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Letter to the Editor

HUMAN EMBRYONIC STEM CELL–DERIVED FIBROBLASTIC AND EPITHELOID LINEAGES AS XENO-FREE SUPPORT?

Dear Editor:

Human embryonic stem (hES) cells have tremendous potential for application in the newly emerging field of regenerative medicine (Gerecht-Nir and Itskovitz-Eldor, 2004). Recently, the first successful derivation of a hES cell line from cloned human embryos (Hwang et al., 2004), as well as the successful derivation of 17 new hES cell lines from donated in vitro–fertilized embryos (Cowan et al., in press), once again placed hES cells at the forefront of international scientific research.

However, a major barrier to the application of hES cells in human clinical therapy is their routine propagation on a feeder layer of mitotically inactivated murine embryonic fibroblasts (Thomson et al., 1998; Reubinoff et al., 2000; Cowan et al., in press). With the advent of newly emerging human diseases that have crossed the species barrier, such as severe acute respiratory syndrome (Normile, 2004) and Nipah virus infection (Chua et al., 2002), there are overriding safety concerns regarding the use of mouse-derived feeder cells for hES propagation. We, therefore, read with great interest the recent studies (Richards et al., 2002, 2003; Amit et al., 2003; Hovatta et al., 2003) that have examined the use of donated human tissues to develop a xeno-free support system for hES cells. It is important to note the various limitations of such an approach. Obviously, the use of fetal tissues originating from human abortuses (Richards et al., 2002, 2003) would raise huge ethical concerns. Donated human adult and neonatal tissues pose much less of an ethical dilemma (Amit et al., 2003; Hovatta et al., 2003). However, there may be problems with their availability for large-scale culture of hES cells. Moreover, the use of donated human tissues would introduce a high degree of variability within the culture milieu, which could confound good-quality control in the laboratory. Although cross-species transfer of pathogens would be eliminated through the use of human-derived feeders, there is still a real risk of contamination with yet unscreened pathogens of newly emerging human diseases. All these would pose significant challenges in the application of hES cells for human clinical therapy.

A novel approach around these problems would be to examine the possible use of hES cell–derived fibroblastic and epitheloid lineages as feeder supports. The rationale is that such lineages derived from adult and fetal tissues have already been successfully used for maintaining the undifferentiated state of hES cells during prolonged in vitro culture (Richards et al., 2002, 2003; Amit et al., 2003; Hovatta et al., 2003). Hence, it is likely that similar lineages derived from differentiating hES cells could possibly possess the same ability. Presently, there are as yet no reported studies on screening the various differentiated lineages within embryoid bodies for their ability to act as feeder supports for undifferentiated hES cells.

Differentiation of hES cells into the fibroblastic and epitheloid lineages could be enhanced through the use of exogenous cytokines, in particular the various isoforms of fibroblast growth factor (FGF) and epithelial growth factor (EGF) (Fernig et al., 1994; Boonstra et al., 1995; Schuldiner et al., 2000). It is likely that a diverse plethora of fibroblastic and epitheloid lineages could be generated by varying combinations of these isoforms of FGF and EGF. These lineages can then be screened for their ability to act as feeder support for undifferentiated hES cells. Once a particular lineage has been identified, the next major challenge would be to establish well-defined and efficient protocols to generate that lineage from hES cells.

Preliminary investigations by the Genome Institute of Singapore have revealed major differences in the transcription profiles and proliferation rates of different hES cell lines, although all of these expressed the common marker genes characteristic of hES cells (B. Lim, pers. comm.). Hence, it may also be necessary to screen differentiated lineages derived from several hES cell lines.

The development of such "autogenic" xeno-free feeder support systems for hES cell culture will then provide the stringent levels of quality control and safety standards that enable the application of hES cells in human clinical therapy. We hope this will be achieved in the near future.

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