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Induced Pluripotent Stem Cells and the Promise of Proliferation

Ruth Williams

Coaxing differentiated cells to become pluripotent is no easy task. But new studies show that switching off a tumor-suppressing pathway can help. So, will these findings bring induced pluripotent stem (iPS) cells one step closer to the clinic?

Embryonic stem (ES) cells have the potential to give rise to all cell types of the adult organism. Researchers and clinicians have thus focused great efforts into harnessing this potential both for the study of cell and tissue development and for the use of these cells in tissue replacement therapies, such as for making cardiomyocytes to repair damaged hearts. Human ES cells are physically and ethically hard to come by. The discovery three years ago that simply adding four transcription factors to differentiated cells could generate ES-like cells thus opened an entirely new in-road to stem cell availability.¹

Shinya Yamanaka created these first so-called iPS cells by introducing the 4 transcription factors (Oct3/4, Sox2, Klf4, and c-Myc) into cells via viral vectors. Earlier this year, 3 reports brought iPS cells leaps and bounds closer to clinical use by describing vector-free iPS generation, which leaves the cells' genome unaltered.²⁻⁴ Despite this advance, the feasibility of iPS cell clinical use has been plagued by the inefficiency of their generation.

Six studies, 1 published last year and 5 published together in *Nature* this August,⁵⁻¹⁰ have now revealed that inhibiting the action of a tumor suppressor protein called p53 can improve iPS cell generation dramatically. Up to 100-fold in fact, depending on the cell type and factors used.^{5,7} But is it wise to turn off this cellular safety mechanism? After all, in the case of ES cells, their oncogenic potential has been a major needle to the balloon of hope for clinical use.

The initial discovery that inhibiting p53 might aid iPS cell generation came from Hongkui Deng and colleagues in 2008, when they tested a panel of factors along with the four Yamanaka factors to find those that improve iPS reprogramming efficiency.⁵ The 5 new studies take that discovery further by examining the mechanism behind the reprogramming block of p53. The studies also confirm what has been suspected for some time: that pluripotency and tumorigenicity are unnervingly close relatives.

Five simultaneous publications from different research groups is quite a feat. "I think this is an example in how it's

possible to do highly competitive research without competition!" boasts Maria Blasco (Spanish National Cancer Research Centre, Madrid, Spain), senior author of the report by Marion et al.

In the study by Li et al, the team looked upstream of p53 and showed that the genetic locus of a p53 activator (the Ink4/Arf locus) is silenced in both iPS cells and ES cells and becomes active as cells differentiate and age.⁷ As a consequence, older cells had higher levels of p53 and this was correlated with their resistance to reprogramming. Indeed, switching the Ink4/Arf locus off in older cells increased iPS cell yield.

Hong et al, in contrast, focused on downstream target of p53.⁶ They found a number of genes specifically regulated by p53 and, through functional analysis of these genes, determined that the p53 to p21 pathway is the active barrier to reprogramming. Indeed, overexpression of p21, itself a cell cycle inhibitor, could counter the reprogramming boost caused by lowering p53.

Consistent with the findings of Hong et al, Kawamura et al showed that iPS cell yield could be raised by inhibiting either p53 or p21.⁸ They also showed that skin cells, which are known to be efficient reprogrammers, had naturally lower levels of both p53 and p21.

As with the above 3 studies, those by Utikal et al and Marion et al both show that reducing p53 activity enhances the reprogrammability of cells.^{9,10} On the face of it, these 5 studies report a relatively simple way to increase iPS cell yield. Excellent news, given iPS cells are arguably the best choice of source material for cell replacement therapies. "The beauty of iPS cells is that they can be easily made from the same individual that's being treated," says Deepak Srivastava, professor of cardiology at the University of California, San Francisco. Before clinicians get too excited about fixing damaged hearts, or other organs, with iPS cells, however, there is a cold helping of caveats to serve up with the piping-hot p53 pie.

Not So Fast

Blasco spells out the risk in no uncertain terms, "I think it is not likely to be safe to inactivate p53, as it is going to allow conversion into iPS cells of parental cells with preexisting DNA damage, something that will compromise the quality control."

Blasco's team had previously shown that cells with short telomeres do not reprogram efficiently,¹¹ and in their present study they show that this is because such cells contain higher amounts of p53.

The transcription factor p53 has long been known as the guardian of the genome, thanks to its ability to shut down the cell cycle and/or induce apoptosis in response to DNA

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Non-standard Abbreviations and Acronyms

ES	embryonic stem
iPS	induced pluripotent stem

damage. "In the case of our study we show that p53 is eliminating suboptimal cells (those with DNA damage) by apoptosis at the time of induction of pluripotency," says Blasco. Blocking p53 lets these risky damaged cells through the security checkpoint.

Given that most human cancers have an impaired p53 pathway, iPS cells generated by removing p53 function must surely be of use only for research. Not necessarily, according to Srivastava. "I think if one permanently did [reduce p53] then there would be a concern, but the reprogramming process just requires transient modulation of these proteins." He is referring to Utikal et al's finding that acute p53 inhibition (for just a few days) was sufficient to improve reprogramming efficiency.

Konrad Hochedlinger (Harvard Stem Cell Institute, Boston, Mass), senior author of the study by Utikal et al, errs on the side of caution. "I think the answer is we don't know yet if transient inhibition of p53 will induce harmful changes to the cells. It just needs to be tested," he says.

Finding alternative means of inducing proliferation and immortality, which seem to be the key requirements for reprogramming, might be safer, suggests Hochedlinger. One way to do this might be to block apoptosis by, for example, introducing Bcl2 protein, an approach that improved reprogramming efficiency in Kawamura et al's study. Alternatively, Blasco's group have shown that immortality can be achieved, and reprogramming efficiency improved, by expressing telomerase in cells.

Perhaps the best bet, though, would be to choose cells that are naturally highly proliferative. "Adult progenitor and stem cells reprogram into iPS cells up to 300 times more efficiently than mature cells," explains Hochedlinger, referring to another recent study by his group.¹²

Pluripotency Paradox

Although cellular immortality appears to be the key to reprogramming, it is also the ultimate hurdle for using stem cells for therapies. "The main impediment to using iPS cells in therapy is the same as using embryonic stem cells: we still do not know how to differentiate them to different cell types with a 100% efficiency," says Blasco, "Efficiency has to be 100%, as these cells can form tumors if left undifferentiated."

Thus, the p53 studies confirm the similarity of stem cells to cancer cells, and it is this similarity that has on the one hand provided a solution, yet on the other continues to be a problem.

"I think differentiation protocols are getting better," says Timothy Kamp (University of Wisconsin, Madison). Kamp works on, among other things, differentiation protocols to turn stem cells into cardiomyocytes. There are ways to remove pluripotent cells from a culture of differentiated cells,

he says. For example, researchers can use magnetic beads coated with antibodies that recognize undifferentiated cells and then pull out the beads with the potential tumor time-bombs attached. He concedes, however, that "it is going to continue to be an issue that we will have to aggressively address before therapies are possible."

With any differentiation protocol, it would be helpful to know exactly where you are starting from, and yet despite their name, there had not been conclusive proof as to whether iPS cells are truly pluripotent. Kamp and colleagues recently showed that iPS cells and ES cells behave almost identically under conditions designed to give rise to differentiated cardiomyocytes.¹³ In the study, the iPS-derived and ES-derived cardiomyocytes demonstrated a similar gene expression pattern, similar organization of their contractile proteins, and similar electric signals and action potentials. "At least for the lines that we tested, they [iPS cells] seemed to behave similarly to the ES cells," says Kamp.

Definitive proof of that similarity came just recently, however.^{14,15} Two groups reported in a recent issue of *Nature* that normal adult mice can be produced from iPS cells by a method called tetraploid complementation. The technique involves generating an early embryo in which every cell is tetraploid (4n). At the morula or blastocyst stage of development, diploid (2n) iPS (or ES) cells are combined with the 4n embryo. The fetus, and ultimately the adult mouse, develops exclusively from the 2n cells, the 4n cells contributing to extraembryonic tissues only. The 2 groups generated their iPS cells by slightly different methods (although with the same 4 Yamanaka factors), confirming that there is more than one way to make truly pluripotent iPS cells.

"[Tetraploid complementation] is the most rigorous test of pluripotency that we have . . . so I think that's a powerful testimonial to the mouse iPS cells," says Kamp. "Hopefully the human ES and iPS cells are comparably pluripotent, but of course we can't do the same assay!"

With their pluripotency confirmed, and the efficiency of their production improving, iPS cells are becoming an increasingly feasible option for therapy. Moreover, both of these advances sit snugly on the back of the reports that iPS cells can be produced vector-free.²⁻⁴ Although there are remaining issues to iron out before iPS therapies become a reality for treating heart disease or other conditions, Kamp surmises positively, "Things are moving forward. These are just growing pains."

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