Confirmation of Parthenogenetic Identity by Recombination Signature in Human Embryonic Stem Cells

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PARTHENOGENETIC EMBRYOS PROVIDE a source of human embryonic stem cells (pESCs) that avoid destruction of a fertilized embryo or cloning by somatic cell nuclear transfer (SCNT). We previously isolated parthenogenetic pESCs from mouse oocytes by chemical activation during meiosis II (MII), thereby preventing extrusion of the second polar body. This procedure causes the haploid genome to undergo diploidization, and the developing parthenogenetic blastocyst can be used as a source of p(MII)ES cells. We subjected the pESC lines we generated from mice to genome-wide single-nucleotide polymorphism (SNP) analysis and found that the cells harbor a characteristic recombination signature related to their parthenogenetic origin. Oocytes activated in MII gave rise to p(MII)ESCs that exhibited increased SNP heterozygosity at the distal ends of chromosomes, with pericentric regions adjacent to centromeres showing predominantly SNP homozygosity. This pESC-specific signature was not observed in ESCs derived from blastocysts of fertilized embryos or SCNT-derived embryos, which show heterozygosity across all chromosome loci [1]. Thus, pESCs have unique and stable recombinant signatures based on their origin that can be easily distinguished by SNP analysis.

With the same principles and techniques used in murine pESCs, we can similarly predict the recombination signature of human parthenogenetic embryonic stem cells (hpESCs) [1]. At the time of preparation of our original manuscript [1], hpESCs generated by conventional parthenogenetic activation using human oocytes were not available; however, we did have SNP data from one line of hpESCs that had been generated accidentally during the SCNT procedure. The major deficit of our previous publication [1] was the lack of a critical hpESC control that would have incontrovertibly demonstrated the ability of the SNP recombination signature to distinguish between hpESCs derived by parthenogenetic activation and those derived by SCNT. Although this approach has been used by another group [2], the scientific community considered the use of the recombination signature to verify the origin of the first hpESCs—which were accidentally established during the SCNT procedure—to be an investigational method that needed to be further validated because of the above deficiency in the previous publication. It should be noted that this hpESC line has since gained patent approval as an SCNT-ESC line in Canada, and approval is pending in other countries.

Multiple hpESC lines have been now been generated by three laboratories [3], providing the critical controls that were missing from our previous publication [1]. Because the re-

combination rate of human chromosomes is 1.25 cM/Mb, we analyzed the SNP data for chromosomes longer than 40 MB (chromosomes 1–12) to reveal the recombination signatures in these hpESC lines (Fig. 1). We then compared these data with those of our first hpESCs to verify the unique recombination signature of each hpESC line and confirmed the origin of the first hpESCs generated during the SCNT procedure. We observed the characteristic pESC recombination signature—predominant heterozygosity at the distal ends and homozygosity at the pericentric regions of chromosomes—thereby confirming that the cell lines generated by multiple independent groups are indeed hpESCs (Fig. 1a). The recombination rate (slope value of the graph of heterozygosity vs. gene distance from centromere) is highly species specific, and these values remain unaltered unless the recombination phenomenon is created by random gene rearrangements. In our previous publication, the normalized graph for the first hpESCs generated during SCNT was defined as $y = 0.0275x - 0.0789$, $R^2 = 0.9783$ [1]. Here, we report comparable values of $y = 0.022x + 0.0609$, $R^2 = 0.9776$, for the new dataset that includes the additional hpESC lines (Fig. 1b). Notably, these values are also comparable to the known human recombination frequency in oocytes. This indicates that the observed recombination pattern is not a random event, but rather a tightly controlled phenomenon dictated by the recombination frequency during human oocyte development.

In others' hands, interpretation of ambiguous imprinting data [4] has confounded the characterization of hpESCs generated during SCNT. We assert here that pESC recombination signatures are stable in their transmission, allowing reliable identification of pESCs; by contrast, epigenetic markers are inherently unstable and become variable with cellular time and culture conditions, and therefore are not faithful markers of pESCs during extended in vitro culture (passage number 35; our previous report [1]; and passage numbers 70 and 140 in a recent report by another group [4]). In addition, imprinted loci manifest gene expression patterns in a cell type-specific manner (eg, IGF2 is highly expressed in muscle); thus, comparing gene expression patterns between 2 different cell types (eg, pluripotent stem cells vs. somatic fibroblasts) or even between tissue types would not faithfully recapitulate the designated expression patterns of imprinting. Therefore, we believe that the use of recombination signatures by SNP analysis and genetic matching between donor tissue and SCNT-ESCs are the most reliable methods

FIG. 1. Single-nucleotide polymorphism (SNP) genotype data for human parthenogenetic embryonic stem cells (hpESCs). **(a)** SNPs of 6 hpESCs are depicted, except that the short p-arm of the human chromosomes projects superiorly, while the long q-arm projects inferiorly. Note pericentromeric regions of homozygosity for each chromosome. Conversion to homozygosity near telomeres is a reflection of the high frequency of double recombination in human chromosomes; **(b)** heterozygosity of SNP markers plotted against SNP marker distance from the centromere for the 6 cell lines. Slope function is indicated. Error bars represent standard deviation.

to distinguish between pESCs and SCNT-ESCs. Recently, human SCNT was performed by Noggle et al. [5], but generated only triploid SCNT-ESCs without enucleation of the oocyte chromosome. We focused our comparative analysis on the recombination signatures and the rates of hpESCs initially isolated from our accidental SCNT procedure and of hpESC lines subsequently generated by others. Once diploid human SCNT-ESCs are available to the scientific community, it will be important to perform additional comparative analyses with the hpESC lines to further confirm our findings.

Author Disclosure Statement

No competing financial interests exist.

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