potentially for years) when exposed to chemotherapy. This is a critical survival mechanism, as cancer therapy requires damaged cancer cells to divide for it to work and to kill the cells. Therefore, the giant PACCs wait until the stress is gone before dividing again.

Dr Pienta has also worked with Dr Max Wicha, a cancer biologist and breast cancer oncologist at the University of Michigan, a founder of solid tumour cancer stem cell theory. This theory suggests that in a cancer cell population, a few cells act as stem cells that reproduce themselves to maintain the cancer and metastasise themselves to disperse around the body. Sounding much like the behaviour of PACCs, Dr Wicha and Dr Pienta were able to demonstrate that PACCs have stem cell-like properties. Dr Wicha noted that while he had commonly observed these cells in cancer, he had not recognised their importance. It has been a typical response from many cancer researchers that have talked to Dr Pienta. In other words, the cells were blatantly hiding in plain sight.

Beginning the Search for the Cure

The next stage for Dr Pienta and colleagues is now to put this new knowledge base together to target the keystone PACCs for destruction, as they believe that by removing the intermediate keystone species, the cancer ecosystem will collapse. Multiple research strands are being planned to provide a deeper understanding of PACCs and to identify ways to eliminate them. One strand is to target how the aneuploid cells with too much DNA reproduce to produce the resistant clones. Such genetically messed-up and damaged cells would normally trigger the body's natural defence systems to initiate cell death. PACCs have figured out a way around this problem. Early research demonstrates that the PACCs switch on a protein normally only used in the embryonic stage of development to assist the cell to perform mitosis, the normal chromosome division to create two cells. The researchers are searching for ways to target this process in the PACCs.

In a second approach, learning again from ecology, Dr Pienta and his team have noted that hibernating animals survive the winter using fat stores, and strikingly, PACCs are also filled with fats. Thus, the researchers propose that targeting fat storage and metabolism may starve the PACC cells in the hibernation stage and thus prevent the cloning of new cells.

The team recognises that targeting the PACCs is a difficult task since they represent such a tiny fraction of the overall tumour cell burden. Furthermore, they acknowledge that they will likely need to combine multiple strategies that include eliminating a large number of the general cancer cells with a strategy to kill the few in number, but critically important PACCs. Again, drawing from ecology, it may take a pesticide (chemotherapy) to kill the many worker bees (cancer cells) with a specific trap to capture and kill the queen bee (PACC) before it can repopulate the hive (new tumour).

Dr Pienta's call to arms will bring together the expertise of investigators drawn from several different institutions and fields. Excitingly, the targeting of keystone PACC cells may ultimately end all metastatic cancers, and in doing so, solve one of medicine's greatest challenges.

Meet the researcher

Dr Kenneth J. Pienta, MD
The Johns Hopkins Hospital
Baltimore, Maryland
USA

Dr Kenneth J. Pienta completed his undergraduate degree in Human Biology in 1983 at the John Hopkins University in Baltimore, USA, followed by his medical doctorate in 1986. Dr Pienta completed further training in internal medicine and oncology before taking faculty positions at Wayne State University from 1991–1993 and the University of Michigan from 1994–2013. In 2013, he returned to the John Hopkins University to take the position of the Donald S. Coffey Professor of Urology and Director of the Brady Urological Institute Research Laboratories. He is also a Professor of Oncology, Professor of Pharmacology and Molecular Sciences, and Professor of Chemical and Biomolecular Engineering. Commensurate with these prestigious appointments, Dr Pienta is recognised as helping pioneer the application of ecological principles to cancer and has international expertise in the development of novel therapeutic programmes for prostate cancer, often accomplished with collaboration with multi-disciplinary teams of scientists and clinicians. He has published extensively, holds a number of consulting positions, and is the well-deserved recipient of numerous honours and awards.

CONTACT

E: kpienta1@jhmi.edu
W: www.kennethjpienta.com
@pienta_brady

KEY COLLABORATORS

Sarah Amend, Johns Hopkins School of Medicine
Joel Brown, Moffitt Cancer Center
Bob Axelrod, University of Michigan
Bob Austin, Princeton University
Emma Hammarlund, Lund University
Laura Buttitta, University of Michigan
Max Wicha, University of Michigan

FUNDING

National Institutes of Health
Prostate Cancer Foundation
Multiple additional donors

FURTHER READING

KJ Pienta, EU Hammarlund, R Axelrod, et al, Poly-aneuploid cancer cells promote evolvability, generating lethal cancer, Evolutionary Applications, 2020, 00, 1–9, DOI: 10.1111/eva.12929.

KJ Pienta, EU Hammarlund, R Axelrod R, et al, Convergent evolution, evolving evolvability, and the origins of lethal cancer, Molecular Cancer Research, 2020, DOI: 10.1158/1541-7786.MCR-19-1158.

SR Amend, G Torga, KC Lin, et al, Polyploid giant cancer cells: Unrecognized actuators of tumorigenesis, metastasis, and resistance, Prostate, 2019, 79(13), 1489–1497.

SR Amend, S Roy, JS Brown, KJ Pienta, Ecological paradigms to understand the dynamics of metastasis, Cancer Letters, 2016, 380, 237–-42.

SR Amend, KJ Pienta, Ecology meets cancer biology: the cancer swamp promotes the lethal cancer phenotype, Oncotarget, 2015, 6, 9669–78.

KJ Pienta, N McGregor, R Axelrod, DE Axelrod, Ecological therapy for cancer: defining tumors using an ecosystem paradigm suggests new opportunities for novel cancer treatments, Translational Oncology, 2008, 1(4), 158–64.

JOHNS HOPKINS
MEDICINE

WORLDWIDE CANCER RESEARCH

The arrival of COVID-19 changed the world as we knew it. Global priorities underwent seismic shifts and measures to prevent the spread of COVID-19 changed our daily and working lives beyond recognition. In this exclusive interview, we speak with Worldwide Cancer Research's Director of Research, Dr Lynn Turner, to hear how the pandemic has impacted the ongoing battle against cancer, and what challenges must now be faced as a result.

Could you explain how individuals with cancer have been affected by the pandemic?

We know from reports, studies and analysis conducted over the past year that people with cancer have been heavily impacted by the pandemic. We are concerned that people with cancer are in danger of becoming collateral damage as a result of everyone's attention being focussed on 'the other C' – COVID-19. Across the UK, the pandemic has led to delays in diagnosis and treatment, which researchers estimate could lead to anywhere between 7,000 and 18,000 additional deaths from cancer in 2021. We also saw early in the nationwide lockdown that there was a significant decline in the number of people being referred to emergency cancer services. This is truly worrying and it's vitally important that people remember to still see their GP if they suspect any sign or symptom of cancer so that they can be referred to a specialist if needed.

How has your work, and research into cancer more generally, been affected by the COVID-19 pandemic?

When the pandemic hit in 2020, we saw a rapid shut down across the world of nearly all our research projects due to university and research institute closures. There were some exceptions, such as in Israel, where our scientists were still able to go to the lab to conduct their research. But even then, they were working at a reduced capacity, with strict social distancing and safety measures in place to protect people.

One thing we felt was important was to make sure that our researchers did not feel pressured in the face of delays caused by lab closures. We offered all our researchers the chance to extend the length of their projects to make up for the lost time. We also allowed the researchers who were just about to start their projects, when the pandemic hit, to delay their start date.

We also had to react quickly internally to shift our annual Big Ideas Gathering, where our Scientific Advisory Committee makes the final decision on which new projects to fund, from a face-to-face meeting to a virtual one. This came with its own set of challenges, but the team did a fantastic job adjusting and we were delighted to be able to make a commitment to fund 16 new projects which will start in 2021.

Can you speculate as to the longer-term consequences of the pandemic for cancer research?

This is a difficult question to speculate an answer to, but we do know that charity-funded medical research is under threat all over the world. In the UK, the sector invested an estimated £1.9 billion in medical research in 2019, which is around half of all publicly

'...people with cancer are in danger of becoming collateral damage as a result of everyone's attention being focussed on "the other C" – COVID-19.'

funded medical research. The sector is now facing a 40% decrease in medical research spend over the next year and a shortfall of £310 million caused by the impact on fundraising. It's expected that it will take four and a half years for the sector to recover – something that could have a devastating impact on people diagnosed with cancer in the future.

Despite the significant and unprecedented challenges, what positives have arisen for cancer research over the last year?

COVID-19 undoubtedly slowed scientific research all over the world in 2020, but science has not stopped completely. Scientists supported by Worldwide Cancer Research have contributed to several important breakthroughs in 2020 – including a new cancer vaccine that could enter clinical trials within the next three years and a game-changing treatment for prostate cancer that could be available to patients within four years.

We were also delighted that, thanks to the generosity of our supporters, we were still able to fund new projects this year. We invested over £3.2million in 16 new projects happening in 11 countries around the world. Knowing that we will continue to start new cancer cures gives us, our supporters and people affected by cancer, much-needed hope for the future.

Finally, how can we ensure that cancer research remains a priority in such challenging times?

The type of research Worldwide Cancer Research funds is what we call discovery research. It's the starting point from which new ways to prevent, diagnose and treat cancer emerge. Without it, we would not have the foundations to build on and we could miss out on future lifesaving discoveries. We must continue to fund this type of research to keep the ideas flowing down the pipeline – now is the time to invest in our future. As we have seen with the amazing response to COVID-19, research is essential to progress. And putting money behind research and those that fund it means that we can make progress faster.

People have woken up to the power of science and research to solve global problems. With 1 in 2 of us in the UK predicted to receive a cancer diagnosis during our lifetime, this is the time to use the momentum we have gained and use it to tackle the 'big C'.

worldwide cancer research

Do you want to become a regular **READER OF SCIENTIA?**

Scientia's mission is to connect people: scientists and educators, policy-makers and researchers, and the public and private sectors. We communicate science into the wider world, providing **high-quality, engaging,** and **relevant information** to our audience of thinkers and explorers.

Register today for your **FREE** subscription at:

www.scientia.global/subscribe/

EDIT FOR IMPACT AND ACHIEVE YOUR PUBLISHING POTENTIAL

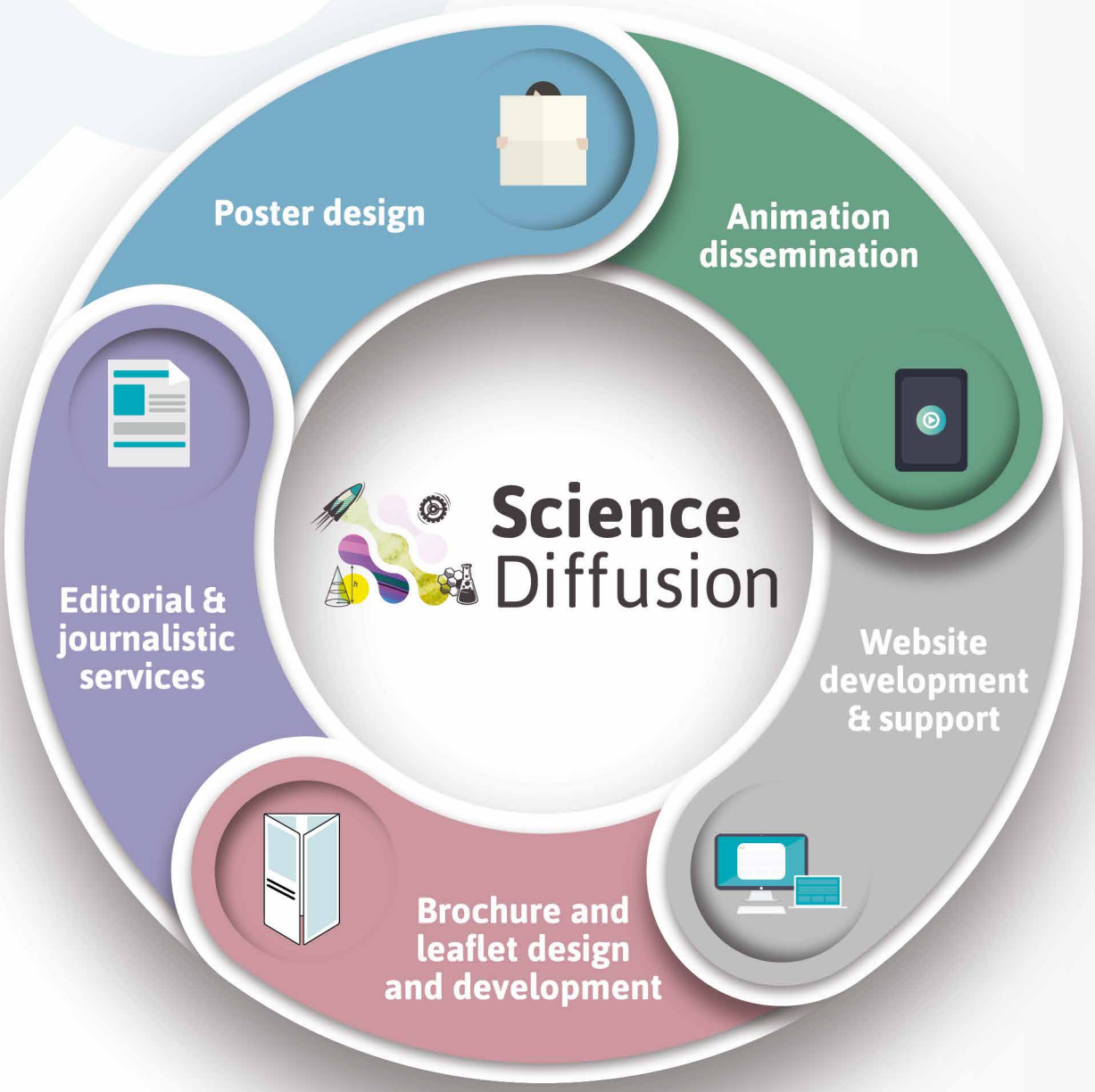
Our team is unique. We are a group of academics, publishers, marketeers, and media specialists who have come together from around the world to help you achieve maximum impact from your research.

We know what makes a great, high-impact, effective paper. We've published our own work in the top listed journals with big impact factors. We know how to sell ideas to leading journals and get research papers successfully published.

Visit: www.diffusionediting.com

Do you want to increase the **visibility** and **accessibility** of your **research?**

Contact us today to discuss your dissemination needs and find out how we can help.



W: www.sciencediffusion.com **E:** info@sciencediffusion.com

worldwide cancer research

EVERY DAY, MILLIONS OF LIVES ARE TURNED UPSIDE DOWN BY CANCER

#LivesTurnedUpsideDown

Every day millions of lives are turned upside down by cancer.

Whether they are one of the estimated 1 in 2 people worldwide diagnosed with some form of the disease or one of those forced to watch a much-loved friend or family member fight it…

However, there is hope. Worldwide Cancer Research funds pioneering research projects around the world, working tirelessly to find better ways to prevent, diagnose and treat the disease.

To find out more, please visit www.worldwidecancerresearch.org