Human embryonic stem cell lines generated without embryo destruction: New strategies, new ethical problems

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Already in 2006 the group of Irina Klimanskaya / Robert Lanza had published a controversially discussed paper on the generation of human embryonic stem cells (hESCs) from cells that had been obtained by blastomere biopsy. Title and abstract of that paper suggested that removal of one or only few cells from an embryo could be sufficient, and that the original embryo might survive undamaged, so that this procedure might avoid the ethically problematrical embryo destruction. However, that paper was critizised because it had indeed not directly demonstrated that embryos survived, and because cells had been combined for culturing.

Now the same group publishes results obtained with new methodological variants along these lines. On one hand some (limited) data are presented on the survival of the biopsied human embryos (although only up to the blastocyst stage). The main part of the data, however, concerns methodological improvements for culturing of the isolated cells which allow to considerably increase the rate of success in hESC derivation. Co-cultivation with pre-existing hESC lines (still found necessary in the first publication) is now found unnecessary if replaced by the addition of the ECM (extracellular matrix) protein laminin (pure or in various combinations with fibronectin which, itself, showed somewhat different effects). The use of laminin in ESC culture is not really new but had already been done by other authors in order to replace feeder cells by better defined (and pathogen-free) alternatives. A major point in the present paper is that the authors conclude, on the basis of a (limited) series of control experiments, that the effect of laminin is to keep the isolated cells in a relatively undifferentiated state, and that it can even push cells back into such a state, and that this "optimized" in-vitro condition somewhat mimics the stem cell niche present in a blastocyst, so that due to this treatment the isolated cells acquire the properties of ICM (inner cell mass, embryoblast) cells of a blastocyst. Removal of laminin, on contrast, pushes cells towards trophoblast differentiation. The same effects are seen not only with the freshly isolated cells but also with established ESCs.

Ethical problems:
The ethical aspects of blastomere biopsy (and of PGD in general) are seen quite differently and are controversially discussed in different countries, but this is not the main point of ethical concern here as I feel. What the authors of this paper do not seem to realize is that their strategy of improving ESC derivation methodology considerably aggravates a problem that is already present (but not equally apparent) in the existing protocols: the (transitory) generation of twin embryos. The declared purpose of the authors is to mimic in their in-vitro conditions, as much as possible, the stem cell niche present for the ICM cells in a blastocyst (citation: "…employed a modified approach aimed at recreating the ICM niche by preventing trophectoderm differentiation."; "… enhanced blastomere differentiation into ICM."). Their control experiments indeed provide some data suggesting that this goal is being reached at least partially. Ethically relevant appears that choosing their methodology means to deviate from the previous concept of ESC derivation, i.e. diverting them as quickly as possible from their original path of development (in the embryo) so that they lose their totipotency (while being forced by the in-vitro conditions to undergo epigenetic changes and to convert into a

cell line, i.e. ESCs). On contrast, what is being done now comes more close to what is going on during the (spontaneous) formation of a monozygotic twin in vivo (cf. textbooks of embryology; an illustration can also be found in: Denker, H.-W., 2003: Embryonale Stammzellen als entwicklungsbiologisches Modell…; Fig. 3: B, p. 38; PDF see List of Publications on this homepage) because it involves isolating, expanding, re-isolating of cell clusters and colony formation of ICM type cells. I have already before discussed the developmental biology behind such phenomena (of twinning, individuation, morphogenesis, basic body pattern formation, gastrulation) in connection with the remarkable finding of highly organized "embryoid bodies" in marmoset monkey ESC colonies (Thomson et al., 1996) (see e.g. Denker, H.-W. 1999, 2004, 2006; PDFs on this homepage) and have speculated that such phenomena may be most typically (and most frequently) found with freshly isolated cells or with cell lines that are cultivated under conditions under which they accumulate only few epigenetic changes. In the present paper Chung et al. not only chose conditions aimed at keeping cells in an equivalent of the ICM niche but also divide them up into many such clusters, starting with the freshly isolated cells. This comes worryingly close to what must be going on during twinning in vivo. Certainly, these authors indicate that by choosing various concentrations of laminin and fibronectin they can modulate, to a degree, the rate of trophoblast or embryoblast differentiation, i.e. the two cell types which in vivo interact in the subsequent embryonic pattern formation processes. However, realistically one has to assume that it will practically never be possible, with the described in vitro culture variants, to make sure that the formation of structures is avoided which can regain organismic wholeness (blastocyst or early postimplantation embryo equivalents). What those authors apparently do not see is, unfortunately, that while making a big effort to save the life of the original embryo (the blastomere donor) they create a new ethical problem with their specific methodology: to risk the life of numerous (potential) embryo equivalents which (without intending to do this) they newly create from the isolated cell.

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