In Conversation With Xin Lu

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Abstract

Xin Lu is Professor of Cancer Biology at the University of Oxford, UK, and Director of the Oxford Branch of the Ludwig Institute for Cancer Research, co-Director of the Cancer Research UK Oxford Centre, NIHR Oxford Biomedical Research Centre Multi-Modal Cancer Therapies Theme Lead and Director of the Oxford Centre for Early Cancer Detection. She has a long-standing interest in mechanisms of tumour suppression and cellular plasticity, centred on studies of p53 and the ASPP family of proteins (Apoptosis-Stimulating Protein of p53; Ankyrin repeats, SH3 domain and Proline-rich sequence containing proteins). Her lab's discovery of ASPPs, regulators or 'molecular switches' of the apoptotic function of p53, led to key insights into the role of cell plasticity in cancer and other diseases, and these could pave the way to new diagnostic and therapeutic interventions. Xin has received many awards and honours in recognition of her significant contributions to cancer biology, including being elected as a Fellow of the Royal Society in 2020. Here, she outlines how her breakthrough discovery of ASPPs came about and its impact on the cancer field, as well as highlighting the importance of mentors including Min Wu, Birgit Lane and David Lane in shaping her early career and helping her to navigate a new research world, having moved to the UK from China in the '80's.

Congratulations on being elected as a Fellow of the Royal Society earlier this year. What was your reaction when you first received the news?

I was very humbled to receive this honour from the Royal Society. I feel very grateful for the fantastic scientists in my laboratory, the colleagues I've had the privilege to work with throughout my career to date, my wonderful mentors, and the long-standing support from the Ludwig Institute for Cancer Research. Without all of these, this recognition would not have been possible.

What was the path that led to your discovery of ASPPs?

My research interests have been centred on tumour suppression, as a detailed understanding of this process will help us to develop strategies for cancer prevention, early cancer detection and therapy. The tumour suppressor p53 is the most mutated gene in human cancer; it's mutated in ~50% of all cancer cases. Understanding how this molecule can prevent us from developing tumours and how to harness its tumour suppressive function to kill cancer cells

are fundamental questions in cancer research. During my post-doc with Sir David Lane - one of the discoverers of p53 - I observed that its tumour suppressive function can be bypassed in cancer cells by the overexpression of oncogenes such as Myc that promote cancer growth. When I started my group, I continued to search for other factors with this function. We knew then that p53 was a transcriptional regulator and its best-known tumour suppressive function at the time was to induce cell cycle arrest or apoptosis (cell death). As cell cycle arrest is reversible whereas cell death is irreversible, I focused my lab on identifying factor(s) that can regulate the cell death-inducing function of p53, with the aim of specifically killing cancer cells.

We initially looked into the impact of p53 post-translational modifications, particularly phosphorylation, on its tumour suppressive function. To our surprise and everyone's disappointment, we showed that phosphorylation only fine-tuned the activity of p53 rather than acting as its on/off switch. Subsequently, we turned our attention to proteins that could bind and potentially regulate p53. We made a scientific "bet" on 53BP2, a protein identified at the time to interact with the DNA-binding domain of p53 and the anti-apoptotic protein Bcl-2, but with little else known about its function.

We raised a panel of monoclonal antibodies against 53BP2 and were surprised to detect a larger protein than previously reported. When this larger protein was co-expressed with p53, we observed its ability to selectively enhance p53's target-selective transcription to promote apoptosis, with less impact on the cell cycle function of p53. A further c-DNA library screen, sequence analysis and functional characterisation led to the identification of its family member, which we named ASPP1 (Apoptosis Stimulating Proteins of p53). The name ASPP also reflects the unique protein domain structures these two proteins share: Ankyrin repeats, SH3 domain, and Proline-rich region containing Protein. We renamed our newly cloned full-length 53BP2 as ASPP2 to reflect its sequence and functional similarity to ASPP1. Searching the available protein database using the unique protein domain structures, we identified the third member of the ASPP family. We later named this protein iASPP, inhibitory member of ASPP, after a fruitful collaboration with Patricia Kuwabara led us to discover that iASPP inhibits the apoptotic function of p53, a property of iASPP that is conserved from worm to human. This contrasts with the stimulatory role of ASPP1 and ASPP2 on p53-induced apoptosis. We now know that there is only one ASPP family member in both the worm *Caenorhabditis elegans* and fly *Drosophila melanogaster*.

What broader impact did discovery of the ASPPs have on the cancer field?

Studies from us and others have now shown that the ASPPs are key regulators of cellular plasticity - the ability of a cell to change from one state to the other. This is partly achieved through their ability to shuttle between the cytoplasm/cell junction and the nucleus to control target-selective transcription and cell fate determination, via both p53-dependent and -independent pathways.

ASPP2 localises to cell junctions in many normal epithelial cells, including gastric epithelial cells, and maintains the integrity of epithelial apical polarity by binding to Par3. Over 80% of cancers originate from epithelial cells and loss of cell polarity is a hallmark. Consistently, ASPP2 is a prime cellular target of one of the most important oncoproteins and virulence factors of *Helicobacter pylori*, CagA, a potent inducer of cellular plasticity and a major risk factor for gastric cancer. Some of the tumorigenic properties of CagA appear to be achieved through its ability to complex with ASPP2 and disrupt cell polarity and induce

epithelial-mesenchymal transition. Loss of ASPP2 is associated with poor survival in hepatocellular carcinoma and breast cancer.

In the nucleus, ASPP1 and ASPP2 function as p53 regulators, controlling p53's transcriptional selectivity. Specifically, they enhance the transcriptional activity of p53 on promoters of pro-apoptotic genes but have much less impact on genes that cause cell cycle arrest. *In vivo*, ASPP2 acts as a haploinsufficient tumour suppressor and a p53 activator in mouse models and in human cancers, including head and neck, and breast cancers.

iASPP blocks p53-induced apoptosis, and the iASPP-p53 complex is often enriched in highly metastatic cancer cells. Inhibiting iASPP-p53 binding using small molecules can restore p53's tumour suppressive property in human melanoma cells. Recently, we reported the iASPP-p53 co-crystal structure, which revealed precisely how iASPP can alter the target selectivity of p53, thus opening new prospects for agents targeting specific properties of p53. However, despite our detailed understanding of ASPP functions, ASPPs are unfortunately not easy cancer targets *in vivo* because they play a critical role in controlling other diseases beyond cancer.

In what other diseases are the ASPPs implicated?

Beyond cancer, ASPPs touch our "hearts and minds". ASPP2 plays a key role in the development of the central nervous system and ASPP2 defects are likely to contribute to brain structural abnormalities in the human 1q41/42 microdeletion syndrome. A genomic sequence analysis identified a point mutation in ASPP2 as a potential genetic cause for open angle glaucoma in a large multiplex family. All these observed pathological phenotypes could be explained by ASPP2's roles in maintaining cell polarity, cell adhesion and facilitating apoptosis.

iASPP mutation or deletion underlies a cardio-cutaneous disorder with sudden cardiac death, due to an essential role of iASPP in controlling the integrity of desmosomes, which are specialised anchoring junctions enriched in cells that endure mechanical stress, such as cardiac myocytes in the heart and keratinocytes in the skin. Our identification of iASPP as a critical regulator of desmosomes has broad implications for understanding unexplained sudden cardiac death. Over 600,000 UK individuals are estimated to experience sudden cardiac death, mostly caused by genetic mutations. Around half of them are caused by mutations in desmosome components, and a key remaining challenge is to identify the faulty genes responsive for the remaining 50% of the patients. Following the initial identification of a homozygous loss-of-function iASPP variant in a large consanguineous family with five cases of paediatric dilated cardiomyopathy by Orly Avni's group, a study led by Elena Zaklyazminskaya showed that biallelic iASPP gene variants, which often cause loss of iASPP expression, are a causal genetic defect for paediatric sudden cardiac death in five more families. The latter study argued for the inclusion of iASPP in the candidate gene panel for genetic testing of paediatric dilated cardiomyopathy.

How can an understanding of 'molecular switches' of cellular plasticity guide the early detection and prevention of cancer?

In my view, cellular plasticity is at the heart of cancer biology and is vital for cancer initiation and progression. It is also crucial in patients' responses to therapy as plasticity enables the cancer cells to escape immune surveillance and to acquire resistance to therapy. In fact, the ability of four transcription factors, including Myc, to reprogram fibroblasts to iPSCs is a

beautiful demonstration of cellular plasticity. The enhanced reprogramming after ablating p53 illustrates that transcription factors, particularly oncogenes and tumour suppressors, are key molecular switches for cellular plasticity. Our lab aims to expand the known repertoire of molecular switches that respond to stress and also to determine how epithelial cells sense external signals and relay these to the nucleus to achieve selective transcription and cell fate determination.

Because ASPP2 and iASPP are detected at cell junctions as well as in the nucleus, we paid special attention to shuttling molecules such as β-catenin and their roles in cell fate determination. The first challenge we encountered was how ASPPs can enter the nucleus without an identifiable nuclear localisation signal (NLS). Our search led us to the unexpected discovery of the second general nuclear import pathway, named the RaDAR pathway. The RaDAR import 'code' we identified enabled us to accurately predict which of the ~250 Ankyrin Repeat-containing proteins in mammalian cells can enter the nucleus via the RaDAR pathway, explaining the import of some of the 50% of nuclear proteins that lack a canonical NLS. An important biological implication of the RaDAR pathway is the finding that the most common familial melanoma-associated mutation results in acquisition of a RaDAR code in the tumour suppressor p16, resulting in its nuclear localisation.

The RaDAR nuclear import pathway also enabled us to propose a group of proteins called STRaNDs (shuttling transcriptional regulators and non-DNA binding), including ASPPs, as potential guardians of cellular plasticity. They are proteins that translocate from the cytoplasm to the nucleus in response to external signals and regulate transcription - selective transcription in particular - without binding to DNA directly. We hope that many of the newly identified molecular switches of cellular plasticity can serve as biomarkers of early cancer diagnosis and/or molecular targets for treatment to prevent cancer progression.

Which cancer types are you particularly interested, and why?

I'm particularly interested in upper gastrointestinal cancers – oesophageal and gastric cancers. These are highly plastic cancers with pre-cancerous high-risk conditions such as Barrett's Oesophagus, gastritis, Epstein Barr virus and *H. pylori* infection, making them ideal for exploring the multiple layers of successive molecular switches needed for cancer initiation. An increased understanding about cancer initiation will help us to develop biomarkers to identify when the crucial switch(es) have occurred that tip the balance towards cancer initiation. This will present opportunities for catching these cancers earlier and cancer prevention.

What tools and methods do you generally employ in your lab?

We have used approaches that involve biochemical, cell biology and experimental model systems in our lab, but the recent technology advancement provided us with great opportunities to carry out experimental medicine-based discoveries. In the last few years, my group has enhanced our experimental medicine research activity. For example, we used single cell RNA-sequencing to study how the inflammatory pre-malignant condition Barrett's Oesophagus arises, revealing a profound transcriptional similarity between cells in Barrett's Oesophagus and oesophageal sub-mucosal glands. These findings urge a re-think of the origins of this disease and may influence treatment and monitoring for early diagnosis or prevention of oesophageal adenocarcinoma.

What role did your early-career mentors play in shaping your research interests? Did they also influence your style of running a lab?

I have been fortunate to have excellent mentors that have played different and important roles in shaping each stage of my research career. My supervisor during my Master's degree at the Cancer Institute, Chinese Academy of Medical Sciences (CICAMS), Min Wu, was very highly respected in China and demanded the highest possible standard from his students. This instilled in me a strong work ethic that I have maintained to this day. My PhD supervisor Birgit Lane gave me the freedom to devise my own scientific approach to the research question she suggested for me and I was free to pursue my own scientific interests within this and related fields. This scientific freedom continued and was encouraged during my postdoctoral work with Sir David Lane. I am grateful for the support from Ludwig Institute for Cancer Research, who have funded my research for my entire independent career, and a long list of people at the Institute who have supported me, especially the late Lloyd Old. Having these fantastic mentors has undoubtedly shaped how I run my lab. I give my lab members the freedom to develop the direction of their research projects, which is a vital skill for an independent research career.

Arriving from China, were there any obstacles you had to overcome to establish your academic career in the UK?

Obstacles?! I landed on a "new planet" and everything was new to me! The first flight I ever took was the Beijing-London flight when I moved to the UK, which lasted almost 24 hours and stopped at three counties. The only words I understood and remembered from various seminars I attended were the few oncogene names such as Myc and Ras that I had read in papers. I was one of only two Chinese people in the former Imperial Cancer Research Fund (ICRF), an organisation of around 700 people. Even though I had come from the top cancer research institute in China at the time, I felt overwhelmed by the high calibre of the scientists I was now mixing with and really doubted my ability to do anything useful. I still remember vividly my opening statement at my PhD interview; "I am from China and my (scientific) background is very bad but I would like to do a PhD with you". Reflecting back, I realised how brave my PhD supervisor Birgit Lane was by taking me on as her PhD student! Her excellent support and the friendly environment provided by the ICRF, the Clare Hall laboratory in particular, enabled me to start finding my feet.

What changes have you seen in the status quo of women in science during the course of your career?

I grew up with the slogan 'women hold up half the sky', so gender bias was not a big part of my early life. I was also lucky to have many female role models in my upbringing and during my early career. My mother was a doctor and a professor, and the CICAMS Director and almost half the CICAMS group leaders were women. In the UK, I even had a female PhD supervisor and my postdoctoral mentor was hugely supportive. It was only later, once I had started my group, that I realised female PIs were rarer and how fortunate I had been to have so many great role models to follow. The Athena SWAN Charter has been important in raising awareness about and support for women in science in the UK, and I hope to see further

improvement over the coming years. We need the very best scientists, male and female, to drive forward discoveries, and right now there still aren't enough women making it to the highest levels.

What is your key advice for early-career researchers seeking to forge a successful career in academia?

Don't give up! Persistence and optimism are key, and every cloud has a silver lining. If someone like myself can have a career in academia, the opportunity is there for everyone.

How do your balance your research commitments with your duties as Director of the Institute and other leadership positions in the University of Oxford?

Balancing these activities is a very difficult task and I don't think I necessarily have an answer. However, an all-inclusive approach is crucial; involve people at all levels when possible. I have been fortunate to have many supportive colleagues who have given me tremendous help. I try my best to achieve as much as I can; however, I have to accept that juggling my research with leadership roles both in the Institute and across the wider cancer research landscape at the University of Oxford will inevitably involve sacrifice.

What is your greatest achievement outside of the lab?

My two daughters.

If you hadn't become a scientist, what would you be doing?

During my teenage years, I practiced the violin for at least 3 hours every day. I grew up during China's Cultural Revolution, and Chairman Mao issued a call for the "Down to the Countryside Movement," in which young students from the city were sent to live in the countryside. Having been brought up in an urban area, I knew I didn't have the skills or stamina to cope with intensive agricultural labour. Instead, I hoped the violin would give me a skill that could ease the intense labour demand on me. But I was one of the lucky ones and right after I graduated from high school, the universities that had been closed during the Cultural Revolution reopened and I was able to sit the university entrance exams. I chose science and have been passionate about the subject ever since. I was fortunate enough to establish my own lab and I've never looked back! I consider myself very lucky to be paid to do my "hobby".

How do you relax away from the lab and have you had the opportunity to do this during this year, during lockdown and other COVID-19 restrictive measures?

Doing regular exercise has been my New Year's resolution for many years but failed to materialise until the lockdown. Daily morning walks in the local park is a new routine I acquired during the lockdown and it sets me up for the day. Since working from home, I've been spending a lot of time on calls and in virtual meetings and so this time outside is increasingly important.

Footnote
Xin Lu was interviewed by Paraminder Dhillon for *The FEBS Journal's* In Conversation With…interview series.

Selected publications and related interview

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