The cancer stem cell field would predict this hierarchical model, as would experiments in which genetic inducers are targeted to NSCs in order to create tumors[13,14]. However, because most genetic manipulation strategies are not designed for clonal analysis in the pre-morbid, premalignant brain, the field is still unable to distinguish between fundamental models of cerebral oncogenesis.

If, in fact, gliomas were found to be of polyclonal origin (having arisen from a population of ostensibly equipotent and identically predisposed progenitors in the same germinal zone), and if some of those clones could also give rise to normal central nervous system structures, then, rather than viewing gliomagenesis as solely a cell-autonomous process, the field might be forced to accept a more stochastic rather than hierarchical view of the process, look for cell non-autonomous influences (such that the ultimate phenotype of even a genetically flawed NSC might be molded by the actions of an oncosuppressive versus oncopermissive niche), and determine how a fate choice by a given NSC is made. For example, investigators might have to assess whether alterations in otherwise fundamental NSC-based developmental programs contribute to or trigger the neoplastic process (for example, might aberrations in normal neurogenic events foreshadow oncogenic ones?) (A.I. Persson, R.J., K. Shchors, G.I. Evan, E.Y.S., W.A. Weiss *et al.*, unpublished data).

Therefore, lineage and clonal analysis of brain tumors is the next problem that must be tackled at the bench. Tracking the process of neoplastic transformation in tumor-initiating cells before morbidity and before malignancy in living subjects holds value not only for oncology (early diagnosis and treatment) but also for regenerative medicine—the caution being to be cognizant not only of the genetic predispositions of a stem cell but also of the niches they will engage following transplantation. Real-time imaging of grafted stem cells harboring inducible suicide genes may prove a prudent fail-safe strategy.

Although the specter of oncogenic transformation of stem cells in regenerative medicine should not be cavalierly dismissed, it also should not paralyze rational forward movement in the field. Conversely, understanding how that undesirable outcome could emerge might not only insure safety but also yield new insights into oncotherapeutics. The other take-home lesson from the events in Russia: patients should not seek 'stem cell therapies' from those not immersed in the intricacies of its biology.

1. Amariglio, N. *et al. PLoS Med.* **6**, e1000029 (2009).
2. Flax, J.D. *et al. Nat. Biotechnol.* **16**, 1033–1039 (1998).
3. Roy, N.S. *et al. Nat. Med.* **12**, 1259–1268 (2006).
4. Stemcells, Inc. <http://www.stemcellsinc.com/news/090608.html> (2008).
5. Lee, J.P. *et al. Nat. Med.* **13**, 439–447 (2007).
6. Redmond, D.E. Jr. *et al. Proc. Natl. Acad. Sci. USA* **104**, 12175–12180 (2007).
7. Aboody, K.S. *et al. Proc. Natl. Acad. Sci. USA* **97**, 12846–12851 (2000).
8. Singh, S.K. *et al. Cancer Res.* **63**, 5821–5828 (2003).
9. Lapidot, T. *et al. Nature* **367**, 645–648 (1994).
10. Keller, C. & Capecchi, M.R. *Cancer Res.* **65**, 7530–7532 (2005).
11. Read, T.A. *et al. Cancer Cell* **15**, 135–147 (2009).
12. Yang, Z.J. *et al. Cancer Cell* **14**, 135–145 (2008).
13. Alcantara, L.S. *et al. Cancer Cell* **15**, 45–56 (2009).
14. Marumoto, T. *et al. Nat. Med.* **15**, 110–116 (2009).

■ BENCH TO BEDSIDE

Inducing pluripotency

Laura Clarke & Derek van der Kooy

The isolation of human embryonic stem cells[1] (hESCs) theoretically gave scientists the starting material to produce any cell type in the body for use in regenerative medicine. One major obstacle to this approach has been the inevitable host immunological response to transplanted foreign tissue. The development of protocols for inducing hESC-like pluripotent cells from mature adult cell types has provided a potential solution to this problem. A series of recent studies[2–5] have now focused on how to produce patient-specific cell lines efficiently and safely enough to bring into the clinic.

In 2006, Takahashi and Yamanaka[6] showed that skin cells from embryonic or adult mice could be reprogrammed into induced pluripotent stem (iPS) cells simply by expressing a combination of four pluripotency genes, which became known as the Yamanaka factors: *Pou5f1* (also known as *Oct4*), *Sox2*, *Myc* and *Klf4*[6]. The Yamanaka group[7] and others[8,9] quickly extended the technique to human skin cells, so that, in theory, a single biopsy would be sufficient material to produce perfectly immuno-compatible mature cell types. These studies generated a great deal of excitement in the field of regenerative medicine: the race was on to produce iPS cells safe enough for clinical trials.

The first studies used single viruses to deliver each gene, resulting in multiple copies of individual viruses present in established iPS lines. Any vector that integrates into the genome has the potential to disrupt the function of a gene at its site of entry, which can lead to a number of undesirable effects due to gain or loss of function, such as tumorigenesis. In addition to this concern over insertional mutagenesis was the question of regulation of expression of the pluripotency genes. Reprogramming factors are required for a certain time period to establish pluripotency but need to be shut off to allow cells to differentiate into desired cell types such as neurons or cardiac muscle. Continued activation or reactivation of the exogenous genes could impair differentiation or transform cells. One particular factor, c-Myc, is a known oncoprotein, and its abnormal expression in chimeric mice derived partially from iPS cells resulted in a variety of tumor types in adults[10].

The only certain way to avoid both insertional mutagenesis and reactivation of transgenes is to produce an iPS cell line that does not contain any alterations to its genomic DNA. Pluripotency genes need either to remain separate from the host genome or to be completely removable—a 'hit-and-run' strategy. With so little known about the mechanisms and time course of reprogramming, it was not known whether transient transgene expression would be effective. Work in mice provided initial proof of principle that reprogramming does not require permanent integration. Adenoviruses, which do not invade the host genome, have been used to reprogram mouse liver cells, albeit with low efficiency[11]. Mouse iPS cells have also been derived from skin cells by multiple transfections with nonintegrating plasmids[12,13].

Two recent studies have used virus-free methods to establish iPS cells from human cells[2,5]. In one, a combination of vectors was used to deliver seven reprogramming genes to skin cells, the Yamanaka factors plus the genes encoding Nanog, Lin28 and large T antigen

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SV40. The Epstein-Barr virus–derived vectors remain separate from the host cell DNA and are gradually lost from proliferating cells. Clones that have lost the vectors can be selected and expanded, resulting in an iPS cell line completely free of foreign DNA sequences. In an alternate approach, the protein products of the reprogramming genes have been directly delivered to cells, with no foreign DNA involved[2]. The authors of this study made use of a short peptide sequence that is permeable to cell membranes, taken from a human immunodeficiency virus protein[2]. When this peptide is linked to each factor, the resulting fusion proteins can enter cells and cause reprogramming. Although promising, this strategy currently requires multiple rounds of factor delivery, and it has a low success rate.

Considerable progress has also been made toward an effective hit-and-run strategy. A collaborative effort[3,4] has combined the use of a sequence containing all four Yamanaka factors separated by spacer elements with a transposon vector known as piggyBAC. The transposon integrates into the host genome to allow reprogramming and derivation of iPS cell lines. After pluripotency has been established, the transposon can be seamlessly removed by addition of a transposase, leaving no traces of foreign DNA. This work did successfully produce mouse iPS cells with neatly excised transgenes; however, it has yet to be demonstrated that the transposase can be easily delivered to human cells and that the removal of the reprogramming genes can be appropriately timed and will not decrease the efficiency of deriving human iPS lines.

The rapid progress toward creating a safer iPS cell promises that, in the near future, facilities worldwide will be able to efficiently produce patient-specific iPS lines free of alterations to their genomic DNA. Once this is a reality, however, the story will be far from finished. Scientists are still left with most of the same issues that initially created a barrier to using hESC-derived products in the clinic. We still do not know how to produce a large, purified population of a desired cell type such as pancreatic beta cells or dopaminergic neurons. Two recent reports that mouse iPS cells can give rise to an entire viable adult through tetraploid chimera technology[14,15] show that, in principle, these cells can give rise to derive every cell type in the body. However, the low frequency of truly pluripotent cells suggests that they may be rare, and no such robust assay exists to test the potential of human iPS cell lines. In addition, genetic defects in patient cells may need to be corrected to produce functional grafts or even to initially derive patient-specific iPS cells[16]. Virus-based correction again subjects cells to insertional mutagenesis, and this risk needs to be eliminated or minimized.

There is also no agreement on the best approach to cell-based therapy—should terminally differentiated cells be transplanted, or are tissue-specific stem cells more likely to result in clinically relevant, functional replacement of dysfunctional or degenerated tissue? One recent report of donor-derived nervous system tumors in an individual who received a graft of undifferentiated fetal neural progenitors[17] serves as a warning that transplanted progenitor cells may become transformed, particularly if poorly characterized. The use of clonal assays to select for cells with appropriate karyotype, epigenetic marks and gene expression will be required, such as the development of a method for clonal derivation of single, expandable neural stem cells from single hESCs (R. Chaddah, M. Arntfield, S. Runciman and D.v.d.K., unpublished data).

The recent examples of derivation of human iPS cells free from permanent genetic manipulation have addressed one major hurdle in bringing this technology into the clinic. The extremely rapid progress in the iPS field fuels optimism that a viable approach to cell-based therapies is imminent.

1. Thomson, J.A. *et al. Science* **282**, 1145–1147 (1998).
2. Kim, D. *et al. Cell Stem Cell* **4**, 472–476 (2009).
3. Woltjen, K. *et al. Nature* **458**, 766–770 (2009).
4. Kaji, K. *et al. Nature* **458**, 771–775 (2009).
5. Yu, J. *et al. Science* **324**, 797–801 (2009).
6. Takahashi, K. & Yamanaka, S. *Cell* **126**, 663–676 (2006).
7. Takahashi, K. *et al. Cell* **131**, 861–872 (2007).
8. Yu, J. *et al. Science* **318**, 1917–1920 (2007).
9. Park, I.H. *et al. Nature* **451**, 141–146 (2008).
10. Okita, K., Ichisaka, T. & Yamanaka, S. *Nature* **448**, 313–317 (2007).
11. Stadtfeld, M., Nagaya, M., Utikal, J., Weir, G. & Hochedlinger, K. *Science* **322**, 945–949 (2008).
12. Okita, K., Nakagawa, M., Hyenjong, H., Ichisaka, T. & Yamanaka, S. *Science* **322**, 949–953 (2008).
13. Gonzalez, F. *et al. Proc. Natl. Acad. Sci. USA* **106**, 8918–8922 (2009).
14. Kang, L., Wang, J., Zhang, Y., Kou, Z., & Gao, S. *Cell Stem Cell* published online, doi:10.1016/j.stem.2009.07.001 (2009).
15. Zhao, X *et al. Nature* advance online publication, doi:10.1038/nature08267 (2009).
16. Raya, A. *et al. Nature* **460**, 53–59 (2009).
17. Amariglio, N. *et al. PLoS Med.* **6**, e1000029 (2009).