Embryonic and extra-embryonic stem cell lines derived from single mouse blastomere

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Several seminal discoveries during the past 25 years can be regarded as major breakthroughs for cell and development biology: the establishment of embryonic stem (ES) cell lines derived from mouse [1] and human [2] embryos; the reprogramming of somatic cells after nuclear transfer into enucleated eggs in animals [3] and humans [4]; and the demonstration that stem cells can generate every cell type in the body, *in vitro* and *in vivo* [5]. These discoveries have opened the new avenue of regenerative medicine. Differentiation of human ES cells into various cell types and successful studies in animal models have demonstrated the 'proof-of-principle' for using human ES cells in cell therapies. However, many problems must be resolved and much remains to be learned before such applications can be implemented. One of the serious problems potentially associated with the use of differentiated cells derived from human ES cells for tissue repair is the immunological incompatibility between donors and recipients that leads to the rejection of mismatched grafts. Although the expression of the major histocompatibility complex I class of molecules on ES cells is low, their expression tends to increase during differentiation [6]. Different strategies have been described that have potential to circumvent this problem: somatic cell nuclear transfer [4,7], the creation of parthenogenic stem cells [8] and the use of single cell (blastomere) method.

In the paper by Chung and colleagues [9], the team lead by Robert Lanza has developed a method of generating ES cells from the single blastomere that leaves the remaining embryo viable. They have used eight-cell stage mouse embryos labelled with *LacZ* to perform a biopsy to remove one blastomere and transferred the remaining seven-cell embryos to the oviduct of 1.5 days post-coitum-synchronized surrogate mothers. Each isolated blastomere was aggregated with a clump of green fluorescent protein (GFP)-positive mouse ES (mES) cells. After co-culture of 24–48 h, a growing aggregate of GFP-negative cells was

observed on the sides of 60% of GFP-positive clusters of mES cells. The GFP-negative clumps were separated from mES cells mechanically or by enzyme treatment under fluorescent microscope, transferred on growth-arrested mouse fibroblast feeder layers and cultured under the standard culture conditions for mES cells. Approximately 50% of these clumps gave rise to ES cell-like colonies that were passaged by excluding any GFP-positive cells in order to obtain cell lines of pure *LacZ* ES cells derived from one blastomere. In four experiments, putative cell lines were obtained that had normal karyotype and maintained markers of pluripotency after up to 50 passages or more. The purity of these ES cell lines was tested by polymerase chain reaction analysis that confirmed the presence of *LacZ* (marker of blastomere-derived ES cells) but not GFP (marker of supportive mES cells) gene sequences. Derived ES cell lines formed the embryoid bodies and differentiated into cells of all three germ layers. To further demonstrate the pluripotency of the derived ES cells, X-gal (5-bromo-4-chloro-3-indolyl- β -D-galactoside)-labelled blastomere-derived ES cells were either injected into CD-1 mouse blastocyst or aggregated with eight-cell stage morulae. These 'mixed' embryos were transferred to recipient females. Staining of the resulting fetuses showed that the X-gal-positive ES cells contributed to all organ systems. Approximately 83% of fetuses were chimeric and had the *LacZ* gene in their gametes. They produced the *LacZ*-positive offspring when crossed with CD-1 females, confirming the contribution of the blastomere-derived ES cells to the germ line. In addition to ES cell lines, these authors were able to generate cells with extra-embryonic stem-cell-like morphology. These putative trophoblast (TS)-like cells contributed to the extra-embryonic lineages in chimeric fetuses generated by aggregation with the *LacZ* TS cells.

In this study, the blastomere-biopsied embryos developed to term without reduction in their developmental potential. The authors have stressed

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the importance of these experiments for the possible future application of this approach in humans. Their results are consistent with human data that embryos that were biopsied to perform the preimplantation genetic diagnosis (PGD) develop into blastocyst and implant with the comparable efficiency to control, non-biopsied embryos.

In summary, this article provides a new approach that, if replicable in humans, may resolve the two main problems in ES cell-based cell therapy: the source of ES cells and immunological rejection. This procedure will generate custom-made stem cells that will allow banking of the autologous ES cell lines from children born from

transferred biopsied embryos. The procedure of single-cell biopsy is widely used in *in vitro* fertilization clinics for PGD. It is possible that some future parents will decide to use this procedure on healthy embryos to provide their children with a unique source of cells that can in the future save their lives or improve the quality of their lives. It seems that this approach may resolve a major current controversy associated with the use of embryo-derived stem cells for therapy: the embryo is not destroyed, but its genetic makeup is made immortal in derived ES cells. These cells can then provide a source of autologous cells for future use by the child generated from the same blastocyst.

Executive summary

Introduction

- Derivation of mouse embryonic stem (ES) cells from single isolated blastomere that does not interfere with the embryo's normal development and birth.
- The blastomere biopsy procedure described in this paper is performed in humans in *in vitro* fertilization (IVF) clinics worldwide without the reduction of pregnancy rate
- This approach, if applicable to humans, can provide the unique source of custom made stem cells for the future use by the individual born following IVF.

Methods & results

- Chang and co-workers performed six separate experiments to determine whether stem cell lines can be generated from single blastomere. Their experimental approach is innovative and very well designed. They used green fluorescent protein (GFP)-labeled mES as a feeder and co-cultured them with LacZ-labeled single blastomere so that they could visualize the clumps of stem cells and separate them from the feeder.
- The blastomere developed into ES cells shared all the characteristics of ES cells derived from the inner cell mass of the blastocyst stage embryo: they formed embryoid bodies *in vitro* and produced *in vivo* chimeric fetuses if injected into mouse blastocyst transferred to the recipient females.
- Two goals were achieved: derivation of stem cells from the single blastomere and normal development of the biopsied embryos.

Conclusion & future perspective

- The work by Chung and colleagues provides a model for generation of the custom-made ES cells that avoid the use of donor oocytes. The biopsy procedure to obtain single blastomere is performed in IVF clinics without the reduction of pregnancy rate. The derived autologous stem cell lines can be stored and banked for children born from transferred embryos. In the near future we expect to see whether stem cell lines can be derived from single human blastomeres obtained by the standard micromanipulation technique and whether feeder ES cells can be replaced with defined matrix.
- Characterization at the molecular level of different custom-made stem cell lines will provide a lot of information about genetic and epigenetic changes and on the variability of their differentiation capacities.

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