doi:10.1038/mt.2010.99

Molecular Therapy

Defining the Probability that a Cell Therapy Will Produce a Malignancy

Cell therapies have tremendous potential, but there is a 700-pound gorilla in the room: the risk that the cells can produce malignancies. The concerns about the gorilla were highlighted by reports[1–3] of malignant transformation in culture of the adult stem/progenitor cells referred to as mesenchymal stem cells or multipotent mesenchymal stromal cells (MSCs). These reports cast a shadow over the 100 or more clinical trials with MSCs currently being contemplated or in progress (ClinicalTrials.gov). The shadow was lifted in part by a recent follow-up report by one of the laboratories[4] revealing that its cultures had been contaminated with malignant cells. The sequence of events is probably instructive for all potential cell therapies. Unfortunately, none of our current technologies provides a definitive test for the presence of small numbers of tumorigenic cells in the large doses required for most therapies.

The limits of our tests for tumorigenicity are severe. There are no hard data on the minimum number of tumorigenic cells necessary to produce tumors in patients, but observations with hematopoietic stem cells suggest that the number could be approximately 100 cells. Some of the clinical trials of cell therapies have delivered 5×10^6 cells per kg (ref. 5), or about 3.5×10^8 cells per average adult. Therefore, a conservative estimate is that we need an assay that will guarantee that a preparation of therapeutic cells contains less than 1 tumorigenic cell per 3.5×10^6. Our present assays fall far short of this level of sensitivity. Classic karyotyping detects only major rearrangements of chromosomes, is subject to cultural artifacts, and samples only a small aliquot of any cell preparation. Tests for tumorigenicity in mice are meaningful only if positive, because many human tumors will not produce tumors if directly injected into immunodeficient mice. Newer techniques such as whole–comparative genomic hybridization or whole-genome sequencing are impressive, but they certainly lack the sensitivity required. In addition, we are far from developing the genomic database required to reliably distinguish neutral from cancer-producing mutations.

At the same time, decades of biological research allow us to classify cells into three broad categories in terms of their potential for tumorigenicity: (i) cells that are immortal in culture and therefore highly likely to be tumorigenic, (ii) cells that escape senescence as they are expanded in culture and become both immortal and tumorigenic, and (iii) cells that have rarely been observed to emerge from senescence as they are expanded in culture and therefore have a very low probability of being tumorigenic.

Cells that begin as immortal cells in culture present serious dangers to patients regardless of how they have been engineered, differentiated, or selected. Therefore, we face a serious barrier in considering therapeutic uses of immortal cells such as virally transformed cell lines, embryonic stem cells, or induced pluripotent stem cells. Newer strategies include introduction of suicide genes or rendering the cells unable to divide mitotically by irradiating them, but such strategies may not circumvent the barrier for some applications of cell therapies. The strategy of differentiating tumorigenic cells, such as embryonic stem cells or iPS cells, and then cloning them does circumvent the problem because most differentiated cells are difficult to expand in culture. Also, expanding a clone cell through multiple population doublings that may be required raises the specter of spontaneous transformation.

Cells that escape senescence in culture have been well characterized since the first attempts to culture fibroblasts from mice. Cultures of murine fibroblasts grow slowly at first, but then undergo a "crisis" in which most of the cells die, leaving a few cells that undergo a transformation that allows them to expand rapidly as tumorigenic cells.[6] Other mouse cells, including MSCs,[7] undergo the same sequence of events.

Cells from human sources generally undergo senescence in culture after expansion through 30 to 50 population doublings. There have been no authenticated reports of human skin fibroblasts spontaneously emerging from senescence in the countless times the cells have been cultured in research and clinical laboratories. The probability of emergence from senescence was estimated as less than one per 10^9 cell divisions.[8] However, the same is not true for all human cells. For example, the probability of emergence from senescence is about 10^{-7} for mammary fibroblasts and 10^{-4} for mammary epithelial cells.[9]

The need for care in preparing cells for administration to patients was emphasized by recent reports on MSCs. The three publications describing the escape of human MSCs from senescence during expansion in culture[1–3] are inconsistent with reports by numerous laboratories that human MSCs consistently pass into senescence.[10,11] The inconsistency was probably resolved by the subsequent report from one of the laboratories that its cultures were contaminated by malignant cells that initially grew slowly in the presence of the human MSCs.[4] Therefore, the field of MSC research has rediscovered the risk of cross-contamination of cell cultures posed by malignant cells, a danger that has been known for many decades but one that still plagues the field of cancer research.[12]

In the end, the development of any medical therapy depends on the careful weighing of risks and benefits to the patient. But we rarely, if ever, have the benefit of hard data on either the risks or the benefits. The risks are always probabilities based on statistical estimates, such as the probability of contamination of an aspirin tablet by a toxin or the probability of contamination of a cell preparation by tumorigenic cells. The benefits are also probabilities based on statistical estimates of the probability of the progression of the disease and the probability of a favorable response in a given patient. The issues are not simple. But we must address them with care as we pursue the potential benefits of cell therapies for the millions of patients whose hopes have been raised and who are now looking over our shoulders.

Darwin J Prockop
Associate Editor

REFERENCES

1. Rubio, D, Garcia-Castro, J, Martín, MC, de la Fuente, R, Cigudosa, JC, Lloyd, AC *et al.* (2005). Spontaneous human adult stem cell transformation. *Cancer Res* **65**: 3035–3039.
2. Wang, Y, Huso, DL, Harrington, J, Kellner, J, Jeong, DK, Turney, J *et al.* (2005). Outgrowth of a transformed cell population derived from normal human BM mesenchymal stem cell culture. *Cytotherapy* **7**: 509–519.
3. Røsland, GV, Svendsen, A, Torsvik, A, Sobala, E, McCormack, E, Immervoll, H *et al.* (2009). Long-term cultures of bone marrow–derived human mesenchymal stem cells frequently undergo spontaneous malignant transformation. *Cancer Res* **69**: 5331–5339.
4. Garcia, S, Bernad, A, Martín, MC, Cigudosa, JC, Garcia-Castro, J and de la Fuente, R (2010). Pitfalls in spontaneous in vitro transformation of human mesenchymal stem cells. *Exp Cell Res* **316**: 1648–1650.
5. Hare, JM, Traverse, JH, Henry, TD, Dib, N, Strumpf, RK, Schulman, SP *et al.* (2009). A randomized, double-blind, placebo-controlled, dose-escalation study of intravenous adult human mesenchymal stem cells (prochymal) after acute myocardial infarction. *J Am Coll Cardiol* **54**: 2277–2286.
6. Rubin, H (2001). Multistage carcinogenesis in cell culture. *Dev Biol* (Basel) **106**: 61–66.
7. Tolar, J, Nauta, AJ, Osborn, MJ, Panoskaltsis Mortari, A, McElmurry, RT, Bell, S *et al.* (2007). Sarcoma derived from cultured mesenchymal stem cells. *Stem Cells* **25**: 371–379.
8. Shay, JW and Wright, WE (1989). Quantitation of the frequency of immortalization of normal human diploid fibroblasts by SV40 large T-antigen. *Exp Cell Res* **184**: 109–118.
9. Romanov, SR, Kozakiewicz, BK, Holst, CR, Stampfer, MR, Haupt, LM and TIsty, TD (2001). Normal human mammary epithelial cells spontaneously escape senescence and acquire genomic changes. *Nature* **409**: 633–637.
10. Bernardo, ME, Zaffaroni, N, Novara, F, Cometa, AM, Avanzini, MA, Moretta, A *et al.* (2007). Human bone marrow derived mesenchymal stem cells do not undergo transformation after long-term *in vitro* culture and do not exhibit telomere maintenance mechanisms. *Cancer Res* **67**: 9142–9149.
11. DiGirolamo, CM, Stokes, D, Colter, D, Phinney, DG, Class, R and Prockop, DJ (1999). Propagation and senescence of human marrow stromal cells in culture: a simple colony-forming assay identifies samples with the greatest potential to propagate and differentiate. *Br J Haematol* **107**: 275–281.
12. Lacroix, M (2008). Persistent use of "false" cell lines. *Int J Cancer* **122**: 1–4.