Deconstructing Stem Cell Tumorigenicity: A Roadmap to Safe Regenerative Medicine

PAUL S. KNOEPFLERA,B

ADepartment of Cell Biology and Human Anatomy & Stem Cell Program, University of California Davis School of Medicine, Sacramento, California, USA; bInstitute of Pediatric Regenerative Medicine, Shriners Hospital For Children Northern California, Sacramento, California, USA

Key Words. Stem cells • IPSC • hESC • Tumors • Regenerative medicine • Safety

ABSTRACT

Many of the earliest stem cell studies were conducted on cells isolated from tumors rather than from embryos. Of particular interest was research on embryonic carcinoma cells (EC), a type of stem cell derived from teratocarcinoma. The EC research laid the foundation for the later discovery of and subsequent work on embryonic stem cells (ESC). Both ESC isolated from the mouse (mESC) and then later from humans (hESC) shared not only pluripotency with their EC cousins, but also robust tumorigenicity as each readily form teratoma. Surprisingly, decades after the discovery of mESC, the question of what drives ESC to form tumors remains largely an open one. This gap in the field is particularly serious as stem cell tumorigenicity represents the key obstacle to the safe use of stem cell-based regenerative medicine therapies. Although some adult stem cell therapies appear to be safe, they have only a very narrow range of uses in human disease. Our understanding of the tumorigenicity of human induced pluripotent stem cells (IPSC), perhaps the most promising modality for future patient-specific regenerative medicine therapies, is rudimentary. However, IPSC are predicted to possess tumorigenic potential equal to or greater than that of ESC. Here, the links between pluripotency and tumorigenicity are explored. New methods for more accurately testing the tumorigenic potential of IPSC and of other stem cells applicable to regenerative medicine are proposed. Finally, the most promising emerging approaches for overcoming the challenges of stem cell tumorigenicity are highlighted.

Disclosure of potential conflicts of interest is found at the end of this article.
The shift to a potentially more tumorigenic state as ICM cells transition to ESC in vitro may be driven by the method of creating ESC via selecting for those unique ICM cells that can be forced to grow in vitro. This selective process is predicted to go hand-in-hand with epigenetic changes that enable such growth. Thus, driving ICM cells to become ESC may be at the same time creating a cell type that is inescapably also pushed toward a tumorigenic phenotype. Just how different ESC are from ICM cells remains an intriguing question. It is important to note that even transplants of whole early embryos can drive teratoma formation as well so some of the forces driving teratoma formation are intrinsic to ICM cells and not because of their in vitro growth to produce ESC.

**The Teratoma Assay: Both a Pluripotency and a Tumor Assay**

It is remarkable that, to date, one of the most common assays for demonstrating and studying the pluripotency of stem cells, including induced pluripotent stem cells (iPSC), is the teratoma assay. Often this is referred to as a pluripotency assay, but of course it is also a tumor assay. The fact that a key assay of “stemness” is also a tumor assay further illustrates the strong link between stem and tumor cells, a reality too rarely discussed in the field when interpreting results from teratoma assays. Even ignoring for the moment the ability of ESC and iPSC to produce malignant tumors in some cases [5, 6], the production of benign teratoma as a side effect in humans given a hypothetical regenerative medicine therapy in the future, would be unacceptable. Such tumors could be numerous and would prove highly destructive to surrounding normal or regenerating tissue. Thus, a key concept is that stem cells, even those with potent self-renewal and pluripotency, will almost certainly never be directly used in regenerative medicine if they cannot be proven to lack the ability to cause teratoma in mice.

**What Is the Molecular Basis of the Tumorigenicity of Normal Stem Cells?**

Recent studies indicate that many of the same master programming elements are at work in both stem cells and tumor cells [7–10]. This shared molecular machinery suggests that untangling the determinants of pluripotency from the programming responsible for tumorigenesis is going to be a major challenge. The links between pluripotency and tumorigenicity are exemplified by the fact that many of the genes used to produce iPSC are either outright established oncogenes such as Myc and KLF4 [11–13] or are in various ways linked to tumorigenesis such as Sox2 [14], Nanog [9], and Oct3 [15]. But perhaps no molecule embodies the intertwined nature of the pluripotency and tumorigenicity programs more than Myc itself. Overexpression of the Myc family of protooncogenes is linked with an array of human tumors and elevated Myc expression may have some role in all human cancer [16]. Not only is Myc expression itself shared between stem and tumor cells, but distinct groups of Myc regulated target genes are coexpressed in both malignant tumors and ESC [10].

Myc was one of the first oncogenes discovered and there are literally thousands of papers studying the function of excess Myc in cancer. However, it has only been more recently that the normal role of Myc in stem cell biology has been discerned. Loss of function models reducing or eliminating expression of Myc genes in stem cells consistently show disruption of the function of those stem cells [17, 18] and c-Myc appears to be essential for normal STAT signaling in mouse ESC (mESC) required for self-renewal and pluripotency [19]. In terms of iPSC, although it is formally possible to create iPSC without Myc [20, 21], the efficiency is dramatically reduced by the omission of Myc and the timeframe for appearance of colonies is greatly extended. In Yamanaka’s groundbreaking iPSC paper, they reported being unable to make iPSC without Myc [22] further suggesting Myc strongly boosts iPSC formation. Thus, the stem cell field is faced with a catch-22 situation in that if one seeks to make stem cells safer by lowering Myc levels, a tandem reduction in the “stemness” of those cells may prove inevitable. The same appears to be true for other master stem cell regulators such as KLF4. Lowered levels of KLF family members including KLF4 substantially impaired ESC pluripotency and self-renewal, forcing ESC to differentiate [23].

**Are Pluripotency and Tumorigenicity Coupled?**

The dualistic natures of Myc and KLF4, linked to both tumorigenesis and normal stem cell biology, highlight the more general dilemma that the regenerative medicine field faces in trying to preserve self-renewal and pluripotency while eliminating tumorigenicity. A fundamental principle of cell biology may be that the greater the pluripotency and self-renewal properties that a stem cell possesses, invariably the higher the probability it will cause tumors (Fig. 1). Conversely, reducing the tumorigenic nature of stem cells may inevitably reduce the self-renewal and pluripotency of stem cells. Unfortunately, this means that one may not be able to completely eliminate the ability of a stem cell to cause tumors without robbing the cell of identity, its stem-like nature. In other words, to make a stem cell completely unable to cause tumors, you may have to make that cell no longer be a stem cell. However, not all stem cells even within the same culture have the ability to form tumors suggesting that pluripotency and tumorigenicity may not be so completely bound together. In this alternative model, “stemness” and tumorigenicity are highly related process, but separable and with important distinct molecular features.

**Animal Safety Models: How to Distinguish Between Lack of Stem Cell Tumorigenicity Versus Host Rejection?**

Part of the challenge of resolving issues related to stem cell safety is that only few in vivo studies have been reported, particularly on human cells such as human ESC (hESC). Some studies with introduction of hESC into animal models have given apparently encouraging results. An example of such a study was one where rats were given a hESC transplant. They not only showed improvement of their Parkinson’s disease symptoms, but at least for the 3 months of the study also did not develop detectable teratoma [24]. On the other hand, another study using an animal model system and stem cell transplant failed because of teratoma formation [25]. In all such studies, particularly with a “negative” result where no teratoma were detected, it is unclear whether the apparent
lack of tumorigenesis is related to the inherent properties of the transplanted stem cells or rather reflects the level of immunosuppression in the animal model being used (i.e., a false negative). Lack of teratoma in animal models with stem cell transplants may most often be reflective of a failure of engraftment due to immune cells in the host killing the stem cells. Fortunately, new, improved humanized mouse models are continually being developed that may be more useful for assessing the tumorigenicity of stem cells [26].

Teratoma is not the only concern as hESC can also form malignant tumors. A recent study found robust malignant tumor-inducing capacity of hESC including H1 and HSF-6 [6]. IPSC can also form both teratoma as well as malignant tumors such as neuroblastoma and follicular carcinoma [5]. Thus, the potential risk to human patients from both teratoma and malignant tumors is quite real, yet remains difficult to estimate as no human trials of hESC or IPSC have been conducted at this time.

**PARALLELS BETWEEN INDUCED PLURIPOTENCY AND ONCOGENIC TRANSFORMATION IN THE FORM OF FOCUS FORMATION IN FIBROBLASTS**

IPSC have been produced from a number of cell types, but most often from fibroblasts. The production of IPSC is eerily similar in a number of ways to a tumor-formation assay called “focus formation” in fibroblasts [27, 28] with both able to be driven by Myc genes. Focus formation is an assay for testing tumorigenicity of specific genes in fibroblasts. In both focus formation assays and IPSC induction, monolayer cultures of fibroblasts are transduced with retroviruses, some of which encode oncogenes. The growth of cultures is continued at high density without passaging. Normal cells undergo contact inhibition and generally remain as a largely quiescent monolayer. In both IPSC and focus formation assays, the expectation is that at some point tightly packed growths of cells will form above the monolayer. In each case, these “colonies” exhibit escape from the normal quiescent state induced by contact inhibition. Both IPSC and oncogenic foci are transferable to form new cultures and both can cause tumors when injected into immunocompromised mice. The parallels between foci formation and IPSC production suggest these processes are perhaps related to some extent. It is quite possible that a fraction of colonies that form during IPSC induction are more similar to an oncogenic focus than an IPSC colony and may very well be essentially tumor colonies. If so, studies of these byproducts of the IPSC process may prove fruitful for furthering our understanding of the links as well as differences between stem and tumor cells.

**WILL NONGENETIC METHODS FOR PRODUCING IPSC REDUCE THE RISK OF TUMORS?**

If one accepts the model that making a cell more stem-like predisposes that cell to cause a tumor, then IPSC are predicted to be inherently more tumorigenic than their nonstem cells of origin such as fibroblasts. However, even beyond this kind of modeling there are compelling reasons for worrying about IPSC tumorigenicity based on actual published data. Of greatest concern is that nearly all IPSC described in published works have been demonstrated to cause teratoma, proving pluripotency but also tumorigenicity, and that mice genetically derived to contain some tissues from IPSC have a malignant tumor incidence of 20% [29]. Genetic changes intrinsic to the IPSC generation process may pose risk of enhancing tumorigenesis through both the introduced genes themselves and in theory via the potential changes at specific integration sites.

The IPSC field is evolving rapidly and moving away from methods of induction that rely on genetic changes. This approach is in its early days with some very promising initial results [30, 31], but predictions are that such a move generally should reduce tumorigenicity and improve safety. However, important questions remain. Will it ever be possible to make IPSC with absolutely no genetic changes? Can IPSC ever totally escape from dependence (whether via genetic or nongenetic approaches) on Myc, KLF4, and other possible oncogenes? Although it may appear that the IPSC field has already answered this affirmatively for Myc in that IPSC can be generated without added Myc, the omission of Myc reduces the efficiency of IPSC generation and yet these IPSC can still produce tumors in the form of teratoma [20, 21, 32]. These studies also do not address the role of endogenous Myc. In IPSC generated without genetic addition of Myc, the cells of origin may well be characterized by unusually high levels of endogenous Myc proteins required for the reprogramming and could make the cells prone to tumorigenesis.

**PROPOSING AN ALTERNATE ASSAY OF TUMORIGINICITY FOR IPSC AND OTHER STEM CELLS**

To gauge the tumorigenic nature of IPSC most often researchers have used what will be termed the derived mouse assay. In this assay, through mouse genetics IPSC are used to contribute to the formation of chimeric mice in which some tissue lineages are derived from IPSC. The mice are then studied for tumor development. Using this assay, IPSC have been reported to cause malignant tumors in up to 20% of such derived mice [5], whereas IPSC generated without exogenous Myc have been reported to not form tumors [20, 32]. The
cause of the tumors has been thought to be most often due to reactivation of Myc from previously silenced viral insertions. The problems with the derived mouse assay are twofold. First, the iPSC must go through early embryogenesis and are subject to the powerful embryonic reprogramming forces that are predicted to dramatically reduce the apparent tumorigenicity of the cells. Second, the derived mouse system for studying iPSC tumorigenicity bears no resemblance to how cells would be used in regenerative medicine where they would either be focally injected in a site to be repaired or administered intravenously (IV). Both focal injection and IV administration of iPSC as a means for studying iPSC tumorigenicity have not been reported in the literature. Unfortunately, these assays are expected to show higher rates of tumorigenicity than the derived mouse assay, but more accurately reflect risk to patients. Another problem with the derived mouse assay is that currently it is unusable for studying tumorigenicity of human iPSC. Researchers appear to be disinclined to put human iPSC into mouse embryos, perhaps due to the ban on putting hESC into a mouse embryo and taking it beyond day 8 of growth. Thus, other than their robust teratoma inducing abilities, the reality is that the stem cell field knows almost nothing about the tumorigenicity of mouse iPSC and essentially nothing about that of human iPSC in a context relevant to regenerative medicine.

**AN UNANTICIPATED RISK FROM INDUCED EPIGENETIC CHANGES?**

Mostly under the radar in the field of stem cell safety are potential undesirable side effects of epigenetic changes in iPSC and hESC that are undetectable by karyotyping, but could have profound effects on cell biology as the biology of normal hESC is regulated by epigenetic programming [38]. Epigenetic changes are also postulated to play a key role in the reprogramming at the heart of iPSC formation [11, 12] and are modeled to be instrumental in creating iPSC both with or without genetic changes, particularly in the latter case. Thus, it is critically important to more fully study the global epigenetic changes associated with pluripotency and especially induced pluripotency, even if iPSC are ultimately produced without genetic changes. Epigenetic alterations may in part confound the efficacy of moving away from genetic changes through promoting tumorigenesis themselves. Such changes also occur during establishment and passaging of hESC [39], whose epigenome is highly unstable [40], almost certainly enhancing tumorigenicity. Characterizing the relationships among the epigenome, pluripotency, and tumorigenicity should prove of great benefit for developing safe regenerative medicine.

**APPROACHES TO SAFE STEM CELL-BASED REGENERATIVE MEDICINE THERAPIES**

The four most promising approaches to make stem cell-based regenerative medicine safer are discussed here (Fig. 2). Some pretransplant screening is applicable to all four approaches. Safety screening could encompass everything from assays of genome integrity (chromosome number, deletions, and duplications) to gene expression array profiling as well as micro RNA patterns and perhaps even epigenetic screening. These approaches should enhance the safety of any regenerative medicine therapy.

**Transplants of Progenitors**

In a general sense, the reason that relatively “normal” stem cells such as ESC can in turn cause tumors is because they are programmed to be robust tissue and organ growers. Tumors are abnormal organs or tissues, which a growing
body of work suggests in many cases, have developed from and perhaps are maintained via their own population of stem cells. The notion that a wide variety of tumors beyond those such as teratocarcinoma and teratoma may contain stem cells is gaining widespread acceptance (reviewed in [41]). These tumor or cancer stem cells, also termed tumor-initiating cells, seem to share many traits with normal stem cells, but are predicted to have at least partially impaired pluripotency. In that sense, tumor stem cells may be akin to racecars with accelerators (indefinite self-renewal potential) but bad brakes (differentiation potential/pluripotency).

The simplest way to slow or even eliminate the tumorigenicity of normal stem cells prior to transplantation may be to take advantage of their natural "brakes" or pluripotency by partially differentiating them into progenitors. Therefore, a promising proposed method for making stem cell-based regenerative medicine therapies safer may seem paradoxical: to not transplant stem cells at all into patients. This avenue has gained wide acceptance as the most promising approach to regenerative medicine. The idea is to use the stem cells to produce progenitor or precursor cells of the desired lineage and then transplant progenitors purified by sorting (Fig. 2). On January 23, 2009 such an approach was given approval by the Food and Drug Administration based on hESC-derived oligodendrocytes. With sufficient purity, weeding out through differentiation coupled with sorting all or nearly all contaminating stem cells that remain, the progenitor transplant should be both safe and effective. The sorting could be either positive (sorting for the progenitors based on markers) or negative (sorting via stem cell markers for their elimination). Because differentiation is a dynamic process, not an "on/off" switch, there will always be residual stem cells remaining in differentiated cultures and sorting is not perfect. Thus, the most practical approach to safe regenerative medicine would be some combination of differentiation, sorting, and one of the two general approaches outlined below (first two subsections) to kill residual stem cells.

It remains unknown just how pure the progenitors must be to be safe. How few remaining stem cells are enough to cause concern about tumors? If the answer is "zero," then it may be difficult to achieve this goal because the reality is that the only pure cell population is that consisting of a single cell. It seems much more probable that the presence of a few stem cells may not pose a serious risk. However, the remaining undifferentiated stem cells may be unique and have a much higher risk of generating a tumor because they in some manner escaped being differentiated, perhaps reflecting a partial impairment of pluripotency that brings them one step closer to a tumorigenic phenotype. The few published studies addressing this question leave the issue largely unresolved. For example, a study of use of hESC for Parkinsonian rats found that differentiation of the cells prior to transplant lowered the incidence of teratoma [42]. However, high levels of natural killer (NK) cells in many rat and mouse animal models appear to kill most or all injected stem cells, questioning the validity of such safety studies when results are negative. Further complicating the story is the observation that ESC are in fact more susceptible to killing by NK cells than are differentiated ESC due to differences in cell surface proteins [43].

---

**Figure 2.** Methods to address stem cell tumorigenicity to develop safe regenerative medicine therapies. Four possible approaches are outlined. "Stemotoxic" refers to agents or methods that are specifically toxic to and hence kill stem cells.
Introduction of a Stem Cell Specific Suicide Gene

The stable genetic introduction of a suicide gene such as thymidine kinase (tk) into stem cells has been reported to be effective in combination with Ganciclovir (Gan) treatment [44]. However, in this study, the treatment was not stem cell specific and would have also killed any differentiated progeny from those stem cells in a hypothetical treatment situation causing it to fail. Differentiated teratoma cells were also readily killed by Gan treatment. Nonetheless, relatively simple modifications such as using oct3 or nanog promoter driven expression of tk, would make the system more stem cell specific to ideally kill only those hESC that have escaped differentiation. Of concern is the fact that it remains unknown if all hESC express what are thought of as the key stem cell factors such as oct3 and nanog. Although populations of hESC do express these seemingly without exception, it is unclear whether small, but functionally relevant subpopulations may not. Another open question is whether transplantation of hESC and engraftment of hESC in the host could lower or shut off expression of suicide genes driven by stem cell promoters either immediately following transplant or at a much later date. Even with efficient stem cell killing, the possibility of patients requiring life-long treatment with Gan or other agents to suppress growth of residual stem cells raises the issue of possible reemergence of proliferating, drug-resistant stem cells possibly in turn leading to tumors at later dates.

The major concern with the suicide gene approach is its requirement for genetically modifying the stem cells, which could raise the risk of tumorigenicity from the beginning. However, a recent study of the safety of viral transduction of human hematopoietic stem cells and MSCs in which animals were followed for up to 18 months found no evidence of tumor formation. Further advances in our understanding of tumor stem cells and tumorigenesis more generally will also provide additional fuel for these advances. Finally, a much more open discussion and investigation of the tumorigenic nature of stem cells than has yet to occur, particularly that of IPSC and hESC, will undoubtedly prove essential for the development of safe and effective regenerative medicine therapies.

FUTURE DIRECTIONS

The road from where we are today to a future with IPSC- and hESC-based regenerative medicine therapies being safe and more common treatment modalities is not a clear, linear one. However, basic and translational studies into the tumorigenic nature of stem cells are going to be collectively an essential bridge to cross along the way. Further advances in our understanding of tumor stem cells and tumorigenesis more generally will also provide additional fuel for these advances. Finally, a much more open discussion and investigation of the tumorigenic nature of stem cells than has yet to occur, particularly that of IPSC and hESC, will undoubtedly prove essential for the development of safe and effective regenerative medicine therapies.

ACKNOWLEDGMENTS

I thank Jan Nolta and David Pleasure for reading the manuscript prior to submission. This work is supported by New Faculty Grant RN2-00922-1 from CIRM.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The author indicates no potential conflict of interest.
REFERENCES

1. Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. Nature 1981;292:154–156.
2. Martin, GR. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. Proc Natl Acad Sci USA 1981;78:7634–7638.
3. Doetschman TC, Eistetter H, Katz M et al. The in vitro development of blastocyst-derived embryonic stem cell lines: Formation of visceral yolk sac, blood islands and myocardium. J Embryol Exp Morphol 1985;87:27–45.
4. Solter D. From teratocarcinomas to embryonic stem cells and beyond: A history of embryonic stem cell research. Nat Rev Genet 2006;7:319–327.
5. Okita K, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. Nature 2007;448:313–317.
6. Shih CC, Forman SJ, Chu P et al. Human embryonic stem cells are prone to generate primitive, undifferentiated tumors in engrafted human fetal tissues in severe combined immunodeficient mice. Stem Cells Dev 2007;16:989–902.
7. Knoepfler PS, Cheng PF, Eisenman RN. N-myc is essential during neurogenesis for the rapid expansion of progenitor cell populations and the inhibition of neuronal differentiation. Genes Dev 2002;16:2699–2712.
8. Wilson A, Murphy MJ, Oskarsson T et al. c-Myc controls the balance between hematopoietic stem cell self-renewal and differentiation. Genes Dev 2004;18:2747–2763.
9. Cartwright P, McLean C, Sheppard A et al. LIF/STAT3 controls ES cell self-renewal and pluripotency by a Myc-dependent mechanism. Development 2005;132:885–896.
10. Nakagawa M, Yamanaka S. Induction of pluripotent stem cells from human fibroblasts. Nat Biotechnol 2006;24:795–797.
11. Waizenegger I, Wahl C, protesters A et al. Expression of OCT-4 and Nanog in oral cancer stem-like cells and high-grade oral squamous cell carcinoma. Clin Cancer Res 2008;14:4085–4095.
12. Ben-Porath I, Thomson MW, Carey VJ et al. An embryonic stem cell-like gene expression signature in poorly differentiated aggressive human tumors. Nat Genet 2008;40:499–507.
13. Yamanaka S. Strategies and new developments in the generation of patient-specific pluripotent stem cells. Cell Stem Cell 2007;1:39–49.
14. Knoepfler PS, Cheng PF, Eisenman RN. N-myc is essential during neurogenesis for the rapid expansion of progenitor cell populations and the inhibition of neuronal differentiation. Genes Dev 2002;16:2699–2712.
15. Weis D, Kanai M, Huang S et al. Emerging role of KLF4 in human gastrointestinal cancer. Cancerogenesis 2006;27:23–31.
16. Chen Y, Shi L, Zhang L et al. The molecular mechanism governing the oncogenic potential of SOX2 in breast cancer. J Biol Chem 2008;283:17969–17978.
17. Palma J, Pena RW, Contreras A et al. Participation of OCT3/4 and beta-catenin during dysgenetic gonadal malignant transformation. Cancer Lett 2008;263:204–211.
18. Edlers M, Eisenman RN. Myc’s broad reach. Genes Dev 2008;22:2755–2766.
19. Knoepfler PS, Cheng PF, Eisenman RN. N-myC is essential during neurogenesis for the rapid expansion of progenitor cell populations and the inhibition of neuronal differentiation. Genes Dev 2002;16:2699–2712.
20. Wilson A, Murphy MJ, Oskarsson T et al. c-Myc controls the balance between hematopoietic stem cell self-renewal and differentiation. Genes Dev 2004;18:2747–2763.
21. Cartwright P, McLean C, Sheppard A et al. LIF/STAT3 controls ES cell self-renewal and pluripotency by a Myc-dependent mechanism. Development 2005;132:885–896.
22. Nakagawa M, Koyanagi M, Tanabe K et al. Generation of induced pluripotent stem cells. Cell 2006;125:315–326.
23. Humphreys D, Eggan K, Akutsu H et al. Epigenetic instability in human embryonic stem cells and in vitro differentiated neuronal cells by defined factors is greatly improved by small-molecule compounds. Nat Biotechnol 2007;25:207–215.
24. Maitra A, Arking DE, Shivapurkar N et al. Genomic alterations in cultured human embryonic stem cells. Nat Genet 2005;37:1099–1103.
25. Baker DE, Harrison NJ, Matlby E et al. Adaptation to culture of human embryonic stem cells and oncogenesis in vivo. Nat Biotechnol 2007;25:207–215.
26. Wernig M, Meissner A, Jaenisch R. C-Myc is dispensable for direct reprogramming of mouse fibroblasts. Cell Stem Cell 2008;2:10–12.
27. Miura K, Mann M, Glassy B et al. Transforming growth factor beta signaling in human embryonic stem cells and in vitro differentiated neuronal cells. Cell Stem Cell 2008;2:101–106.

28. Yancopoulos GD, Nisen PD, Tesfaye A et al. N-myc can cooperate with ras to transform normal cells in culture. Proc Natl Acad Sci USA 1985;82:5455–5459.
29. Okita K, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. Nature 2007;448:313–317.
30. Shi Y, Do JT, Desponts C et al. A combined chemical and genetic approach for the generation of induced pluripotent stem cells. Cell Stem Cell 2008;2:525–528.
31. Huangfu D, Maehr R, Guo W et al. Induction of pluripotent stem cells by defined factors is greatly improved by small-molecule compounds. Nat Biotechnol 2007;25:207–215.
32. Giordano A, Galdersi U, Marino IR. From the laboratory bench to the patient’s bedside: An update on clinical trials with mesenchymal stem cells. J Cell Physiol 2007;211:27–35.
33. Bershadsky AD, Olgivie TE, Bosse M, Stewart M et al. Characterization of human embryonic stem cell lines with features of neoplastic progression. Nat Biotechnol 2009;27:91–97.
34. Bernstein BE, Mikkelsen TS, Xie X et al. A bivalent chromatin structure marks key developmental genes in embryonic stem cells. Cell 2006;125:315–326.
35. Allegrucci C, Wu YZ, Thurston A et al. Restriction landmark genome scanning identifies culture-induced DNA methylation instability in the human embryonic stem cell epigenome. Hum Mol Genet 2007;16:1253–1268.
36. Hummers I, Songdon R, Aspinall P et al. Protein array analysis of mouse embryonic stem cells and in vitro differentiated neuronal cells is controlled by the recipients’ immune response. Plos One 2008;3:e2622.
37. Schuldiner M, Itskovitz-Eldor J, Benvenisty N. Selective amination of human embryonic stem cells expressing a “suicide” gene. Stem Cells 2003;21:257–265.
38. Choo AB, Tan HL, Ang SN et al. Selection against undifferentiated human embryonic stem cells by a cytotoxic antibody recognizing podocalyxin-like protein-1. Stem Cells 2008;26:1454–1463.
39. FDA. Food and Drug Administration: Cellular, Tissue and Gene Therapies Advisory Committee. Available at http://www.fda.gov/ohrms/dockets/ac/08/slides/2008–0471S1andS2–00-index.html. Accessed April 10–11, 2008.
40. Alvarnas JC, Forman SJ. Graft purging in autologous bone marrow transplantations: A promise not quite fulfilled. Oncology (Williston Park) 2004;18:867–876; discussion 876–881, 881, 884.