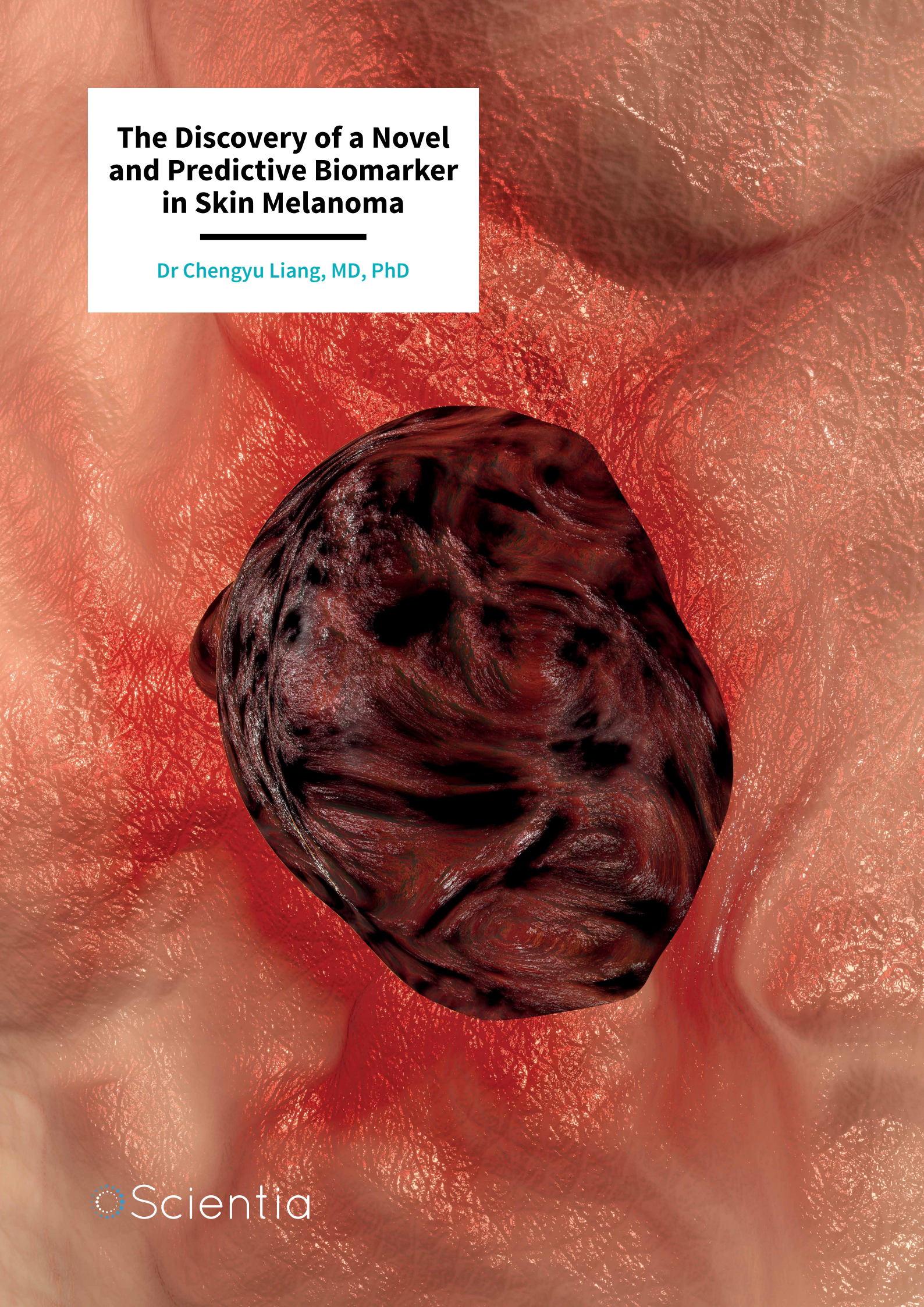
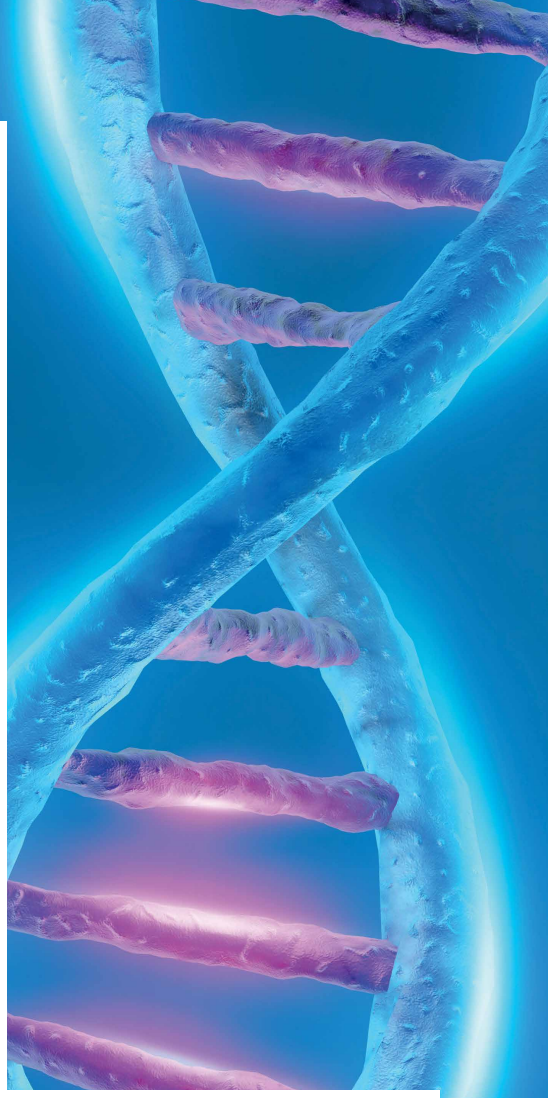
The Discovery of a Novel and Predictive Biomarker in Skin Melanoma

Dr Chengyu Liang, MD, PhD



Scientia

THE DISCOVERY OF A NOVEL AND PREDICTIVE BIOMARKER IN SKIN MELANOMA



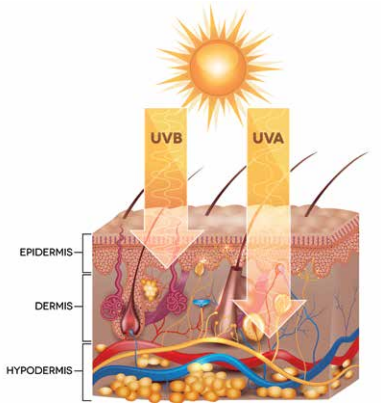
Ultraviolet radiation (UVR) from sunlight has been identified as a leading risk factor for the development of melanoma. Despite numerous research studies, the molecular mechanisms underlying the link between UVR and melanoma remain still poorly understood. **Dr Chengyu Liang**, from The Wistar Institute 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.

THE WISTAR INSTITUTE