Cell Stem Cell
Commentary

Teratoma Formation Assays with Human Embryonic Stem Cells: A Rationale for One Type of Human-Animal Chimera

M. William Lensch,[1,2,4] Thorsten M. Schlaeger,[4,5] Leonard I. Zon,[1,3,4,5,7] and George Q. Daley[1,2,4,5,6,*]
[1] Division of Hematology/Oncology, Children's Hospital Boston and Dana-Farber Cancer Institute, Boston, MA 02115, USA
[2] Department of Biological Chemistry and Molecular Pharmacology
[3] Department of Pediatrics
Harvard Medical School, Boston, MA 02115, USA
[4] Harvard Stem Cell Institute, Cambridge, MA 02138, USA
[5] Stem Cell Program, Children's Hospital Boston, Boston, MA 02115, USA
[6] Division of Hematology, Brigham and Women's Hospital, Boston, MA 02115, USA
[7] Howard Hughes Medical Institute, Boston, MA 02115, USA
*Correspondence: george.daley@childrens.harvard.edu
DOI 10.1016/j.stem.2007.07.019

Despite a long and valuable history, human-animal chimera research has often been questioned. Among the moral issues raised by chimeras is the concept that integration of human cells into anatomical locations such as the brain might endow animals with "human-like" capacities including self-awareness. We present a justification for one type of human-animal chimera experiment: the evaluation of hES cell developmental potency via teratoma formation in immunodeficient mice. We argue that this experiment raises no significant moral concerns and should be the jurisdiction of animal care and use committees and exempt from formal review by the stem cell research oversight process.

Introduction

The classical representation of a chimera, such as that described in Homer's *Iliad*, is a mythical beast bearing the body parts of several different creatures combined into a single animal. The term chimera has a more tangible usage in modern science and medicine, where it indicates organisms comprised of cells from two or more individuals of the same or different species. Today, the most common usage describes cellular combinations at the preimplantation blastocyst stage of development. That said, the term also accurately reflects other entities created by introducing cells at later developmental stages including in adult recipients. The fractional percentage and physiological integration of contributing cells will vary depending upon when and where during development chimerism is established, being high when early embryos are fused (Tarkowski, 1961; Yu et al., 2002) and low when more differentiated cells are introduced later in development, as in clinical hematopoietic transplantation. Chimeras made by transferring cells and tissues between different species (xenotransplantation) have a lengthy history. The earliest documented xenotransplants into humans used lamb or calf blood and took place in mid 17th century Europe (e.g., Lower and King, 1667), while the reverse (human into animal) has been practiced as far back as the mid to late 18th century (see Deschamps et al., 2005).

In the laboratory, animal hosts have long been used to test human cells or tissues for basic research, particularly in the study of malignancy (e.g., Hegner, 1913), as well as in preclinical models for the evaluation of therapeutics. One of the best examples of human cell engraftment into animal hosts is the use of immunodeficient mice as transplant recipients for human stem and progenitors cells of the hematopoietic system (Kamel-Reid and Dick, 1988; Behringer, 2007).

Regulation of Human-Animal Chimera Research

The propriety and utility of evaluating human cells in animal models has been questioned (Karpowicz et al., 2004; Robert, 2006; Streiffer, 2005). Experiments involving human-animal chimeras have further been the subject of restrictive legislation (for example, in the U.S.A., see Brownback [2005]) and raise the issue of how scientists justify research in a particularly controversial area. To begin with, the scientific objectives of every experiment must be clearly reasoned, as a poorly articulated experimental goal is unworthy of pursuit. Beyond this, studies involving human-animal chimerism present a wide range and depth of ethical issues (Hyun et al., 2007). However, the transfer of human cells into postnatal, adult recipients is likely the least contentious due to a low probability that human cells introduced at such a late developmental stage may integrate appreciably into existing structures (see Greene et al., 2005; Robert, 2006; Streiffer, 2005). The prospect that certain chimeras might adopt human features would necessarily compel our moral interest in their fate and prohibit certain experimental objectives (Robert, 2006), a concern most pertinent to studies in which human neurons or gametes might be incorporated into the brain or gonads, respectively, of a closely related primate.

Considering the number of nations engaged in human embryonic stem (hES) cell research worldwide, the topic of how human-animal chimera research should be regulated is of international relevance (e.g., House of

Commons Science and Technology Committee on Guidelines for Human Embryonic Stem Cell Research, 2007). Within the United States, a National Academy of Sciences (NAS) panel has formulated guidelines for research on hES cells that include specifications for experiments involving human-animal chimeras and recommends that Embryonic Stem Cell Research Oversight (ESCRO) committees be formed to interpret the guidelines for local experiments (National Research Council, 2005; National Research Council, 2007). In their 2005 report (National Research Council, 2005), the NAS made the following recommendation:

"All research involving the introduction of hES cells into nonhuman animals at any stage of embryonic, fetal, or postnatal development should be reviewed by the ESCRO committee. Particular attention should be paid to the probable pattern and effects of differentiation and integration of the human cells into the nonhuman animal tissues."

The International Society for Stem Cell Research (ISSCR) has also compiled guidelines that further consider issues relating to the conduct of stem cell research beyond national borders (Daley et al., 2007; International Society for Stem Cell Research Guidelines Taskforce, 2006) (downloadable at http://www.isscr.org/guidelines/). In contrast to the NAS panel recommendations, the ISSCR guidelines stipulate that the assay of hES cells by teratoma formation be accepted as routine and be exempt from Stem Cell Research Oversight (SCRO) review. This commentary explores the scientific rationale upon which such recommendations are based.

The NAS and ISSCR guidelines invite thoughtful consideration. Any type of human-animal chimera experiment should be scrutinized for which tissues will be chimerized (either purposefully or inadvertently), for the degree of chimerism that might result, and for the state of physiologic integration of the engrafted cells. Although the guidelines are most relevant to the purposeful chimerism of specific tissues like the brain, we must consider inadvertent chimerism in the context of other assays and determine the likelihood that human cells might migrate and establish themselves in other locations. Indeed, the ESCRO committee at our home institution raised concerns about the prospects for even a small contribution of cells to the mouse brain or germline during routine hES cell teratoma formation assays and asked that we justify these experiments in scientific terms.

The Assay of hES Cells In Vivo via Teratoma Formation, One Type of Human-Animal Chimera

For murine ES cells, the truest demonstration of cellular pluripotency is assayed in vivo via blastocyst chimerism or tetraploid aggregation followed by gestation (Brinster, 1974; Nagy et al., 1990). When considering human ES cells, such experiments are clearly ethically proscribed. The adult murine host, however, contains permissive niches that support the growth and differentiation of hES cells into a wide variety of cell and tissue types and may be employed in a less robust, though informative, surrogate assay: in vivo teratoma formation (Brivanlou et al., 2003; Thomson et al., 1998). When introduced into adult, immunodeficient mouse hosts by subcutaneous, intramuscular, or intratesticular routes, hES cells will spontaneously form teratoma-like masses containing ectoderm such as nerve and skin; mesoderm including bone, blood, and muscle; and endoderm or gut tissue (Thomson et al., 1998). Formation of differentiated cells from the three somatic germ layers within the teratoma is taken as the best indicator of the pluripotency of hES cell lines (Brivanlou et al., 2003). Of note, while some recently reported "pluripotent" cell lines from nonembryonic sources have proven capable of forming multiple tissue types in vitro, they were incapable of forming teratoma-like masses in vivo (De Coppi et al., 2007), an indication of incomplete potency when compared to hES cells and a challenge to the accuracy of their description as pluripotent.

Paraffin sections of hES cell-derived teratomas are highly informative, and though variable degrees of differentiation are often present, many tissue types as well as three-dimensional organ architecture may be noted (see Figure 1). A "gold standard" for evaluating the quality of hES cells includes the idea that a cell line must form a well-differentiated, teratoma-like mass (Brivanlou et al., 2003). Recently, a multinational study organized to evaluate hES cell lines worldwide included teratoma formation as a feature of their analysis (Adewumi et al., 2007), and a working group of the ISSCR has further formulated a set of "standard conditions" for hES-derived teratomas (Gertow et al., 2007). If a hES line fails in this most basic of developmental tests, it may still remain a valuable research tool, albeit a restricted one, due to an impaired capacity to form the many tissues under study by scientists. Such a differentiation failure might also suggest a dangerously flawed cellular resource for use in future therapies. As such, the need for teratoma assays with hES cells is compelling. That said, special ethical as well as regulatory caveats are to be considered when introducing hES cells into animal hosts.

History and Classification of Naturally Occurring Pluripotent Cell Tumors

In responding to the specific question of whether or not experimental teratoma derivatives in fact migrate beyond the tumor margins and significantly integrate distally, one may literally draw upon centuries of information regarding the pathogenesis of pluripotent cell tumors in both human patients and, more recently, experimental animals. The term "teratoma" was introduced by Rudolf Virchow in his 1863 collection "Die krankhaften Geschwülste," although such tumors were well known prior to his work, including an illustrated contribution to the Philosophical Transactions of the Royal Society from the year 1683 (Birch and Tyson, 1683). In general, pluripotent cell-derived tumors are categorized by their developmental potential, cellular origin, and anatomic location and include sacrococcygeal teratoma, gonadoblastoma, and dermoid cysts of the ovary, among others. Teratomas are a subset of pluripotent tumors associated with germ cells (Andrews, 1988; Askanazy, 1907; Teilum, 1965),

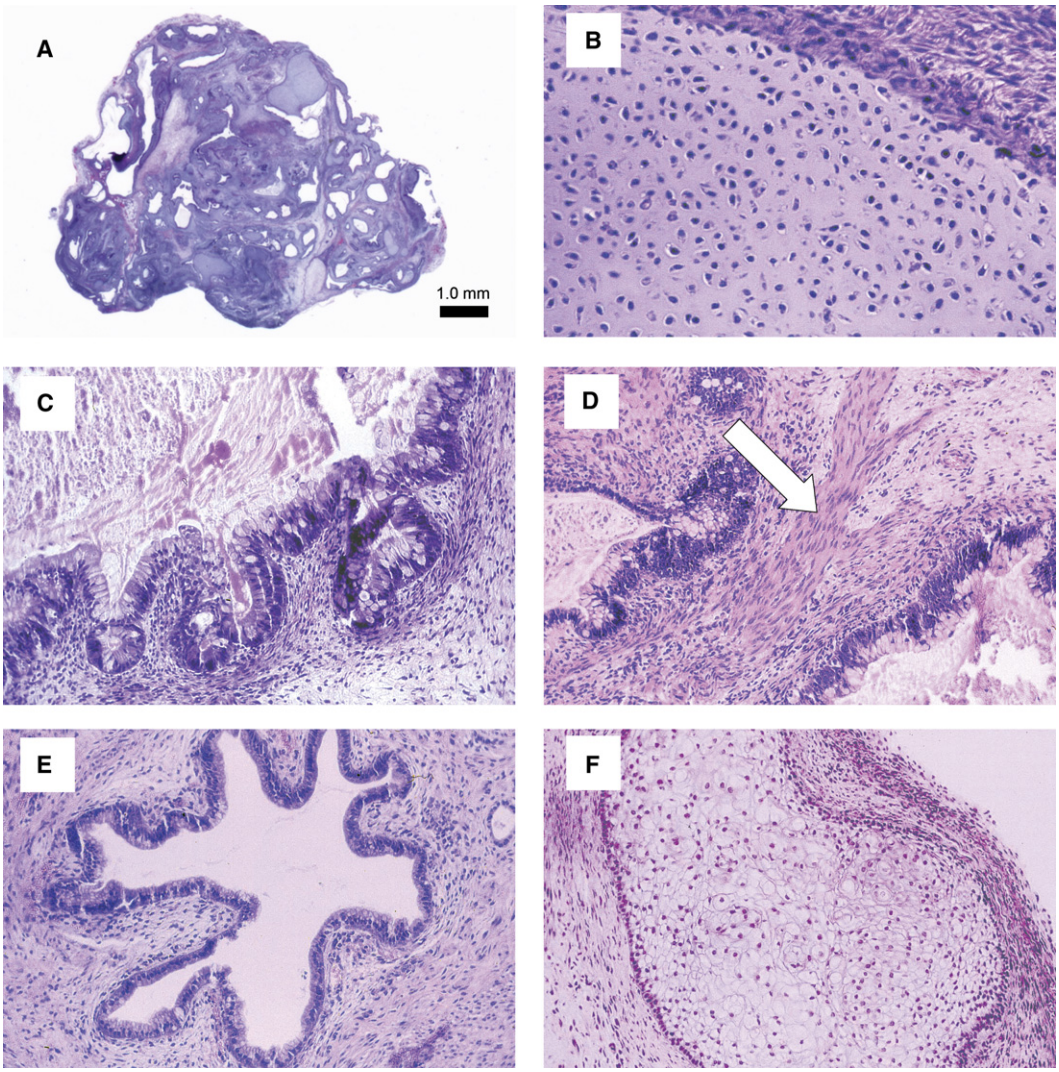

Figure 1. Histopathology of a hESC-Derived "Teratoma"
Histology of a human teratoma formed by subcutaneous injection of the NIH-approved human embryonic stem cell line H9 (Thomson et al., 1998) into an immune-deficient mouse host. Teratomas are pluripotent tumors, i.e., containing derivatives of all three embryonic germ layers (ectoderm, mesoderm, and endoderm), as illustrated here. Though chaotically arrayed within the mass, functional, organ-specific architecture may develop as demonstrated in (C), where layers of gut endoderm secrete muscinous material into a central lumen toward the upper left. (A) Low-resolution image of a whole teratoma demonstrating a complex, highly cystic mass (scale bar, 1.0 mm). (B) Cartilage (mesoderm). (C) Colonic gut epithelium (endoderm). (D) A rivulet of muscle at arrow (mesoderm). (E) Respiratory, brush-border, epithelium (endoderm). (F) Glycogenated squamous epithelium (ectoderm). (B) was photographed at 200× magnification and (C)–(F) at 100× and represent different areas within a single teratoma. All images were obtained from formalin-fixed and paraffin-embedded teratoma sections stained with hematoxylin and eosin.

although they may also arise in extragonadal areas key to fetal primordial germ cell migration (Witschi, 1948) including sites throughout the mediastinum, the central region of the thoracic cavity.

With regard to histopathology, teratomas are characterized as either mature and benign (i.e., containing well-defined somatic structures) or as immature and malignant (bearing a large degree of embryonic neural derivatives or masses of undifferentiated, "embryonal carcinoma" [EC] cells). Though regions of benign, multidifferentiated tissue may be noted in immature teratomas, tumors containing EC cells are appropriately termed "teratocarcinomas" (Andrews, 2002) to reflect their tendency to evolve toward malignancy. The distinct natural history of teratocarcinomas also correlates with their transplantability in experimental systems (Kleinsmith and Pierce, 1964).

The majority of naturally occurring teratomas are well-defined, benign masses (Isaacs, 2004) that fail to metastasize, can be eliminated surgically, and typically do not reoccur. When metastasis is noted, it is most often a result of overlooked microscopic inclusions of a more malignant phenotype such as yolk sac tumor within an otherwise benign-appearing teratoma. There are also overtly malignant primary tumors that contain a paucity of well-differentiated elements and include seminoma and nonseminomatous germ cell tumors in males, dysgerminoma in

females, and teratocarcinoma and embryonal carcinoma in both genders. The behavior of germ cell-related tumors varies substantially between the sexes; e.g., certain tumor types have a much higher incidence in females, and male gonadal tumors are more likely to behave as a malignancy than their female counterparts. Naturally occurring teratomas and related tumors are abnormal, neoplastic pathologies bearing genetic defects. In contrast, experimentally induced teratoma-like masses, including those formed from hES cells, result from normal pluripotent cells transplanted to growth-permissive, ectopic sites.

Early Teratomas in the Laboratory

Experimentally, the first robust teratoma studies were conducted by Stevens and Little in the 129 inbred strain of laboratory mouse, which they noted was genetically predisposed to testicular teratoma (Stevens and Little, 1954). These observations permitted the eventual isolation of the stem cell component of the teratoma (Kleinsmith and Pierce, 1964; Martin and Evans, 1974), the aforementioned EC cell, thus founding the field of pluripotent stem cell research as we know it. Additionally, transplantation of entire egg cylinder or genital ridge stage embryos to ectopic sites in host recipients initiates noninvasive and nonmetastatic teratoma-like tumors (Solter et al., 1970; Stevens, 1968). These ectopic masses have at times been referred to as teratocarcinomas in the literature due to foci of undifferentiated stem cells (EC) and occasional serial transplantability with or without the capacity to establish cell lines when explanted into tissue culture. Such cell lines (e.g., P19, McBurney and Rogers [1982]) are perhaps neither EC nor ES cells but, rather, outgrowths of primordial germ cells (PGCs) and may further be genetically abnormal (McBurney, 1976).

A logical extension of such work asked whether pluripotent cells could be isolated from nontumor tissues, and to date, ES cells have been obtained from the normal, preimplantation embryos of several species including mice (Evans and Kaufman, 1981; Martin, 1981) and humans (Thomson et al., 1998). The fullest developmental potential of mouse pluripotent cells is assayed via the formation of blastocyst or tetraploid chimeras that are then gestated in the uterus of host mice (Brinster, 1974; Nagy et al., 1990). Pluripotency is demonstrated when the progeny of the input cells are found in all tissues, including the germline. Obvious ethical prohibitions preclude similar work with hES cells. Thus, the range of hES tissue fates is assessed using the surrogate assay of transfer into adult immune-deficient mice, where the resulting teratoma-like outgrowths offer important insights into the development plasticity of individual cell lines. The majority of such masses resemble naturally occurring teratomas that would be classified as mature, reflecting the normal, nonmalignant origins of the ES cells.

Summary

Considering these observations, several key points emerge. First, the origins of hES cells are not as isolates from abnormal, neoplastic tissues (as is the case with EC cells) but rather from normal blastocysts. Human ES cell-derived tumors that form following injection into immune-deficient mice are mature, encapsulated (Gertow et al., 2007; Reubinoff et al., 2000; and see Figure 1A), easily resected, and do not invade beyond the local tissue. Analogous, naturally occurring masses (i.e., true teratomas) in human patients respond well to surgical treatment and do not metastasize. While the initiation of hES cell-derived masses is not biologically equivalent to naturally occurring teratomas, the growth, presentation at necropsy, and histopathology of each are quite similar, and we conclude that the behavior of experimentally produced teratomas is well informed by their clinical counterparts. Thus, hES cell migration beyond the tumor mass and into the host brain is not expected in the experimental setting, and the inadvertent migration of normal hES cells into the adult mouse gonad, a niche permissive for pluripotent cells, is likewise unexpected. Furthermore, the species barrier between humans and mice prevents crossfertilization of gametes. Moreover, it is a simple matter to segregate transplant recipients according to sex or to use only a single sex for all experiments. The remainder of our analysis will focus on the potential for neural integration.

Second, while an unexpected result could occur wherein an aberrant or genetically perturbed hES cell line yielded a metastatic tumor with neural elements, the tissue in such tumors is primarily of a very immature, embryonal nature (Paterakis et al., 2005). Absent additional neuronal maturation, such tissue is unlikely to form the integrative networks that participate in higher-order neural interactions including those required for cognition or perception. Experimental systems have been developed that make use of human teratocarcinoma-derived embryonal carcinoma cells such as the EC line NTERA2 (Andrews et al., 1984). NTERA2 bears a robust propensity for neural progenitor differentiation following treatment with retinoic acid (Andrews, 1984). While these neural derivatives (termed NT2N) are immature in vitro (Pleasure and Lee, 1993), it has been shown that they are capable of long-term survival and neuronal process extension in an in vivo model, demonstrating markers of additional maturation within 4 to 6 months following engraftment into immune-deficient murine recipients (Kleppner et al., 1995). However, it is important to stress that while the host environment was capable of refining the maturation of the NT2N cells, antecedent neuronal differentiation with retinoic acid was required prior to engraftment. The concern for relevant integration into the host brain during teratoma assays is further lessened by the fact that experimental hosts are adult mice with well-developed and intact central nervous system architecture. Additionally, while certain structures found in experimental and naturally occurring teratomas can exhibit remarkable completeness of differentiation such as complete teeth (Birch and Tyson, 1683) the degree of tissue organization required for organ function has not been observed for even the simplest concerted actions (e.g., chewing), let alone higher brain function.

Third, even if we assume that ES cell derivatives could somehow migrate to the brain and create complex neural networks, the possibility of the manifestation of any higher human-like brain function confined within a mouse's body is remote. This conviction is based upon recent studies in which functional human-rodent brain chimerism was the explicit experimental aim (Brustle et al., 1998; Flax et al., 1998; Kerr et al., 2003; Mueller et al., 2005; Nistor et al., 2005; Tabar et al., 2005). Such work indicates that graft-derived human neurons and support cells respond to the mouse host environment, integrating into existing structures rather than patterning the host to have more human-like brain architecture. Not a single case has been reported in which host brain function was improved beyond control levels following human neuron or precursor cell grafting, even when functional improvement of the nervous system was the objective (Kerr et al., 2003; Mueller et al., 2005). It seems quite unlikely that small numbers of metastatic teratoma derivatives could prove more effective at functional neuronal engraftment. This conclusion is in accordance with a small but growing body of literature on the ethics of human-animal chimeras that agrees on the general notion that the use of remotely related, noninjured, adult animal recipients presents the least moral concern, particularly if tissue replacement or restoration of function is not the experimental objective. This is in contrast to the injection of human cells or tissues into closely related, developing, or injured organisms (see Greene et al., 2005; Karpowicz et al., 2004; Robert, 2006; Streiffer, 2005; Hyun et al., 2007).

Finally, our human experience perhaps serves as the strongest argument against the possibility that scant neural derivatives of hES cells might serve to "humanize" the mouse. We are aware of the latency between the formation of the most nascent neural structures in utero during human development and the onset of consciousness, the ability to consider abstract principles, and the capacity to articulate (either verbally or physically) individual will or need. The possibility that even extensive chimerism of the mouse brain by human neurons would result in anything remotely approaching an ethically concerning state seems extremely unlikely indeed (Karpowicz et al., 2004), especially considering the short duration of teratoma assays (typically less than 3 months).

Taken together, we believe that the risk of inadvertently creating a rodent chimera with higher, human brain function is negligible. Indeed, a recent analysis by a multidisciplinary working group exploring the moral issues surrounding human/nonhuman primate (NHP) neural grafting concluded that it is "…unlikely that the grafting of human cells into healthy adult NHPs will result in significant changes in morally relevant mental capacities" (Greene et al., 2005). In our opinion, the risk that teratoma formation assays might endow higher cognitive function in an even more distantly related species such as the mouse is so improbable as to obviate the need for experimental limitations beyond those normally in place for other types of animal experimentation. Simple notification that teratoma formation experiments are to be conducted should prove sufficient for regulatory bodies charged with reviewing hES cell research, as the full oversight of such experiments will continue to be the jurisdiction of animal care and use committees.

Conclusion

The formation of hES cell-derived teratomas in experimental rodents represents the most robust and ethically permissible standard for evaluating the developmental versatility of hES cells. This form of human-animal chimera yields unique experimental insights of irreplaceable value (Brivanlou et al., 2003). Teratoma formation is described in instructional materials made available to federally funded investigators using National Institutes of Health (NIH)-approved hES cell lines (see http://stemcells.nih.gov/). Furthermore, the important experimental insights gained by such studies are of enormous worth in our efforts to understand the developmental biology of human tissues, experiments that are only possible due to the availability of hES cells. In conclusion, it is our opinion that human teratoma formation studies in adult mice are justifiable and should be routinely approved by animal care committees with a minimal need for regulation by the stem cell research oversight process.

ACKNOWLEDGMENTS

The authors wish to acknowledge the following individuals for generous comments and assistance: Peter Andrews, Jon Draper, Tan Ince, Asmin Tulpule, Lindsay Frazier, Heather Rooke, Lucretia McClure, Insoo Hyun, Louis Guenin, Carlos Estrada, Phillip Karpowicz, John Dick, and Anne McLaren. The constructive suggestions offered by three anonymous reviewers also refined the manuscript considerably. This work was supported by grants from the Harvard Stem Cell Institute, the Stem Cell Program of Children's Hospital Boston, and the NIH Director's Pioneer Award of the NIH Roadmap for Medical Research (G.Q.D). M.W.L. is a recipient of a Seed Grant from the Harvard Stem Cell Institute. L.I.Z. is supported by the Howard Hughes Medical Institute. G.Q.D. is a recipient of a Burroughs Wellcome Fund Clinical Scientist Award in Translational Research.

REFERENCES

Adewumi, O., Aflatoonian, B., Ahrlund-Richter, L., Amit, M., Andrews, P.W., Beighton, G., Bello, P.A., Benvenisty, N., Berry, L.S., Bevan, S., et al. (2007). Nat. Biotechnol. *25*, 803–816.

Andrews, P.W. (1984). Dev. Biol. *103*, 285–293.

Andrews, P.W. (1988). Biochim. Biophys. Acta *948*, 17–36.

Andrews, P.W. (2002). Philos. Trans. R. Soc. Lond. B Biol. Sci. *357*, 405–417.

Andrews, P.W., Damjanov, I., Simon, D., Banting, G.S., Carlin, C., Dracopoli, N.C., and Fogh, J. (1984). Lab. Invest. *50*, 147–162.

Askanazy, M. (1907). Verh. Dtsch. Pathol. Ges. *11*, 39–82.

Behringer, R.H. (2007). Cell Stem Cell *1*, in press. Published online August 16, 2007. 10.1016/j.stem.2007.07.021.

Birch, S., and Tyson, E. (1683). Philos. Trans. R. Soc. *13*, 281–284.

Brinster, R.L. (1974). J. Exp. Med. *140*, 1049–1056.

Brivanlou, A.H., Gage, F.H., Jaenisch, R., Jessell, T., Melton, D., and Rossant, J. (2003). Science *300*, 913–916.

Brownback, S. (2005). Human Chimera Prohibition Act of 2005 (S.1373), United States Senate, 109th Congress.

Brustle, O., Choudhary, K., Karram, K., Huttner, A., Murray, K., Dubois-Dalcq, M., and McKay, R.D. (1998). Nat. Biotechnol. *16*, 1040–1044.

Daley, G.Q., Ahrlund Richter, L., Auerbach, J.M., Benvenisty, N., Charo, R.A., Chen, G., Deng, H.K., Goldstein, L.S., Hudson, K.L., Hyun, I., et al. (2007). Science *315*, 603–604.

De Coppi, P., Bartsch, G., Jr., Siddiqui, M.M., Xu, T., Santos, C.C., Perin, L., Mostoslavsky, G., Serre, A.C., Snyder, E.Y., Yoo, J.J., et al. (2007). Nat. Biotechnol. *25*, 100–106.

Deschamps, J.Y., Roux, F.A., Sai, P., and Gouin, E. (2005). Xenotransplantation *12*, 91–109.

Evans, M.J., and Kaufman, M.H. (1981). Nature *292*, 154–156.

Flax, J.D., Aurora, S., Yang, C., Simonin, C., Wills, A.M., Billinghurst, L.L., Jendoubi, M., Sidman, R.L., Wolfe, J.H., Kim, S.U., and Snyder, E.Y. (1998). Nat. Biotechnol. *16*, 1033–1039.

Gertow, K., Przyborski, S., Loring, J., Auerbach, J.M., Epifano, O., Otonkoski, T., Damjanov, I., and Ahrlund Richter, L. (2007). Curr. Prot. Stem Cell Biol., in press.

Greene, M., Schill, K., Takahashi, S., Bateman-House, A., Beauchamp, T., Bok, H., Cheney, D., Coyle, J., Deacon, T., Dennett, D., et al. (2005). Science *309*, 385–386.

Hegner, C.A. (1913). München Med. Wchnschr. *60*, 2722–2724.

House of Commons Science and Technology Committee on Guidelines for Human Embryonic Stem Cell Research. (2007). Government proposals for the regulation of hybrid and chimera embryos; Fifth Report of Session 2006–07, The House of Commons, ed. (London: The Stationery Office Limited), pp. 74.

Hyun, I., Taylor, P., Testa, G., Dickens, B., Jung, K.W., McNab, A., Robertson, J., Skene, L., and Zoloth, L. (2007). Cell Stem Cell *1*, 159–163.

International Society for Stem Cell Research Guidelines Taskforce (2006). In Guidelines for the Conduct of Human Embryonic Stem Cell Research, G.Q. Daley, ed. (Northbrook, IL: ISSCR).

Isaacs, H., Jr. (2004). J. Pediatr. Surg. *39*, 1003–1013.

Kamel-Reid, S., and Dick, J.E. (1988). Science *242*, 1706–1709.

Karpowicz, P., Cohen, C.B., and van der Kooy, D. (2004). Nat. Med. *10*, 331–335.

Kerr, D.A., Llado, J., Shamblott, M.J., Maragakis, N.J., Irani, D.N., Crawford, T.O., Krishnan, C., Dike, S., Gearhart, J.D., and Rothstein, J.D. (2003). J. Neurosci. *23*, 5131–5140.

Kleinsmith, L.J., and Pierce, G.B., Jr. (1964). Cancer Res. *24*, 1544–1551.

Kleppner, S.R., Robinson, K.A., Trojanowski, J.Q., and Lee, V.M. (1995). J. Comp. Neurol. *357*, 618–632.

Lower, R., and King, E. (1667). Philos. Trans. R. Soc. *2*, 557–559.

Martin, G.R. (1981). Proc. Natl. Acad. Sci. USA *78*, 7634–7638.

Martin, G.R., and Evans, M.J. (1974). Cell *2*, 163–172.

McBurney, M.W. (1976). J. Cell. Physiol. *89*, 441–455.

McBurney, M.W., and Rogers, B.J. (1982). Dev. Biol. *89*, 503–508.

Mueller, D., Shamblott, M.J., Fox, H.E., Gearhart, J.D., and Martin, L.J. (2005). J. Neurosci. Res. *82*, 592–608.

Nagy, A., Gocza, E., Diaz, E.M., Prideaux, V.R., Ivanyi, E., Markkula, M., and Rossant, J. (1990). Development *110*, 815–821.

National Research Council (2005). Guidelines for human embryonic stem cell research, First Edition (Washington, D.C.: National Academies Press).

National Research Council (2007). Amendments to the National Academies' Guidelines for Human Embryonic Stem Cell Research, First Edition (Washington, D.C.: National Academies Press).

Nistor, G.I., Totoiu, M.O., Haque, N., Carpenter, M.K., and Keirstead, H.S. (2005). Glia *49*, 385–396.

Paterakis, K.N., Karantanas, A.H., Barbanis, S., Hadjigeorgiou, G.M., and Karavelis, A. (2005). Clin. Neurol. Neurosurg. 108, 67–72.

Pleasure, S.J., and Lee, V.M. (1993). J. Neurosci. Res. *35*, 585–602.

Reubinoff, B.E., Pera, M.F., Fong, C.Y., Trounson, A., and Bongso, A. (2000). Nat. Biotechnol. *18*, 399–404.

Robert, J.S. (2006). FASEB J. *20*, 838–845.

Solter, D., Skreb, N., and Damjanov, I. (1970). Nature *227*, 503–504.

Stevens, L.C. (1968). J. Embryol. Exp. Morphol. *20*, 329–341.

Stevens, L.C., and Little, C.C. (1954). Proc. Natl. Acad. Sci. USA *40*, 1080–1087.

Streiffer, R. (2005). Kennedy Inst. Ethics J. *15*, 347–370.

Tabar, V., Panagiotakos, G., Greenberg, E.D., Chan, B.K., Sadelain, M., Gutin, P.H., and Studer, L. (2005). Nat. Biotechnol. *23*, 601–606.

Tarkowski, A.K. (1961). Nature *190*, 857–860.

Teilum, G. (1965). Acta Pathol. Microbiol. Scand. *64*, 407–429.

Thomson, J.A., Itskovitz-Eldor, J., Shapiro, S.S., Waknitz, M.A., Swiergiel, J.J., Marshall, V.S., and Jones, J.M. (1998). Science *282*, 1145–1147.

Witschi, E. (1948). Contrib. Embryol. *32*, 67–80.

Yu, N., Kruskall, M.S., Yunis, J.J., Knoll, J.H., Uhl, L., Alosco, S., Ohashi, M., Clavijo, O., Husain, Z., Yunis, E.J., et al. (2002). N. Engl. J. Med. *346*, 1545–1552.