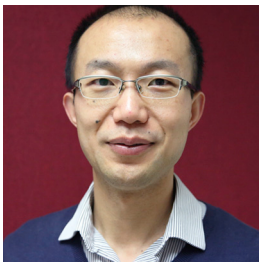
Cell

The Future of Genome Editing

The power of CRISPR is undeniable, but where is the field heading? *Cell*'s April Pawluk caught up with Jia Chen, Weizhi Ji, and Prashant Mali to discuss the successes and challenges we can expect in the coming years. Annotated excerpts from this conversation are presented below, and the full conversation is available with the article online.



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April Pawluk: I just want to kick it off by asking a big picture question: what do you see as the really big challenges remaining to bring CRISPR to the clinic?

Weizhi Ji: Yes, maybe because I'm working on non-human primates, I should be first to answer your question. For CRISPR or base editing or TALEN techniques, it is really useful to understand disease mechanisms, or in the future, we can use these techniques in the clinic. However, I think people are more concerned about the safety and efficiency about these gene editing techniques in human beings. I think non-human primates are maybe a bridge from basic studies to the clinic.

Jia Chen: I come from a background of DNA repair and base editing. I really think base editors are the next generation of gene editing tools. We can use base editor to induce base substitutions with very high frequency, which is hard to be achieved by CRISPR-Cas9-mediated HDR.

Prashant Mali: When I think of moving CRISPR to the clinic, there's two things that are going to be important in the long run. Of course, safety is important. But I think equally important is: can we do this again and again and again? Yes, we want to go after genetic diseases, but I also see a future where we can also start to address chronic ailments. Being able to directly target a protein or a gene just provides that exquisite control on cellular machinery. But of course, if you have to enable both safety and repeat dosing, one of the big challenges is the immune system itself. In fact, pretty much for the whole genome editing community, one of the biggest challenges is delivery. So I would say that now we're sort of engineering the supporting cast, which is this whole delivery system, and delivery is going to be one of the biggest challenges that we're going to have to really circumvent. To give an analogy, if I get a headache and take an aspirin, four hours later if I still have the headache, I'll take another aspirin. And that's the beauty of small molecules—that you can take them again and again and again, and you can take them for a spectrum of disorders. But I think CRISPR could provide you that same benefit. You don't have to do it every day, but perhaps do it every few months. Not just genetic disorders but even chronic ailments is where I see the future of CRISPR just impacting everything in the clinic.

AP: We've seen so many demonstrations of amazing efficiency of CRISPR in embryonic stem cells, in cultured cells, and in mice, for example, but how do we translate that into efficacy in primary cell types and post-mitotic cells? That still seems to be a huge challenge for somatic editing for actual therapies. What are the big challenges there?

PM: So I think, actually, what Jia mentioned right here. He's working on the base editing system. And I think that system sort of highlights one of the big challenges that we have when we target post-mitotic cells, which is a large part of the human body: you don't have any homologous recombination. So we really need to have a different generation of tools there. That's why base editing is very powerful. I still believe the ultimate genetic tool is the programmable recombinase, which has been a very notoriously hard system to engineer, and we haven't yet seen it in a very robust form. But I feel those are the systems of the future, where we can be DNA repair machinery independent. We still leverage the endogenous proteins to some extent, but we are able to do things in these primary cells that may not have all the regular machinery of dividing cells, which we have the luxury of in a cell-culture setting, in the lab, for instance.

(L to R) April Pawluk, Jia Chen, Prashant Mali, and Weizhi Ji

AP: That could apply to base editing and also possibly epigenome editing, right? So what are your thoughts on that?

WJ: I think that because there is a species difference, some techniques may be helpful in mice or rodents; however, they may not work on humans. So I have to say again: we have a need to use non-human primates to test every technique's efficiency for the future in humans.

JC: And I agree with Prashant that base editing will be a future direction for the relevant studies. But the one challenge for base editing is delivery. For most of the *in vivo* gene editing, AAV will be the best vector. But, you know, AAV has a limited capacity. Base editors are even longer than Cas9, so I guess new packaging systems have to be developed to deliver base editors into primary cells or into animals.

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AP: And do you think that the public, or scientists in general, should be worried about CRISPR's safety, or is that not so much of an issue?

WJ: That's hard to say. I think that's a hard topic in the world so far. In general, I believe CRISPR gene editing techniques should be very helpful in the future. So far it's very hard to say to the public that we have a very clear idea of where we really want to do it clinically. So we still need to wait for some time until we can clearly answer the public.

PM: I think this is a great point, because I think that tied to safety is also the ethical use of the tool. Really important. But the nice thing about the CRISPR community, I would say, is we've already brought these issues right at the forefront already. If I were the general community or the general public, what I would be very upset with is if something just crept up on me, right? But here there's already a very open discussion on the powers of the tool, the safety issues of the tool, and what are the other aspects about the tool that we should be mindful of. We also have this very robust discussion on what to edit and what not to edit. When to edit and where to edit, which also become very relevant aspects to consider. So I think yes, as far as safety goes, from a gene-therapy perspective, I think it's important for the end patient to be aware of what could actually happen, and that is important. One can make an educated discussion on how to use the tool for a certain type of editing. Similarly, we don't understand enough about all genes to know

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to what degree to change them, where to change them, how to change them.

AP: We're just not quite there yet.

PM: I think we just. . . . More knowledge of genetics is just going to be critical. So I think that will also inform safety; that will also inform ethical use.

JC: We understand there are people always concerned about the safety of gene editing, so I think we should do it in steps. . . . Let's say we just demonstrated to the public all the gene editing tools are very specific in animal cells, next in primary cells, next maybe in animal models—let's say mice and monkeys—and finally we can do some *in vivo* editing in primates to show that it's safe in tissues, then finally . . . someday we might try that, and we can use that to treat disease.

AP: Right. So since we have researchers from China and from the US here, I was wondering if you know of any big differences in public perception or regulation of CRISPR technology, of gene editing, maybe with respect to crops and GMOs, or maybe with respect to therapeutics, that might make unique challenges for each of your respective communities of research.

WJ: Yes, some reports in China were not so good about transgenic crops, and things like this made the public in China very worried about transgenic foods and other things. Actually, I think recently in China, the public understands transgenic issues more than before. So now the public may think, if you use gene editing techniques to treat some serious diseases such as cancer or maybe HIV, it should be good.

JC: I agree with that. People are concerned about gene editing to treat disease 'cause they don't want to use gene editing to experiment on themselves. But for gene-modified foods, I think it's easier to be accepted by the public, and I think the scientists should let the public know that gene-modified crops or foods are not dangerous. And actually for crops, the food we have now is a lot different from let's say a thousand years ago. It's a kind of evolution, so we just use the gene editing tools to accelerate this process, so it's not monsters or something.

AP: And make it more targeted, right?

JC: That's right, that's right.

AP: Public perception is everything, and it sounds like outreach is everything. What about in the US, Prashant?

PM: I think I agree with all of this. I think it's important for us to articulate our science and the changes that we make to systems and convey that to the public. And I think if we succeed in that, we'll get them on board. If we fail on that, then of course, we're shooting ourselves in the foot.

AP: So what sorts of applications or diseases might CRISPR not be able to solve? Any thoughts?

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PM: I mean, if there are defined alleles and defined targets to go after, of course CRISPR is your most precise tool for going about doing that. But I think for polygenic disorders, CRISPR is probably not the right route. But really these high-throughput screens will really inform us on what are the right targets to go after. So yes, I think CRISPR can impact all diseases, but in just different ways. One to actually directly target them, and the other is to probably find underlying targets to go after.

WJ: And maybe in the future, maybe we will have more advanced techniques than CRISPR. Modern science develops very fast, so I cannot say exactly in the future which techniques it will be instead of CRISPR.

AP: Right. Okay, especially since a couple of you are relatively new independent investigators here, what would be your advice for young researchers entering the CRISPR field these days?

WJ: I have a lot of young folks interested in CRISPR techniques. We really want to attract a lot of young scientists to focus on monkey CRISPR techniques. And compare with maybe 20 years ago when we start something, it's really difficult. But now with CRISPR techniques working very smoothly in monkeys, I believe that monkeys are the bridge between basic research and clinical applications. I hope more young scientists can come to work on monkeys.

JC: Actually, I'm a very young scientist, but from my experience I think if you want to do well in the gene editing field, the first thing: you have to work hard. You know, the field evolves so fast you have to work hard to follow. And the second thing is because gene editing is a pretty big field, you can fit your background into this field. I have a background of DNA repair, so actually my background profile fits into this base-editing field.

PM: The nice thing about CRISPR systems is it's put us in a place where we are no longer too limited, so it is truly sparking the era of big biology. We've had the era of big physics; now we have the era of big biology. So I think it's really exciting for any CRISPR researcher to get into because you have the ability to go after basic biology more powerfully. But, from my perspective, I also have a strong interest in technology development, and there's still so many hard problems to tackle. And I think those are very meaningful problems, and if one goes after them, I think there's a lot of avenues for researchers to dive into. How to more strategically do it, I don't think I have the formula for it. I wish I did!

AP: Okay, well, we'll catch up with you in a couple of years and see if you've figured it out yet!

PM: I think just trying what we love is probably the key of everything.