RESEARCH HIGHLIGHT

More new lines of human parthenogenetic embryonic stem cells

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Two papers [1, 2] published in a recent issue of *Cell Research* describe the derivation of pluripotent human embryonic stem (hES)-like cell lines from parthenogenic blastocysts. These two papers complement and substantiate two other papers published a few months earlier [3, 4], which described seemingly two classes of parthenogenetic hES cell lines. Together, these 4 papers mark the beginning of a new era in hES cell research: parthenogenetic hES cell lines come of age.

Differentiated cells derived from pluripotent hES cells offer the promise of new transplantation therapies. In the past decade, hundreds of hES cell lines have been derived from the blastocyst stage of surplus embryos following *in vitro* fertilization (IVF). Human ES cells and their differentiated progeny express highly polymorphic major histocompatibility complex (MHC) molecules that serve as major graft rejection antigens to the immune system of allogeneic hosts. To achieve sustained engraftment of donor cells, strategies are needed to overcome graft rejection without broadly suppressing host immunity. At least two types of strategies are pursued. The first one is to create a patient-specific ES cell line if we may. The second is to reduce or avoid graft rejection by using an existing hES cell

line in a large hES cell bank with a closest match of MHC molecules.

Parthenogenesis and somatic cell nuclear transfer (SCNT) represent two major strategies for generating histocompatible hES cells potentially for therapeutic use. To date human SCNT has not been successfully used to generate a hES cell line.

Parthenogenesis is a process used by some lizards and birds to reproduce without the need of a male. For mammals, the term is commonly referred to embryonic development of eggs activated artificially or aberrantly without fertilization by a sperm. Although mammalian parthenocytes normally can not develop into a full organism, they could, in theory, provide a source to derive ES cells with an exact match to the oocyte donor's genome (both nuclear and mitochondrial). It is also possible that parthenogenesis could provide a source of cells that are homozygous for major histocompatibility alleles (such as HLA-A, B or DRB), thereby allowing partial MHC matching to a substantial population of unrelated transplant recipients. It was found that recombination indeed occurs during meiosis I and II preceding parthenogenesis, and many alleles are not identical (or homozygous) in derived mouse parthenogenetic ES (PES) cells [5]. Nonetheless, estab-

lishing human PES cells (referred as hPES cells here) is an important milestone, not only for a potential source of human stem cells with improved histocompatibility, but also for future basic studies of epigenetic regulation and developmental/stem cell biology. The two *Cell Research* papers describe multiple new hPES cell lines established in China [1, 2], following two other papers published a few months earlier [3, 4]. Together, these four reports suggest that derivation of parthenogenetic hES cells may be easier or more efficient than what was previously thought.

Mai *et al.* [2] used 19 oocytes that were donated by 10 different patients seeking assisted reproduction treatment and activated them purposely by a two-step procedure (electric and chemical stimulations sequentially). 16 oocytes were apparently activated, each with a pseudo-pronucleus 18 hours after. 14 activated oocytes cleaved and progressed to various stages. Four reached the blastocyst stage and 3 had visible inner cell mass (ICM), which was extracted to make ES cell lines. One culture failed and other two eventually gave rise to hES cell lines by standard procedures. The two parthenogenetic blastocyst-derived ES cell lines, named hPES-1 and hPES-2, were propagated and analyzed for characteristic hES cell

properties at various passages. While hPES-1 was reported to meet most if not all the criteria (see below) and maintain a normal karyotype after >100 passages, hPES-2 with similar markers expressed failed to maintain a normal karyotype after 50 passages or form teratomas in immuno-deficient mice as hPES-1 did. The efficiency to derive hES cell lines from parthenogenetic blastocysts appears high (2 out of 3 or 4 blastocysts), as compared to regular blastocysts from IVF embryos (4 out of 9).

Lin *et al.* [1] reported a parthenogenetic hES cell line from an activated oocyte following the standard IVF procedure. The initial intention was to investigate when hES cell lines could be derived from poor-quality or clinically “unwanted” embryos after IVF. 18 hours after insemination, the treated oocyte had one pronucleus in the center of ooplasm with a second body extrusion. Five days after culture, it developed into the blastocyst stage although morphology was poor. Nonetheless, an hES cell line was established and propagated (called chHES-32). Molecular analyses revealed that the derived hES cell line is parthenogenetic (see below) and highly homozygous. For example, Short tandem repeat (STR) analysis that is normally used in parentage and forensic identification revealed that the 16 loci examined are homozygous as compared to primarily heterozygous nature of the oocyte donor. Similarly HLA typing (for HLA-A, HLA-B and HLA-DRB) revealed that chHES-32 cells are homozygous in these alleles as compared to the oocyte donor, and unrelated to that of the sperm donor used for the IVF procedure. This is somewhat similar to the SCNT-HES-1 that turns out to be the first parthenogenetic hES cell line [4]. The HLA type of SCNT-HES-1 is found to be homozygous (in HLA-A, B, C, DRB1, and DQB1 loci), although single nucleotide poly-

morphism (SNP) analysis revealed that a crossover event occurred telomeric to the MHC-gene cluster [4]. Moreover, SNP analysis of chHES-32 showed that each of 23 chromosomes (including the X-chromosome) is highly homozygous (>99%) by the 500K loci array analysis [1]. In this paper, the authors reported that chHES-32 displayed a normal diploid karyotype when analyzed at passage 6 and 49. The authors also mentioned in a recent international symposium [6] that the derived human ES cell line was diploid at passage 3. Thus, it seems that the oocyte that eventually formed a blastocyst was not fertilized by a sperm but somehow activated by the IVF procedure. It is unclear how duplication of the haploid genome of the activated oocyte occurred (after the secondary meiosis), and when the diploid genome was formed before the first 3 passages. Many questions remain, but their potential applications in biology are tremendous.

Both parthenogenetic hES cell lines, chHES-32 and hPES-1, have been characterized extensively, but not fully. For example, both cell lines display a characteristic morphology and surface marker expression profile: positive for SSEA-3, SSEA-4, TRA-1-60, TRA-1-81, and negative for SSEA-1. They express unique genes such as OCT4, NANOG and SOX2 as did conventional hES cells (via IVF embryos). Both are able to form teratomas containing various cell types derived from the 3 embryonic germ layers. Lin *et al.* further analyzed the potential to differentiate to various cell types *in vitro* via embryoid body formation [1]. They observed various cell types expressing a marker either for ectoderm, mesoderm or endoderm derived cells. However, more characterization of derived parthenogenetic hES cells reported in all the 4 papers and comparison with conventional hES cells are needed. For example, conventional hES cells readily form

trophoblast cells either spontaneously with embryoid bodies or upon induction by BMP4 [7]. It is of interest to determine whether parthenogenetic hES cells can form trophoblast-derived cells. More importantly, it is critical to compare maturely differentiated progeny derived from parthenogenetic hES cells with those from conventional hES cells in assays of cellular functionality and tumor formation, if the former is destined to clinical applications. Because of the differences in expression of paternally and maternally imprinted genes, one may expect differences in gene expression related to cell differentiation and other key cellular processes between these two types of hES cells.

As predicted, two alleles of imprinted genes are both maternally inherited in parthenogenetic hES cell lines. The expression of paternally-associated genes such as *IGF-2* and *SNPRN* is absent and that of maternal-associated genes such as *H19* is doubled [1, 2], similar to two other reports [3, 4]. Aside from the application as a potential source of customized stem cells in future cell therapy, the uniparental, and in the case of chHES, highly homozygous hES cell lines provide a unique tool to investigate epigenetic regulations such as imprinting that remain poorly understood.

With the advent of induced pluripotent stem (iPS) cells that are reprogrammed from human somatic cells [8, 9], one may ask whether there is a future to make and analyze more parthenogenetic hES cell lines. I believe the answer is yes. Even after the generation of human iPS cells becomes routine and without increased risk of tumorigenesis, parthenogenetic hES cell lines as well as conventional (IVF) hES cells still have important role to play. The triad has more to offer to human cell biology.

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