People & Ideas

Lin He: "Junk" DNA isn't

He studies how the noncoding genome shapes development and cancer.

The child of Western-trained engineers, Lin He grew up in a Beijing very different from today's skyscraper megalopolis. She recalls the freedom of the outdoors, the remnants of ancient city walls, and the flying kites. Like many others born in that era, He aspired to become an important scientist like the mathematicians and technologists who were then China's national heroes.

Having long planned to pursue her studies abroad, He leapt at the chance when offered a scholarship at Stanford University for her PhD. The revelation that microRNAs (miRNAs) impact mammalian gene expression brought He to the forefront of efforts to decode the function (1–3) and regulation (4, 5) of noncoding genomic elements. It's becoming increasingly clear that we can no longer afford to think of these portions of the genome as "junk" DNA, He explained when we called her at her lab at the University of California, Berkeley.

WHAT WE ARE

What links your graduate work on mouse pigmentation with your current work on miRNAs?

I feel that technology is a key driving force that shapes how science develops. When I worked with Greg Barsh, one major technology in mammalian genetics—particularly mouse genetics—was using positional cloning to correlate genetic alterations and phenotype. Gene targeting was not easy back then, so people relied on spontaneously generated mouse mutants to study development and disease. Mouse pigmentation is a particularly enriched trait; mouse genetics started with mouse fanciers and their collections of mice with distinct pigmentation patterns, and coat color regulation utilizes similar molecular pathways as many other biological processes. So, one can gain insights into diverse developmental and physiological processes by identifying coat color genes using positional cloning.

> "We have come a long way to realize that miRNAs truly play an important role."

But it takes three or four years of hard work to identify a gene and study its function by this method, so I really desired a technology that would allow us to do this more efficiently. [*Laughs*] When I graduated from Stanford in 2003, the emerging RNA interference (RNAi) technology promised to allow the systematic knockdown of every single gene in the genome and screening for a particular phenotype. I wanted to do that so badly! So after interviewing at all the major RNAi labs I joined Greg Hannon's lab to work on RNAi screens in mammalian systems.

RNA interference, not miRNAs?

I worked on RNAi for about a year but my main project didn't go anywhere because we were not quite ready to use RNAi as the technology for a genome-wide screen. I was also unlucky that my project was following up on a published study that was later retracted. I found out in a painful way that my study was based on findings that were false. After a year, nothing had worked out, and then by pure serendipity Greg was invited to write a review on miRNAs, and he asked me to work with him on the review. I gladly agreed and started reading all the papers in the miRNA field—which was not so many at the time. I realized that this class of novel noncoding RNAs could have unexpected functional importance in development and disease. I made an overexpression library to identify important miRNAs in the oncogene and tumor suppressor networks.

IS WHAT WE AREN'T

That was a more fruitful effort…

When we were first looking at whether miRNAs could play an important role in cancer, we used two major approaches. One was to investigate which miRNAs are enriched or depleted in cancer. That's how we discovered the *mir-17-92* polycistron

PHOTO COURTESY OF LIN HE

Lin He

as the first miRNA oncogene. The other approach was to see what miRNAs are engaged in cross-talk with the major oncogene and tumor suppressors. Towards that end, we identified the *miR-34* miRNAs as bona fide p53 transcriptional targets that mediate its downstream effects.

At the time of our discovery, some scientists were dubious about the importance of these small RNAs. When Victor Ambros and Gary Ruvkun discovered miRNAs in *C. elegans*, it was regarded as a weird phenomenon specific to worms. We have come a long way to realize that miRNAs truly play an important role in development and disease. I'm proud to be among those who have made this contribution.

What aspects of miRNA biology did you first choose to pursue in your own lab?

One of the topics that initially intrigued me was the polycistronic structure of *miR-17-92*. Unlike most protein-coding genes that follow the one-transcript-for-one-protein paradigm, many mammalian miRNAs give rise to a single precursor transcript that generates multiple mature miRNAs. They're called "polycistronic" because they resemble the polycistronic operon structure in bacteria. Our studies revealed a complex mode of functional interactions among the polycistronic *mir-17-92* components. The mechanisms of how polycistronic miRNA components work individually, how they interact, and how they are differentially regulated provide important insights into the unique structural functional features of miRNA genes.

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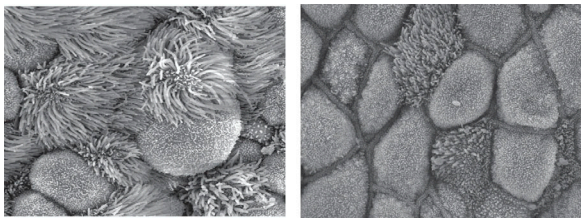

IMAGE COURTESY OF LIN HE

Complete deficiency of *miR-34* miRNA families (including miR-34a, miR-34b/34c and miR-449, right panel) leads to defective motile ciliogenesis.

Our second objective was to demonstrate the functional importance of miRNAs in development and cancer using mouse genetics. At the time, I was assigned to teach advanced developmental biology at Berkeley. I wasn't a developmental biologist so teaching this class was a huge challenge for me. The teaching experience really reinforced my confidence to pursue the developmental phenotypes that go hand in hand with the cancer phenotype.

BUT PROVING THAT IS TRICKY

What are the principal barriers you face?

One thing that is very frustrating when studying miRNA function is that a lot of the individual miRNA knockouts don't have very obvious phenotypes. Earlier in my career, someone commented, "If you want me to think miRNAs are important, show me a dead mouse after you knock one out."

We've been through ups and downs in trying to reveal the functional importance of miRNAs because their unique expression regulation and genome redundancy makes them functionally very robust. The *miR-34* family, for example, is made up of six mature miR-34/449 miRNAs, with copies distributed at three loci, and if you knock out one *miR-34* locus, the other loci have compensatory induction. So, it's challenging to reveal their essential phenotype unless you're determined and completely remove this redundancy.

Indeed, when we remove all *miR-34* miRNAs, these mice exhibit respiratory distress, sterility, and partial postnatal lethality. We've traced all these problems to a defect in motile ciliogenesis. There's an array of exciting developmental and cancer phenotypes waiting to be discovered when the redundancies in miRNA families can be removed.

It turns out that many miRNAs that have essential developmental phenotypes are those that are extremely redundant in the genome. They are also often dominantly expressed; sometimes just one miRNA gene can contribute around 70% of miRNAs expressed in a given cell type. By contrast, the most highly expressed protein-coding mRNA probably constitutes less than 2% of all the mRNAs. I believe there are many more developmentally essential miRNAs that are out there, so we're expanding our studies to new miRNA families.

Inspired by our success with miRNA studies, another direction we're following is to identify other noncoding elements in the genome that can play important roles in development and cancer. A major challenge we face in characterizing novel noncoding RNAs is that there are simply too many uncharacterized ones in the genome to choose from. Thus, it requires some serious thinking to select specific noncoding RNAs that are likely to be essential for development and disease. Of course, the newly developed CRISPR genome-editing technology has significantly lowered the technical barrier for genetics in mice and humans, yet the ultimate success of this endeavor will depend on how smart we are about choosing the right candidates and the right biological systems for the study.

> "There's an array of exciting developmental and cancer phenotypes waiting to be discovered."

Which noncoding RNAs do you have your eye on now?

We're particularly excited about retrotransposons. People think that retrotransposons are remnants of invading detrimental foreign nucleic acids that somehow propagated in the host genome. The traditional wisdom is that the genome is always trying to silence retrotransposon expression. However, we found that specific retrotransposons have very regulated expression during particular developmental stages, and can have profound impacts on gene expression, so we're developing new CRISPR-based genome-editing technology to study these fascinating noncoding elements. These types of studies were extremely difficult before CRISPR because retrotransposons are very repetitive in structure. So the ultimate goal for my lab is to show that the noncoding genome does contain many functionally important elements, and to elucidate the molecular basis underlying their functions.

This is a really exciting field to be in. If you compare the number of protein-encoding genes in worm and human, for example, humans don't have that many more protein-coding genes than worms. The noncoding genome scales up much better with the developmental and pathological complexity of an organism, so I think the big challenge ahead will be to identify and characterize the functionally important noncoding elements in development and disease, and to understand the intricate interactions between protein-coding genes and noncoding RNAs.

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Lin He, husband Kai Jiang, and their son, Gabriel Jiang, in their northern California home.