A series of recent articles have examined the genomes and epigenomes of induced pluripotent stem (iPS) cell lines. The results appear discouraging for the clinical future of iPS cells, but researchers don’t seem worried. In August 2006, Kazutoshi Takahashi and Shinya Yamanaka reported that just four specific factors were sufficient to convert a fully differentiated adult cell into a pluripotent stem cell. Not only was the concept scientifically gripping, but also the implications for the technique’s clinical application were tremendous. A patient’s own cells might be used to generate replacement cells for therapy, avoiding the worry of transplant rejection, as well as the ethical concerns and tricky techniques of embryonic stem (ES) cell generation.

So began a proliferative burst of related research studies. Publications on iPS cell research have become so plentiful, in fact, that it is hard to believe the field is not yet five years old. New approaches to improve safety and efficiency have been suggested, different adult cell types have been converted, and numerous disease model cell lines have been made, and yet, as the fifth birthday draws near, a series of recent research articles threaten to spoil the party. The reports reveal that the genomes of iPS cells exhibit chromosome abnormalities, residual epigenetic marks from the parent somatic cell type, and a higher than normal number of coding sequence mutations.

In one recent article, for example, Pasi et al report that genomic deletions and amplifications (copy number variations) are abundant at fragile sites in the genomes of mouse iPS cells. In another, Hussein et al show that such copy number variation exists in human iPS cell lines too, and that, on average, copy number variation is two-fold higher in iPS cells compared with ES cells and cultured fibroblasts. Interestingly, this copy number variation dropped to levels similar to ES cells and fibroblasts when the cells were grown for longer periods, suggesting the iPS reprogramming process itself prompted the genomic variability and that this variation was selected against during prolonged culture. An article by Laurent et al indicates that loss of tumor suppressor genes from the iPS genomes is associated with the reprogramming process, while amplification of such genes can occur during iPS (and ES) cell passaging, again suggesting that reprogramming and culturing exposes iPS cells to different genomic pressures.

In other recent articles, Mayshar et al report that aneuploidy is common in human iPS cell lines, and Lister et al report that iPS cells have aberrant DNA methylation patterns—some of which are reminiscent of the adult cell from which they were derived, some of which are unique to iPS cell lines (i.e., not shared with ES cell lines). Last, Gore et al, who performed whole exome sequencing on twenty-two human iPS cell lines derived by five different induction methods, reported that all had coding region mutations, an average of six coding sequence mutations per cell line.

This list of reports might seem like a barrage of blows to the iPS field. But, says Timothy Kamp (Director of the Stem Cell and Regenerative Medicine Center, University of Wisconsin Medical School, Madison, WI), “It is not a death knell. It’s not going to scare us out of the field.” Rather, he says, “It’s a bit of a wake-up call. . . The personalized therapies that people dream of using human iPS cells for may be a little further away than we would like.”

Konrad Hochedlinger (Associate Professor in the Department for Stem Cell and Regenerative Biology, Harvard University, Boston, MA) is similarly positive, “It is hard to know at this point whether it is bad news at all.” Because, he explains, “[the researchers] didn’t do follow-up studies to see whether any of the mutations were functionally relevant.”

Hochedlinger points out that previous mouse studies have shown iPS cells are functionally very similar to ES cells. For example, a study from his own group revealed that ES and iPS cell lines made from the same genetic background and devoid of viral transgenes had almost indistinguishable mRNA expression profiles. Furthermore, Hochedlinger adds, “What is known from the mouse is that some iPS cell lines fulfill the most stringent developmental assay available, which is tetraploid complementation.” In a tetraploid complementation assay diploid (2n) iPS cells are injected into an early stage embryo (a mere ball of cells) that consists entirely of tetraploid (4n) cells. The 4n cells are unable to develop into a fetus, but the 2n cells can. Thus, a fetus derived in this way can be determined to have developed from iPS cells only. The fact that some iPS cell lines have given rise to fetuses and ultimately fully formed adult mice by this method suggests that their genomes are unlikely to be severely damaged or disorganized.

Of course, this does not mean the reports of genetic abnormalities can be ignored. “We want to bring this up to the community that this is important information,” says Kun Zhang (Assistant Professor of Bioengineering, University of California, San Diego, La Jolla, CA), senior author on the Gore et al study. “But that does not mean that we cannot solve the problem. . . This is really not the end of the world.”

The reported evidence of potential genomic abnormalities affects all areas of iPS research, not just direct clinical use. Disease model iPS cells derived from patients, for example,
have shown great promise toward investigating disease mechanisms in vitro. But, in light of the new reports, findings from such cells will have to be interpreted carefully. “If we stumble onto unexpected findings, we will have to make sure there aren’t other potential mutations contributing to it,” explains Kamp.

One solution might be to sequence the genomes of all iPS cell lines so that researchers know exactly what they are dealing with. But is that really feasible? And, even if it is feasible for researchers—where one cell line might be used by multiple laboratories—would it ever be feasible for screening individual patient-derived iPS cells for therapeutic use? Deepak Srivastava (Director of the Gladstone Institute of Cardiovascular Disease, San Francisco, CA) thinks so. “It’s very feasible, because genome-wide sequencing is already becoming trivial and will most certainly be trivial by the time any iPS cell lines are ready for clinical use. By then it will be inexpensive and fast.”

Srivastava works on, among other things, transdifferentiation of fibroblasts directly into cardiomyocytes. He uses a mixture of factors in much the same way that iPS cells are induced and says that the recent reports have prompted him to see if similar mutations are present in the genomes of his induced cardiomyocytes.

Both Srivastava’s transdifferentiation technique and iPS cell generation are extremely inefficient—only a tiny percent of starter cells go on to adopt the induced phenotype. It is this inefficiency that Gore et al predict leads to the genetic abnormalities. The reprogramming process essentially selects for genetically plastic cells, which therefore have a higher chance of containing mutations. And because reprogramming events are so few, iPS cell lines are picked and clonally expanded, thus fixing the mutations in the entire progeny. Zhang calls it a “clonal bottleneck.”

If reprogramming efficiency could be improved such that there was no bottleneck, says Zhang, there’s every chance iPS cells would be free of mutations.

Hochdellinger thinks that improving reprogramming, and thus avoiding selection for abnormal subclones, is a real possibility. “We are continuously finding new culture conditions and small molecules that enhance reprogramming efficiency, so I am convinced that over the next few years the problem of introducing aberrations through subcloning iPS cells will be solved,” he says.

One way researchers might find efficiency-improving molecules and pathways is to do functional follow-up studies on the gene mutations that Gore et al have found. If these mutations have indeed been selected for by the reprogramming process itself, as Gore et al predict, “then you could likely devise strategies to address those pathways,” says Srivastava. “It points you in the direction of which pathways to tweak in order to make it more efficient.”

Thus, the very stories that at first glance seemed like a setback to the iPS field may in fact offer clues as to how to make the process of iPS generation more efficient, and safer. “The power of iPS cells is still great,” says Kamp. “Like most fields of science, it takes a while for it to mature and for us to really understand and utilize it to its full potential.”

References

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