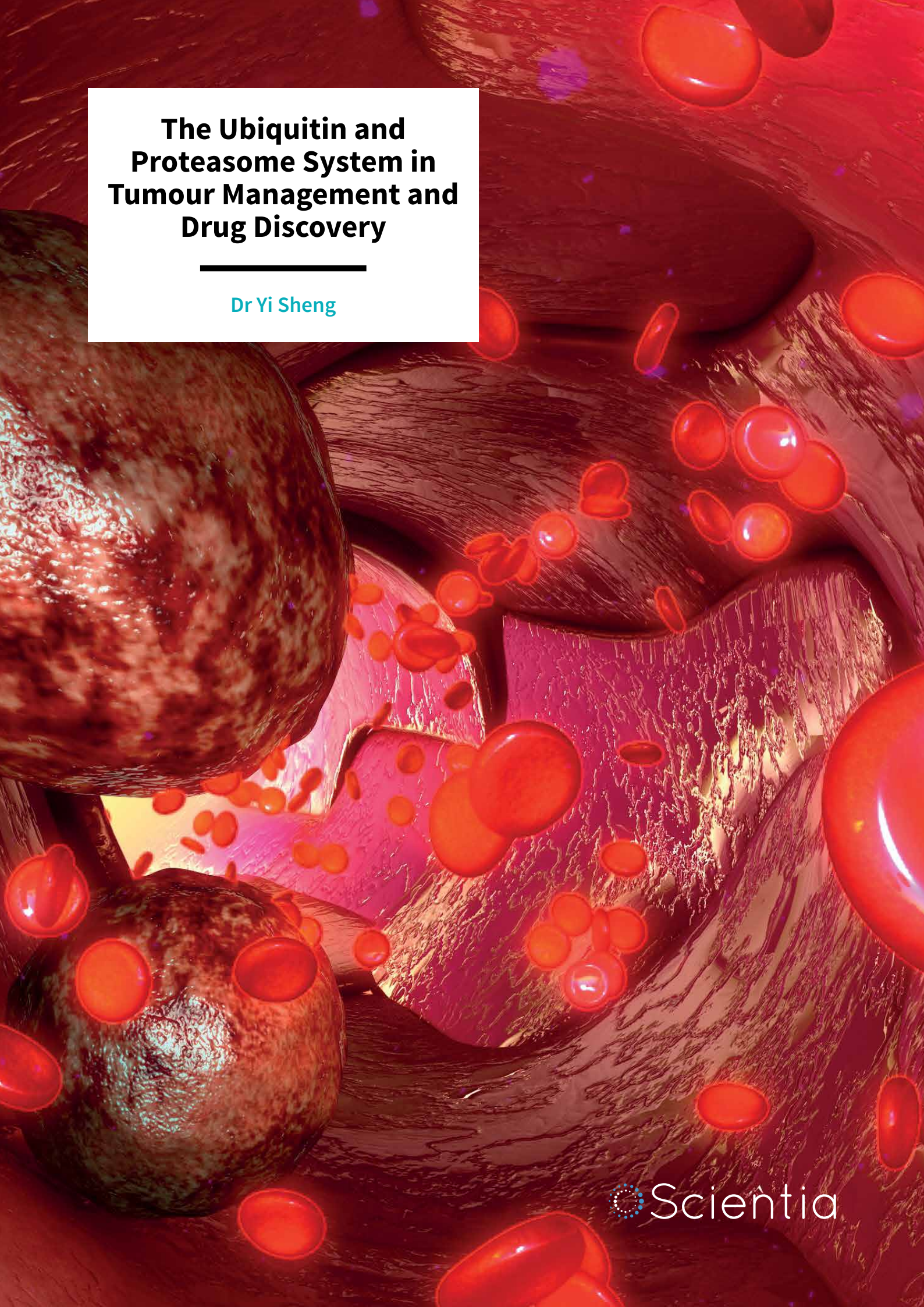
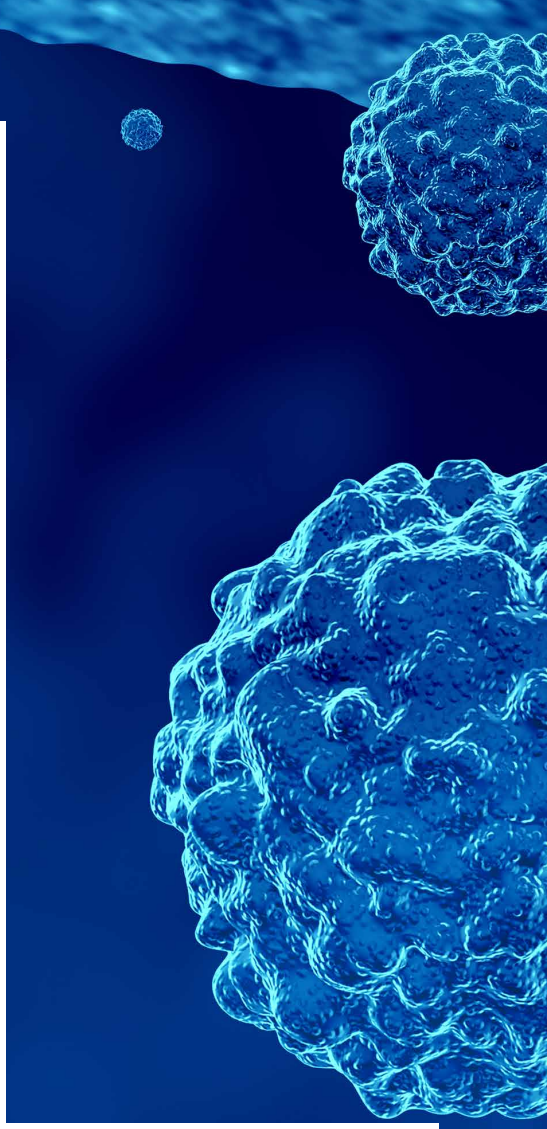


The Ubiquitin and Proteasome System in Tumour Management and Drug Discovery

Dr Yi Sheng

Scientia

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.

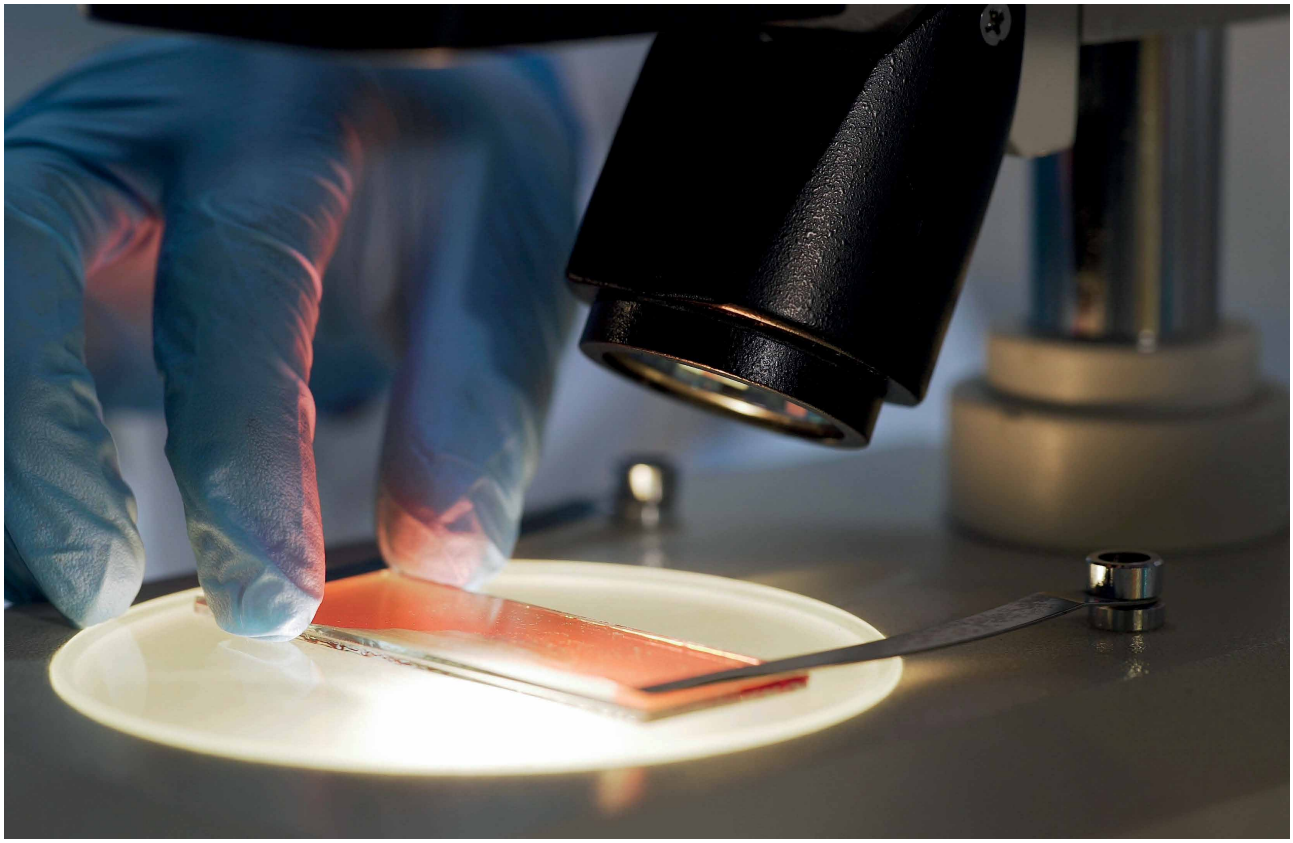
Dr Sheng and her team found that MDM2 performs as a more effective ubiquitylation enzyme for both auto- and p53-ubiquitylation than Pirh2. During their testing, the team discovered that these two E3 ligases, MDM2 and Pirh2, target different sites on the p53 ubiquitin tagging. While the significance of 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

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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.

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