INTERVIEW

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Tian Xu is professor and vice chairman of Genetics at Yale University and a Howard Hughes Medical Institute investigator. His lab is working to understand the mechanisms of tumorigenesis and metastasis using Drosophila and mouse models. Notably, Dr. Xu has developed the powerful piggyBac transposon mutagenesis system, which has allowed investigators to perform forward genetics in mammalian models. This approach should prove instrumental in the identification of important genes involved in human diseases.

A lot of recent cancer research has been focused on tumorigenesis and the initial steps in tumor formation. However, your lab also works on metastasis, or the spread of cancer to multiple organs. What has hindered the study of metastasis and why aren’t more labs working to understand the mechanism of cancer spread?

What kills cancer patients is metastasis of the cancer cells to other parts of the body. If you look at how we’re doing in terms of treating cancer patients, we’re doing poorly. We’ve made significant advances in treating other diseases, but with cancer treatment, the mortality rate is flat. This is mostly because we don’t have an effective way to deal with metastasis.

Here is the problem: Cancer has the second highest mortality rate in the past 25 years, and we haven’t made much progress, even when we’ve made significant advances in other diseases like cardiovascular diseases and even pneumonia or infection in general. That is mostly because we still don’t have an effective way of stopping metastasis. In order to treat any disease, you need to understand mechanism, and we know very little about the mechanism of metastasis because it is a very complicated process. Most of the studies in the past have focused on tumorigenesis, that is, how the tumor starts. If you look at it, probably 99 percent of the cancer research labs are focused on tumorigenesis and not metastasis. But metastasis is what kills cancer patients. It’s very difficult to actually prevent tumorigenesis because we’re living longer now and are able to accumulate more mutations. Also, we pollute the environment, which is getting worse and worse, and we smoke and that induces lung cancer. I don’t think you can prevent cancer, but once you have it, most deaths are because cancer cells spread. More labs don’t study metastasis because it is very difficult to attack.

The most successful areas in cancer research have been in tumorigenesis, because human genetics has been very powerful in identifying the genes that cause it. Human geneticists can find families with a high incidence of cancer and who have inherited mutations that cause the tumor to occur. Using genomic and other approaches, they can identify tumor suppressor genes that are mutated and cause these events to happen. It is very difficult to use a similar ap-
proach to study metastasis. In order to have metastasis, you need to have a tumor first. In other words, if you want to identify a family with inherited mutations that cause or promote metastasis, you actually need to have a family that also has inherited mutations in tumor suppressors. The chance of finding a family that has inherited mutations in multiple genes that cause tumorigenesis and promote metastasis is very small.

Besides the multiple mutations, the difficulty lies in several other areas of studying metastasis. You could ask why can’t we retroactively identify metastatic tumor cells and then compare the mutations in the metastatic cancer cells vs. the benign tumor cells. If you do that, maybe you can provide some clue. And the answer is yes, you can do that, and indeed other groups are doing that and looking at gene expression. However, researchers have been met with limited success, because once the cancer cell becomes malignant, the genome destabilizes so there are thousands of genes mutated. It’s very difficult to look at a malignant cancer cell and ask which mutations are actually bystanders and which are causative.

The third level of difficulty comes from the fact that the actual process of metastasis itself is extremely complicated. Metastasis involves the tumor cells growing in one place, where they first need to degrade basement membranes that engulf every organ and then come out and have to travel — either to the lymph nodes, blood vessels, or other direct extensions into trouble. Once the cells reach another target, they need to degrade the basement membrane again to invade. As you can see, this is a multi-organ, multi-system problem in which the whole organism is involved. You cannot simply recreate metastasis in the Petri dish, because multiple organs are involved.

The fourth reason that metastasis is difficult to study is that it takes a long time to progress. You need accumulating mutations, and the process involves the degradation of basement membranes, cell migrations, and invasion of other tissues for secondary tumor formation. It’s a very elaborate process, and in mammals it usually takes a long time to occur. It’s very difficult to monitor this process. Because of these four reasons, it has been difficult to make progress.

A lot of cancer research has been dedicated to establishing a mouse model of tumorigenesis. Your lab has successfully established a Drosophila model for tumor progression and metastasis. Can you explain the reasoning behind going away from the mammalian system and to the fruit fly in order to study cancer?

If you look at these four problems, you realize that humans are difficult to work with. Most cancer research in tissue culture is also not sufficient. The mouse is a much better model for studying metastasis. However, it is still very difficult to create models to systematically look for genes that have multiple mutations. Right now, the metastasis models in mice have mostly been mutating tumor suppressors or oncogenes, then waiting for spontaneous mutations in the primary tumors driving them to progress. Also, as I already mentioned, it is very difficult to monitor these processes because they take a long time. For example, the basement membrane is very difficult to monitor in the animal. That is why we are actually developing models to study metastasis in the fruit fly. I want to give credit to a very talented former student, Ray Pagliarini. When he joined the lab, we talked and I tried to sell him a project on tumorigenesis, looking at the genes involved in regulating organ size and organism size. I was very excited, but after talking to him multiple times he was not that excited. In a desperate attempt to try to trick him, I said, “Well … maybe we can work on crazy projects.” He said, “Yeah, crazy projects sound great!” One crazy project was metastasis. He actually spent more than two years using the power of Drosophila genetics to set up a system that now allows us to systematically interrogate the genome and look for mutations that can promote metastasis.

This ability has taken two years to set up even in Drosophila, and it is impossible to do in other organisms right now. You can follow the tumor progression and actually see
the degradation of the basement membrane and things like that, and it’s just not possible to do this type of genetics in any other organism. Right now, it’s very exciting because these somatic cell tumors that develop in flies basically exhibit all of the characteristics observed in malignant human cancer: aggressive proliferation/overgrowth, then degradation of the basement membrane, then migration and invasion, then secondary tumor formation. We are very excited to understand the molecular and cellular mechanisms underlying these invasive behaviors.

Flies are not going to be the same as humans; they will be different — even mice are different from humans, we know that — but I think they will help us understand some basic fundamentals about the metastatic phenomenon and help us develop potential therapeutics. Actually, our past experience in studying tumorigenesis and tumor suppressor genes in flies has helped us a lot. We identified somatic mutations that caused tumors in flies, and by studying this, one of the things we have shown in the past several years is that these are tuberous sclerosis complexes, two tumor suppressors identified in humans by geneticists. However, people do not know why mutations in these genes cause tumors, and they don’t have effective therapeutics to combat these mutations. We have shown using fly genetics and biochemistry that these genes actually function in the PI3K/Akt and S6K pathways. Not only that, but if you block S6K activity, you can actually alleviate defects associated with tuberous sclerosis. These results basically suggest that these downstream components are effective therapeutic targets for this disease and other cancers. This is very exciting, because we are the first ones to show this pathway using Drosophila genetics and biochemistry that these genes actually function in the PI3K/Akt and S6K pathways. Not only that, but if you block S6K activity, you can actually alleviate defects associated with tuberous sclerosis. These results basically suggest that these downstream components are effective therapeutic targets for this disease and other cancers. This is very exciting, because we are the first ones to show this pathway using Drosophila genetics and biochemistry, but now the pathway has been shown to be conserved in mice, rats, and human patients as well. More than 50 percent of human cancers have mutations in this pathway; it’s a major cancer pathway. Furthermore, the FDA has approved a clinical trial for these compounds originally used for immunosuppression because we have shown that targeting this pathway can alleviate tuberous sclerosis-associated defects and the pathway has been conserved in humans and mice.

You can see that fly studies can help define major cancer pathways and also lead to clinical trials and therapy. We hope that an understanding of the metastatic phenomenon, which is a very difficult phenomenon that needs the whole organism to study, can be studied in the fruit fly to start to understand the biology. Hopefully, some of the pathways will be conserved from fly to mouse to human, and this knowledge can be translated to help develop effective therapies against metastasis.

Do you envision that the genetic techniques that make Drosophila an advantageous model system to study biology and diseases like cancer can one day be made available in higher organisms such as mice?

That’s exactly our dream! The answer is definitely yes. Fruit flies are invertebrates, and the only reason we use organisms like fruit flies and C. elegans is because of the genetic power. We can do forward genetic screens. We can do whole organism studies. Imagine if we could do these things directly in a mammal. Then we could learn a lot more. The problem is that in the past, we could not do that. One of the limitations is that we could not do forward genetic screens in mammalian systems. The mouse is a powerful genetic model and a powerful disease model, but the main approach has been reverse genetics. That is, using homologous recombination to knock out a gene in an embryonic stem cell and injecting those cells into blastocysts, transplanting chimera embryos into false mothers by doing surgeries, and hoping they will transmit. That process is fantastic and has revolutionized mouse genetics and allowed us to manipulate the mouse genome. However, it still has limitations, because it’s too expensive, laborious, and technically challenging. Also, reverse genetics relies on a lot of speculation, guessing which genes are important for this process and then knocking them out and studying them. We think we’re smart, but we’re not smart enough. When we knock these genes out, past experience tells
us we’re wrong more than 50 percent of the time and these genes do not exhibit the phenotypes we expect. We’re just not as smart as the organism.

If you look at the past several decades, we have learned the most about modern biology from an approach called forward genetics or forward genetic screens. That is, not to be biased in thinking which gene is important in a process, but rather to look for organisms or human beings with a phenotype or a disease. After that, you can identify which genes are causing the defect and which are causing the disease. You can dissect those genes and study molecular mechanisms. It is really painful to see that in mammals such a screen is not possible. Although people are conducting chemical mutagenesis in mice, once you have the mutation it is very difficult to map it and know which genes cause it. We have wanted to do this type of genetics in mammals for a long time; we’ve tried for 10 years. In 1996, when I applied for Investigatorship at the Howard Hughes Medical Institute (HHMI), I proposed that we wanted to improve the mouse system so we could do the type of genetics we were doing in fruit flies and in \textit{C. elegans}. The wonderful thing is I think I’m extremely lucky that we have a collection of very talented and brave young men and women in the lab, and they continue to try all kinds of different methods. Even with all of the failures, they’re still willing to take risks and try crazy projects. A year and a half ago, that led us to a major breakthrough when we discovered a modified transposon that can effectively work in mammalian systems, in human cells, mouse cells, and the mouse germline. Now for the first time, we can systematically mutate genes and look for a phenotype.

\textit{Transposon technology saw wide use in Drosophila, and now you have shown that piggyBac mobilizes in mice. Could you talk about its potential influence on cancer research?}

I think the technique will really offer fantastic opportunities for many diseases, not just cancer. This will be the first time in mammals that we will be able to systematically, on a large scale, mutate genes and know which genes are mutated and look for a phenotype. That opens wonderful opportunities. Just imagine, all of the screens, all of the discoveries you have never made — now you can just compress. More than 6,000 human diseases are estimated to be caused by a single gene mutation. We’ve identified very few; I think the last number was 1,700 known gene mutations causing disease. You mentioned cancer, which, of course, is a major disease. Hypertension is another. Alzheimer’s. These are the things on people’s minds. What about other diseases? For example, one third of the patients in our Yale-New Haven Hospital neurosurgery wards are actually hydrocephalus babies. These are the babies that have swollen brains. We do not know which gene alterations actually cause this. We know there are genetic factors, but we do not have the names of the genes. Approximately 1 out of every 5 women will develop polycystic fibroids and almost 10 percent of women have endometriosis. The genetic determinants for these conditions remain unknown. That’s incredible to me. The hope is that now you can systematically mutate genes in a mammalian system and identify the genetic basis for many diseases, which is very exciting. Not only that, but there are about 5,000 to 7,000 orphan diseases. These are diseases affecting small percentages of the population. Although it’s small, if you add the numbers up, it’s a huge percentage of people affected. Pharmaceutical companies are not interested in developing therapies because there’s not enough of a market. The National Institutes of Health doesn’t have enough money. What’s the solution? By systematically mutating genes, we can identify the cause of many of these orphan diseases. Our hope, our dream, is that we will be supported, and hundreds of young, talented men and women with Ph.D. degrees and M.D. degrees will join us and use screens to focus on a particular disease, identify the genetic cause, and take this wisdom to study the mechanism and develop therapeutics. That really would be a dream come true.
Looking for genes in this manner can contribute to the study of diabetes, obesity, cardiovascular disease, and tumor spread. For example, we are interested in longevity, in how long you can live. In *C. elegans* and in flies, experiments tell us that a single gene manipulation can increase longevity tremendously. In *C. elegans*, a single gene alteration can increase longevity by six times. If we extrapolate that to humans, that means 600 years old. So the real questions are: Can we identify these gene alterations in mammals that can significantly prolong longevity and how long can we really live? Can we live 600 years? A very exciting experiment. Of course, if we can truly live 600 years, other problems will occur, but the biology will be very cool. Now it’s possible because we can systematically mutate these genes. All we need is a little bit of money to keep all of the mice and all of the mutant mice living, and we can look to see which ones double, triple, or six times their longevity. How much would it cost? Thirty million dollars. Not much, if you think about the question. We can do it. I think if many people knew this, they would want to do it. The technology is really here, and all we need are two things: young, talented brave people that can take a run and work on things they’re interested in, either disease or biology, and we also need people who can financially help to say here is a disease, or here is a biological process that you should work on. That would be very exciting.

**What do you see as being the next big breakthrough in cancer research?**

I do not know. If you look at the past successful history, breakthroughs often come through unexpected areas, and that’s why we need to support basic research, even for people who work with fruit flies. It’s very difficult to predict where the breakthroughs will come from, but I think therapeutic breakthroughs will come from conquering metastasis. I think that’s the ultimate goal. If we can manage or control metastasis — not even completely stop it but just manage the situation — I think we will turn cancer into a chronic disease, and that’s a success. In my mind, I don’t think we can cure cancer. I think most likely we’ll make cancer a chronic, controlled disease. Plus, I think more and more people are conscious about the environment, and hopefully we’ll do less harm to ourselves in the future. I’ve already seen that the number of smokers has dramatically dropped in the United States, and that’s a big help. Hopefully, the rest of the world will do the same.