YANG SHI
LUDWIG OXFORD

The epigenetic explorer

Yang Shi has always associated science with a sense of freedom. This is one reason he enjoys his job so much.

"I'm happy to go into work every day," says Shi, who joined the Oxford Branch of the Ludwig Institute for Cancer Research in the summer of 2020. "You can pursue things that you find interesting. You could be listening to a talk when something just clicks, and you come up with an idea and think to yourself, 'Oh, I have a unique perspective on this. I could try it.' Your discovery might have an impact on human health, or answer a very important fundamental question in biology."

Shi knows this better than most. In 2004, he and his colleagues identified and characterized an enzyme, LSD1, that erases methyl marks from histones, the bead-like proteins DNA spools around in the nucleus of the cell. The discovery by Shi's team upended a 40-year-old dogma that considered this particular kind of epigenetic modification—as the chemical tagging of DNA and histones is known—to be irreversible. It forced a reconsideration of existing models of genomic regulation, since epigenetic marks help determine which stretches of the genome are available for reading by the gene expression machinery of the cell—and that, in turn, controls every aspect of a cell's identity and function. As might be expected, aberrations in the distributions of genetic marks are common drivers of disease, especially cancer.

Shi's laboratory went on to identify many other histone demethylating enzymes with roles in a wide array of biological processes. More recently, he and his colleagues have

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discovered several enzymes that methylate RNA and possibly influence RNA splicing and the translation of gene transcripts into proteins, another level of regulation in the expression of the genome. On the translational front, Shi's work contributed to the development of LSD1 inhibitors for cancer therapy, and these drugs are already in clinical trials for the treatment of cancer and neurological disorders. Meanwhile, Shi's laboratory continues to contribute studies to that end—most notably on the potential use of such drugs to enable and improve the efficacy of immune checkpoint blockade (ICB) therapy, and to achieve sustained reinvigoration of T cells for ICB. His team is also exploring the pharmacological targeting of epigenetic modifiers for the treatment of pediatric brain cancers and the blood cancer acute myeloid leukemia, both of which are especially characterized by epigenetic dysfunction.

AN UNLIKELY START

Shi's journey into cancer research was an unlikely one. In high school, Shi was interested in many different topics, including science.

He ultimately opted to indulge his interest in biology, joining the graduate program at New York University, where he explored the regulation of a multi-gene family in mice for his graduate studies in Eva Derman's laboratory, earning his PhD in 1987. The

following year, he began a postdoctoral fellowship in the laboratory of Princeton University researcher Thomas Shenk.

Working out of Shenk's lab in 1991, Shi discovered YY1, a mammalian transcription factor (a regulator of gene expression) that can, rather uncommonly for proteins of its ilk, both activate and repress transcription (the reading of a gene for its expression). YY1 was discovered by three independent groups around the same time, but Shi's name for it, short for Yin Yang 1, was broadly adopted because his work captured the functional quirk of the protein as both an activator and repressor.

Work done by multiple labs has since shown that YY1 helps regulate many important biological processes, including cell proliferation, DNA repair and programmed cell death, or apoptosis—all of which can play major roles in the genesis of cancer. Recent evidence suggests that, perhaps due to its dual function, YY1 can operate as either a promoter or suppressor of cancer.

LSD1

Following his postdoctoral fellowship, Shi joined the faculty of Harvard Medical School in 1991 as an assistant professor. He was granted tenure and made a full professor in 2004.

That same year, Shi's group reported the discovery for which it is best known: histone demethylation. Owing to its chemical stability and the failure of researchers to find an enzyme capable of stripping it from histones, the methyl mark had long been considered irreversible. "People had always thought methylation was a static modification, and therefore not as interesting as phosphorylation, which is reversible and plays a very important regulatory role because it's dynamic," says Shi.

The discovery of LSD1 was somewhat unexpected. Shi and postdoctoral fellow Yujiang Shi were studying the role of metabolic enzymes and their cofactors in epigenetic regulation when they grew curious about how the homolog of a metabolic enzyme, nPAO—which they had discovered in a scrum of proteins involved in transcribing genes—might function in such processes.

> "There are so many different types of RNAs and they are methylated by different enzymes. … These enzymes ultimately will be tied to human diseases, I'm sure of it."

They hypothesized that nPAO regulates chromatin structure either through a reaction called polyamine oxidation or demethylation of histone. But months of experimentation failed to detect polyamine oxidase activity. It was only when they switched the substrate in their experiments from polyamine to one of the histones—H3—that they discovered nPAO's ability to strip specific methyl groups from histone proteins, and gave their enzyme the name that stuck: lysine-specific histone demethylase 1, or LSD1.

When Shi and his colleagues reported their landmark discovery in 2004, the response from the scientific world was immediate. "I got phone calls before the paper was officially out from people who had heard rumors about the discovery and wanted to learn more," Shi recalls. "The thought did cross my mind that if I got this wrong, my career would be over."

He was, of course, far from wrong. His

Photo by Flynn Larsen

discovery prompted a profound rethink of existing models of genomic structure and regulation, since epigenetic modifications alter the packaging of DNA, either unfurling it for reading, or tucking it away, inaccessible to the cell's transcriptional machinery. Further, the specificity of the LSD1 enzyme immediately suggested the existence of other demethylases with different specificities, spurring a broad search for those enzymes. Shi was a major contributor to that search as well, identifying other histone demethylating enzymes with roles in a diverse array of biological processes.

Today, more than 20 histone demethylases are known that catalyze the demethylation of almost all major histone lysine methylation sites in the histone proteins. "People soon realized that these enzymes could be a very interesting area to pursue for drug development," Shi says. Work by his group led to the development of LSD1 inhibitors that are currently in clinical trials for the treatment of cancer.

More recently, Shi and his colleagues have explored the potential role of LSD1 in anti-tumor immunity. They reported in 2018 that the ablation—or removal—of LSD1 in cancer cells leads to the accumulation of double-stranded RNA within the cells. This, they found, induces the activation of an immune factor known as type I interferon, which stimulates anti-tumor immunity mediated by T cells of the immune system. Depleting LSD1 also led to increased infiltration of tumors by T cells, and inhibiting the enzyme made a mouse model of melanoma that otherwise resists immunotherapy susceptible to checkpoint blockade.

In a more recent study examining the effects of LSD1 inhibition on checkpoint blockade therapy, Shi and his colleagues showed in mouse models that the intervention indeed led to an infiltration of T cells into tumors. This desirable effect was, however, countered by the increased production of TGF-β, a signaling protein that suppresses the ability of infiltrating T cells to kill cancer cells. They demonstrated that a combination therapy that depletes both LSD1 and TGF-β during anti-PD-1 checkpoint blockade immunotherapy results in a significant increase in immune cell infiltration, the killing of cancer cells and elimination of tumors in syngeneic mouse models.

On an entirely different tack, Shi and his colleagues have also been investigating methylation regulation on RNA. In 2017 they discovered a biological role for the methylation of RNA—a molecular transcript of DNA—at a specific spot on the base adenosine. The researchers reported that this methylation event, known as m6A, plays an important and specific role in the cell's repair response to DNA damaged by ultraviolet light. That discovery opened a whole new area of research in the Shi laboratory. Harnessing new technologies to identify nucleic acid modifications, Shi's team has identified several RNA methylating enzymes and is now engaged in the exciting endeavor of describing their biology.

AT LUDWIG

At Ludwig Oxford, Shi continues to explore how epigenetic modifications to chromatin impact cancers. One of his main goals is to more broadly examine the role of epigenetic regulators in anti-tumor immunity. Through these studies, he and his colleagues hope to uncover effective means for turning so-called "cold" tumors, which are not inflamed, or infiltrated with cancer-targeting immune cells, into "hot" ones that are, and are thus more likely to respond to immunotherapy.

His lab is also focusing on the epigenetic aspects of two cancers—diffuse intrinsic pontine glioma (DIPG), an aggressive pediatric brain cancer, and the blood cancer acute myeloid leukemia (AML)—where epigenetics has been shown to play a crucial role. An overarching goal is to identify epigenetic regulators whose perturbation can lead to differentiation of tumor cells that can be clinically beneficial. In June, for example, his team reported in a study done in collaboration with Ludwig Harvard investigators that a combination therapy targeting metabolic pathways in combination with LSD1 inhibition might one day serve as a new AML treatment approach. In 2019, he and his colleagues reported evidence that the dual inhibition of LSD1 and another epigenetic enzyme, histone deacetylase, holds some promise for the treatment of DIPG.

Work on RNA methylation too proceeds apace and, in some ways, resembles the early days of research on histone-modifying enzymes—a landscape wide open for discovery. "There are so many different types of RNAs and they are methylated by different enzymes," Shi says. "What do these modifications do? These enzymes ultimately will be tied to human diseases, I'm sure of it."

Finding out if that's true will be made considerably easier with Ludwig support. Shi is excited by the core funding provided by Ludwig, which frees him of some of the burden of grant solicitation, giving him more time to think through scientific problems and their solutions. "Ludwig not only provides the necessary financial resources, but has also created an exciting intellectual environment where like-minded investigators with diverse backgrounds and skill sets come together to tackle cancer, one of the greatest medical threats that humans face," Shi says. "I feel very fortunate to be a member of the Ludwig family."