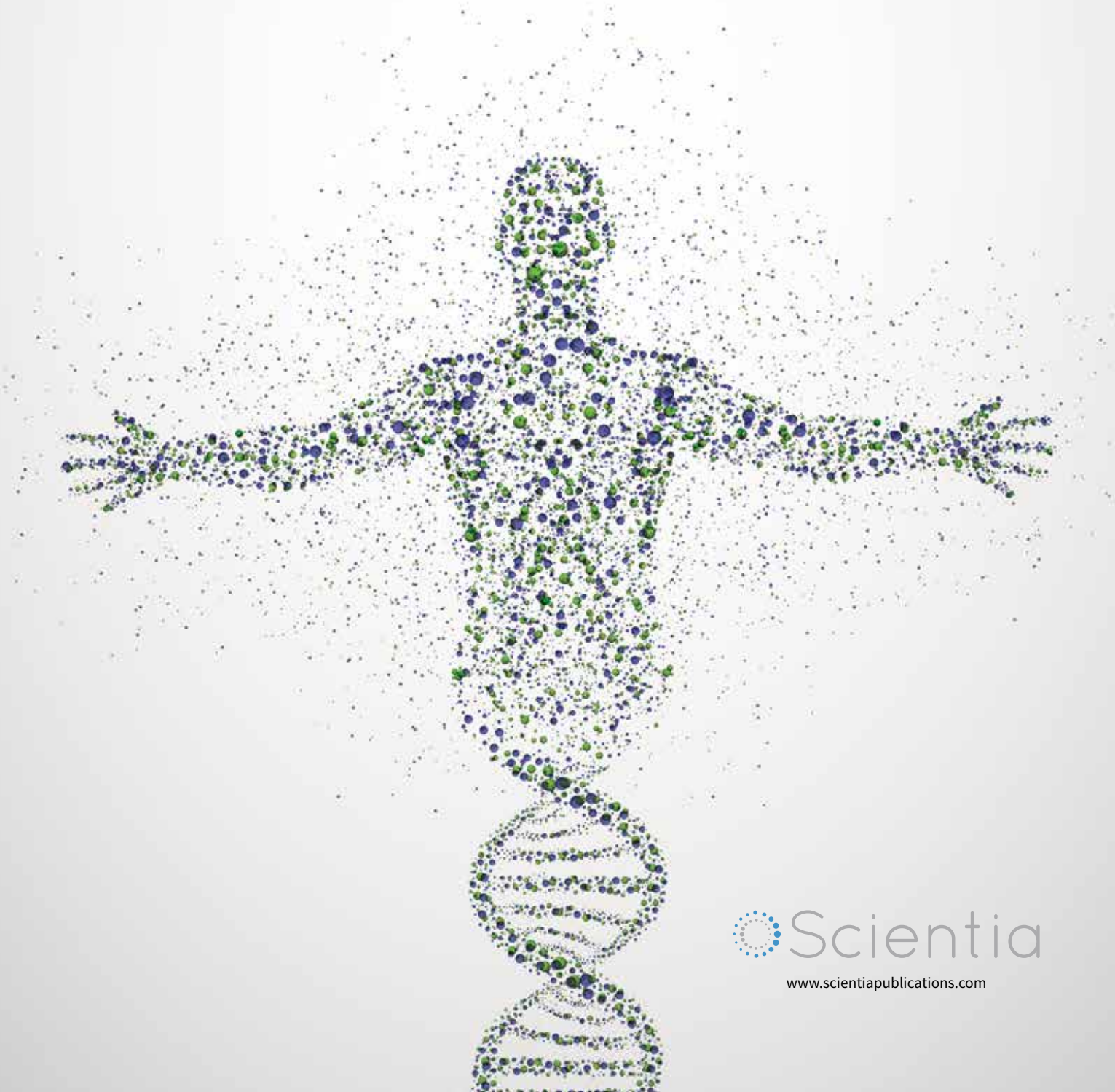
The Biology of
Telomere Chromatin in Stem cells and Cancers

Dr. Lee Wong



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Dr. Lee Wong is a molecular biologist at Monash University, who has been studying the mechanisms regulating the role of specific chromosomal structures known as telomeres in enabling stem cells and cancer cells to develop and replicate. The work has implications for anti-cancer and stem-cell based therapies.

First, what is your academic background, and how did you start your career in biomedical research?

I have obtained both my undergraduate and postgraduate degrees from Monash University, Australia. In 1995, I have completed a Bachelor of Science Degree and I undertook an Honours year in the Interferon Laboratory, Department of Biochemistry and Molecular Biology under the supervision of Dr. Stephen Ralph. Later, I started my PhD studies on the same project. The focus of my PhD generally involved the interferon treatment in Cancers. After obtaining my PhD in 1999, I worked as a postdoctoral fellow with Prof. Andy Choo at the Murdoch Childrens Research Institute to study chromosome biology. I have recently established a new research group at the Department of Biochemistry and Molecular Biology. Our vision is to identify new molecular players and fundamental mechanisms that control chromosome structural stability through research on stem cells and cancer cells. This is important as chromosome function is vital for proper inheritance of genetic material and has significant implications on human health.

Your recent publications address the role of the cellular protein H3.3 in the regulation of telomere stature and integrity in stem cells and cancer cells. What are the most important findings and what are their possible implications for human medicine?

A continual maintenance of telomere length is required for cell growth as critically short telomeres induce replicative senescence and cell cycle arrest. Cancer cells bypass this arrest mostly by activating telomerase (the enzyme responsible for synthesis of telomeres). However, a subset of cancers (~15%) uses a DNA recombination based Alternative Lengthening of Telomeres (ALT) mechanism. ALT is prevalent in cancers such as bone and brain cancers.

H3.3 is highly evolutionary conserved protein and normally localises to telomeres, but very little is known about the molecular functions associated with this chromatin modification. Our studies show that H3.3 distribution in the genome and its specific modification are severely disrupted in ATRX null cancers. This is one of the few chromatin modifications which is known to distinguish ATRX null cancers from normal cells.

Throughout your research have you collaborated with other research groups? If so, what has been their role and how did they contribute to the progress of your research?

I have collaborated with Professor Philippe Collas from the University of Oslo. Collas heads a lab with strong expertise ranging from cell imaging, chromatin studies to bioinformatics analysis. He has ~160 publications in the chromatin field, including in highly ranked prestigious journals. Collas has a strong interest in studying the role of H3.3 in gene regulation, particularly in cancers and has a long-term collaboration with our group. Collas and his team will provide their expertise in chromatin studies using advanced technologies.
I have also a long-term collaboration with Dr. Jeffrey Mann from Monash University, who is a co-author of several of my publications. Mann has vast experience in gene-editing and producing mouse models using modern molecular technologies. Mann has and will continue to help us in the establishment of stem cells with specific H3.3 mutation, which is a key tool in our research.

Are you planning to continue your research on the regulation of telomere function by chromatins? What might be the next step?

One of the next immediate steps is to perform a series of experiments to determine the molecular role of H3.3 in both normal and cancerous cells. This will contribute to an understanding of core chromatin pathways, and reveal how defects in these pathways can lead to cancer. This knowledge will be instrumental to the development of effective and targeted therapies for these cancers, which have proven to be refractory to standard chemotherapeutic interventions.

The other step we will take is to define the non-genetic factors involved in ALT suppression. It is unclear how ATRX mutation leads to ALT. We will perform methodical and targeted disruptions of factors which contribute to telomere chromatin formation in order to identify the components which act in concert with ATRX to suppress ALT. This work will yield a better understanding of the non-gene defects associated with ALT activation and thus, potentially lead to new diagnostic and treatment targets for ATRX mutation-driven ALT cancers.

Principles and Biomedical implications of the Chromatin Regulation of Telomere Integrity

Telomere length is directly linked to the ability of stem cells and cancer cells to live and replicate infinitely. Here we discuss the principle in light of the findings of Dr. Wong on the role of H3.3 histone protein and its chaperone ATRX on maintaining telomere integrity, and their possible therapeutic implications.

TELOMERE FUNCTION IN CELL BIOLOGY

The DNA held within the nuclei of our cells is packaged and arranged in biological structures known as the chromosomes. The latter carry all the genes necessary to control the various functions performed by the cell. During cell division, our chromosomes undergo duplication, a process undertaken by specialized DNA-building enzymes to ensure that both of the daughter cells receive equal sets of new chromosomes. However, in each time a cell divides these enzymes do not copy the chromosomes to their full length, making the daughter chromosomes of the new cells shorter than their predecessors (a phenomenon known as chromosome truncation). To avoid the loss of essential genes due to chromosome truncation, the terminal ends of the chromosomes possess a non-gene-coding region of repetitive DNA units known as 'telomeres'. Upon subsequent cell divisions, a number of these telomeres are abraded, accounting for shorter daughter chromosomes. Adult body cells (referred to as somatic cells) lack efficient mechanisms for telomere replenishment. Thus, at some point in the cell lifespan where the telomeres reserve is fully consumed (after 50-70 divisions), the cell stops dividing and eventually dies. Indeed, scientists have established a link between telomere chromosomal truncation and cell ageing. On the other hand, some types of eukaryotic cells, namely embryonic cells (include stem cells) and cancer cells, possess a specific enzyme termed telomerase, which is capable of replenishing the chromosomal telomeres, allowing a nearly infinite ability of division. Although telomerase is also present in somatic cells, its activity is regulated to very low or even undetectable levels under normal circumstances.

Although telomerase is a primary biological factor that drives telomere lengthening, other factors also influence the capacity for telomere renewal. Chromosomes are not typically found on their own in the nucleus, but are rather complexed with structural proteins. The chromosomal DNA-protein complexes are termed chromatins. One of the chief proteins that are found in close association with chromosomal DNA is known as histones. The latter has been found to play a major role in controlling the integrity and stability of telomeres. Dr. Wong and her research team have been studying the role of chromatins in telomere biology, in terms of maintaining structure and length. These studies will help the scientific community to better understand the basis of the diseases associated with telomere mutation, malformation or dysfunction.

DISEASE IMPLICATIONS OF TELOMERE BIOLOGY

As discussed, there is a well-established correlation between the lifespan of cells with telomere shortening. Shorter telomere lengths have been linked to human health conditions, many of which are age-related. They have been reported in patients suffering from age-related diabetes, cardiovascular disease, migraines and increased risk of neurodegenerative diseases. There are also direct evidences for the importance of telomere length in human disease derives from patients with mutations in the genes encoding the functional components of the telomerase enzyme. These diseases are characterized by rapid telomere attrition, and thus have shorter telomeres and exhibit compromised regenerative capacity of tissues particularly in highly proliferative tissues such as bone marrow, epithelial cells and liver. One example of such human diseases is Dyskeratosis congenital, a premature aging syndrome linked to mutations in the telomerase complex resulting in decreased telomerase stability and shorter telomeres. Patients with Dyskeratosis congenital develop numerous different disease conditions, including short stature,

bone marrow failure, skin defects, blood-cells regeneration defects, infertility and premature death. These patients are also susceptible to develop cancer. Another example of a human disease involving telomerase mutation is Aplastic Anaemia. Individuals with Aplastic Anaemia also show accelerated telomere shortening and die young. Consistently, mouse models of telomerase deficiency also show affected maintenance and regeneration of tissues that undergo extensive proliferation, further implicating the impact of the short telomere length on health.

TELOMERE CHROMATIN AND STEM CELLS

Stem cells are undifferentiated cells that retain an exceptionally high capacity for unlimited replication and the ability to differentiate into specialized organ cells, such as muscle or liver cells (a characteristic referred to as pluripotency). The capacity for continual telomere replenishment is important to the

maintenance of pluripotency in stem cells, but neither the detailed telomeric chromatin structure nor the mechanism for regulating the continual telomere length renewal have been defined. This knowledge is particularly valuable for better understanding of the pluripotent nature of stem cells, which can be the basis of therapies for many diseases related to telomere length and integrity. For instance, research on a class of stem cells, known as induced pluripotent stem cells, which are artificially obtained from somatic cells, show that upon their development, a telomerase-dependent telomere elongation occurs, which continue post-reprogramming until reaching an embryonic cell telomere length. Interestingly, induced pluripotent stem cells generated from dyskeratosis congenital patient cells, which have short telomeres and suffer from premature senescence, could restore telomere integrity. In somatic cells, telomeres are mostly enriched with histone modifications characteristic of 'silenced chromatin', which remains in a permanent strong association with telomeres. However, Dr. Wong and her fellow researchers have shown for the first time that embryonic stem-cell telomeres are enriched with an 'active' histone variant termed H3.3, which gradually dissociates from telomeres upon cell differentiation. Moreover, depleting embryonic stem cells of H3.3 resulted in telomere-dysfunction and deregulation of telomere chromatin, indicating that H3.3 plays important role in maintaining telomere chromatin integrity. These findings raise the question of whether telomere chromatin remodelling might be a requisite for telomerase-dependent telomere elongation during cellular differentiation.

TELOMERE CHROMATIN AND CANCERS

As discussed above, somatic cells in absence or insufficiency of telomerase can stop dividing and die. However, if telomerase is re-activated, cells escape telomere attrition crisis and can become immortalized. The majority of human cancers reactivates telomerase expression, which makes it a potential biomarker for most cancers. Human ALT (Alternate Telomere Maintenance) cancer cells, cells characterized by remarkable high telomere length, do not contain any telomerases, but rather adopt other mechanism to maintain telomere length. ALT mechanism is observed in at least 15% of human cancers, ALT is a common phenomenon in tumours including those from the brain/central nervous system and bones. Brain and bone cancers represent the leading cause of cancer related mortality respectively, in young people (15-29). In addition, brain cancers are the leading cause of cancer mortality in all age groups under 40. .A recent study has linked ALT cancers to a common mutation of ATRX, an enzyme recently identified by Dr. Wong and other researchers to be essential to the H3.3 histone assembly and deposition. The frequency of ATRX mutation is as high as 90% in human ALT tumours. Thus, currently the work of Dr. Wong is focused on determining whether mutations of ATRX affect establishment of chromatin marks at telomeres in ALT cancer cells. The loss of a proper inheritance of chromatin marks at telomeres in ALT cancers may drive indefinite telomere elongation, hence promoting an unlimited cellular lifespan in these cancers.

The existence of ALT for telomere maintenance in cancers raises the possibility that telomerase-positive tumours might escape anti-telomerase therapies by activating the ALT mechanism. For these reasons, it is important to delineate the ALT mechanism to have a clearer picture of the tumorigenic process and the development of specific chemotherapeutic interventions targeting ALT-specific cancers. The work of Dr. Wong will unveil a novel telomere maintenance mechanism that is central to the replication of ALT cancer cells. It will have an impact on our understanding of the behaviour of ALT cancers in terms of the underlying mechanism for telomeric alterations accompanying malignant transformation, and potential target for the clinical treatment of these cancers.

THE PROMISES OF THE TELOMERE CHROMATIN RESEARCH

In the future, more detailed studies of the dynamic chromatin changes at telomeres that occur as cells undergo differentiation, nuclear reprogramming, or cancer development will be key to understanding how telomeres and their epigenetic maintenance are crucial to human health. Furthermore, many questions endure about the role of telomeres and the aging process. A full understanding of how telomeres impact the proliferative capacity and pluripotency of embryonic stem cells and induced pluripotent stem cells holds great promise for stem cell therapies of disease. Thus, the complete understanding of telomere biology will have broad-ranging implications for human health.

Researcher Profile

Dr. Lee Wong
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Dr. Wong is a group leader at the Department of Biochemistry and Molecular Biology, Monash University. She leads an internationally competitive group of researchers in the fields of chromosome and epigenetic research in stem cells and cancers. Her research is mainly focused on understanding the mechanisms underlying the establishment and regulation of centromere and telomere chromatin in stem cells and cancers, and the epigenetic reprogramming during stem cell differentiation and early embryo development.

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