—Mini Review—

Histocompatible Parthenogenetic Embryonic Stem Cells as a Potential Tissue Source for Regenerative Medicine

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Abstract: *Parthenogenesis is the process in which an oocyte develops into an embryo without fertilization. Parthenogenetic activation can be performed at various stages of meiosis, yielding embryos with distinct genetic patterns of homozygosity and heterozygosity. Parthenogenetic embryonic stem (pES) cells derived from such embryos have heterozygous patterns that can be identified using genome-wide single nucleotide polymorphism (SNP) analysis, to determine whether extrusion of the first or second polar body has been inhibited. Heterozygous pES cells carrying the full complement of major histocompatibility complex (MHC) antigens matched to the oocyte donor may provide a potential source of immune-matched cells and tissues for cell replacement therapy. In this review, we summarize the process of deriving heterozygous MHC-matched pES cells using mouse and human models.*

Key words: *Parthenogenesis, Embryonic Stem cells, Major histocompatibility complex antigen*

Introduction

Embryonic stem (ES) cells can be isolated from the inner cell mass (ICM) of blastocyst stage preimplantation embryos. These cells can self-renew and they retain the ability to differentiate into one or more specialized cell types *in vitro* and *in vivo*. Because of their unique properties, ES cells hold great promise for use in cell replacement therapy and in drug discovery. Creation of cell replacement products from ES cells that are genetically matched to patients' tissues is, however, hampered by immunological barriers created by the major histocompatibility complex (MHC; or human leukocyte antigen, HLA, in humans) antigen. Parthenogenesis and somatic cell nuclear transfer (SCNT) are two methods by which ES cells can be derived that are genetically matched to the oocyte donor and somatic cell donor, respectively. Several studies have demonstrated that autologous ES cells can be derived via SCNT in mice [1–3], monkeys [4, 5] and humans [6]; however, the technology used to obtain ES cell lines by SCNT is largely inefficient. Parthenogenesis provides a more efficient means of deriving MHC matched ES cell lines.

Parthenogenesis is the event by which an oocyte is activated to develop to the blastocyst stage without fertilization. Ovulated mature oocytes can be activated parthenogenetically by heat shock, electric pulses, or chemical inducement (e.g., calcimycin, strontium chloride); together with inhibition of microfilament production, this results in inhibition of polar body extrusion and a diploid oocyte. Derivation of parthenogenetic ES (pES) cell lines from these embryos was first demonstrated in the mouse in 1983 [7, 8], just 2 yr after the first establishment of mouse ES cell lines from fertilized blastocysts [9]. Mouse pES cells can be chimerized to nearly all tissues, but rarely to germ cells, when injected into mouse blastocyst embryos [10]. When injected into immunodeficient mice, pES cells generate teratomas, contributing to all three germ layers [11]. Mouse ES cells derived from fertilized embryos can develop into pups when they are injected into tetraploid blastocysts [12]. In contrast, although recently Kono *et al.* recently generated live-born pups from mouse parthenogenetic embryos, by altering imprinted genes via nuclear transfer [13], pES cells can only develop to the

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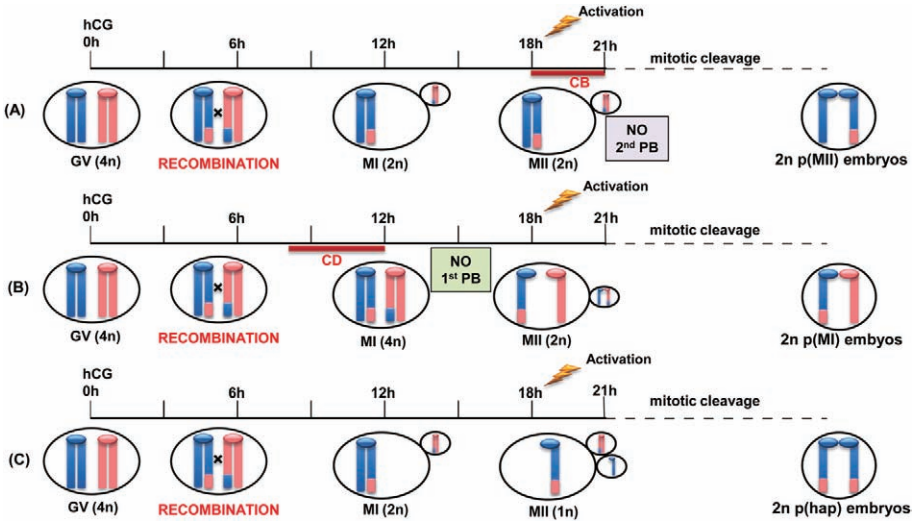

Fig. 1. Chromosome dynamics in oocytes during parthenogenetic activation at various stages of meiosis. (A) p(MII) embryos: MII-arrested oocytes are activated in the presence of cytochalasin B (CB) which blocks the second polar body extrusion, yielding p(MII) embryos with distal heterozygosity. (B) p(MI) embryos: MI oocytes are activated in the presence of cytochalasin D (CD) which blocks the first polar body extrusion, yielding p (MI) embryos with proximal heterozygosity. (C) p(hap) embryos: MII oocytes are activated in the absence of CB or CD, yielding haploid embryos. Homozygous embryos are produced when diploidization occurs during mitosis. hCG: time frame after hCG administration; GV: germinal vesicle oocyte; MI: MI stage oocyte; MII: MII stage oocyte.

early heart beating stage as a result of impaired paternal imprinted genes [14]. Parthenogenetic activation can be performed at meiosis I or meiosis II, which results in distinct patterns of homozygosity and heterozygosity in the resulting embryos [15]. The pattern of homozygosity and heterozygosity of pES cells derived from these embryos can be identified by genome-wide single nucleotide polymorphism (SNP) analysis, which determines whether the first or second polar body extrusion has been inhibited. Importantly, heterozygous pES cells that carry the full complement of MHC antigens of the oocyte donor may serve as a potential source of histocompatible cells or tissues for cell replacement therapy.

In this review, we summarize the mechanisms underlying the distinct heterozygous patterns of pES cells from embryos that have been activated parthenogenetically during the various stages of meiosis and describe the creation of histocompatible mouse pES cells derived from such embryos. Human pES cell lines have been established [16, 17] and similar mechanisms dictating heterozygosity have been observed in these cells [18]. Therefore, we anticipate the future use of human pES cells as a source of histocompatible cells and tissues for cell replacement therapy.

Derivation of p(MII) ES Cells

Immature oocytes arrested at the diplotene stage of meiosis I carry 20 sets of two homologous chromosomes (bivalents). During meiosis I, chromosome crossing over events occur distant from the centromere; then homologue segregation occurs and the paternal and maternal chromosomes segregate into the first polar body. Mature oocytes enter meiosis II and arrest at the metaphase stage until fertilization (MII oocytes). When MII oocytes are activated parthenogenetically and the second polar body extrusion is prevented by cytochalasin B, an inhibitor of microfilament production, the oocyte becomes diploid and develops to the blastocyst stage. pES cells derived from these blastocysts, called p(MII) ES cells, retain pericentric homozygosity with distal heterozygosity at specific loci (Fig. 1A). For example, based on the total length of chromosome 17 (83.10 cM) [19], the mouse H-2 MHC region is located on the proximal region of chromosome 17 (position 18.40–20.43 cM) [20]. Theoretically, the H-2 MHC region in p(MII) ES cells will be homozygous at either the paternal or maternal locus. To confirm this, we collected unfertilized MII oocytes from C57BL/6 × CBA F1 mice and activated parthenogenetic embryo

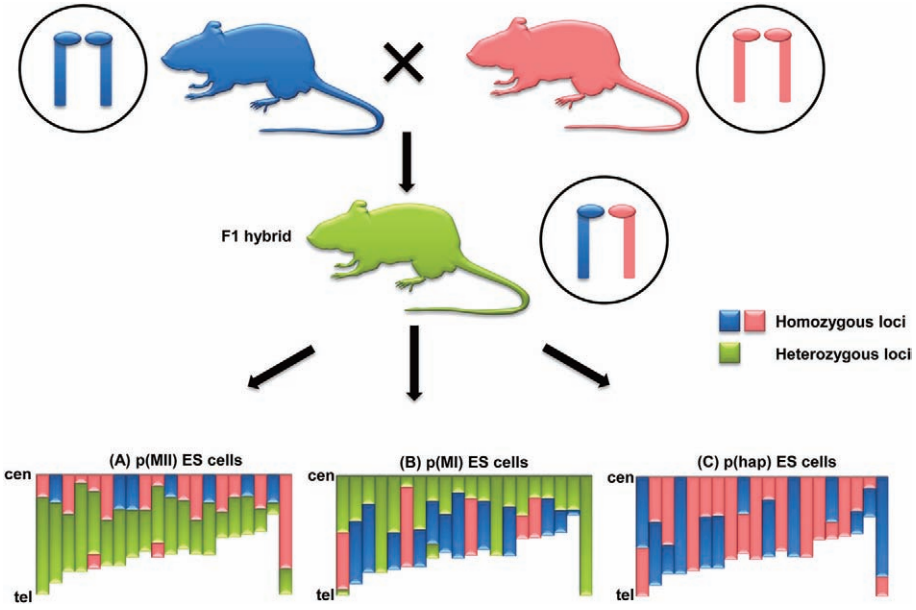

Fig. 2. Determination of homozygosity and heterozygosity by SNP analysis. (A) p(MII) ES cells: pericentromeric homozygosity with distal heterozygosity. (B) p(MI) ES cells: pericentromeric heterozygosity with distal homozygosity. (C) p(hap) ES cells: homozygosity at all loci, but retention of both parental genomes. cen: centromere; tel: telomere.

development by preventing the second polar body extrusion [15]. Of 72 p(MII) ES cell lines derived from these embryos, 24 lines (33%) had restored heterozygosity, confirmed by sequencing of SNPs within the H-2 loci. We selected 17 p(MII) ES cell lines for high-resolution genome-wide SNP analysis using a standard panel of 768 mouse markers spaced across the genome. This analysis revealed that p (MII) ES cells showed a high degree of heterozygosity, and the heterozygosity increased in proportion to the genetic distance from the centromere (Fig. 2A). Interestingly, the p(MII) ES cells showed a much lower degree of homozygosity than we anticipated, indicating that recombination events before meiosis II render the majority of loci in p(MII) ES cells heterozygous. When p(MII) ES cells were injected into immunodeficient mice, they were engrafted and formed teratomas. When these cells were injected into C57BL/6 × CBA F1 mice, p(MII) ES cells were engrafted, though not to C57BL/6 and CBA inbred mice. Thus, the p(MII) ES cells were completely immune matched to the oocyte donor.

Derivation of p(MI) ES Cells

If extrusion of the first polar body is inhibited by treatment with cytochalasin D after chromosome crossing over events in meiosis I during oocyte maturation, the parental chromosomes fail to segregate and the oocytes carry two pairs of parental chromosomes at the metaphase stage of meiosis II (tetraploid). When the oocytes are activated parthenogenetically, they then extrude the second polar body to become diploid. pES cells derived from embryos developed from such oocytes are called p(MI) ES cells. Because parental chromosome pairs fail to segregate, p(MI) ES cells retain pericentromeric heterozygosity and distal homozygosity at various loci (Fig. 1B). We found that 21 out of 23 p(MI) ES cell lines (91%) showed heterozygosity within the H-2 MHC loci according to SNP analysis [15]. Furthermore, p(MI) ES cells showed a predominant pattern of heterozygosity flanking the centromere, with distal telomeric regions of homozygosity (Fig. 2B). The engraftment pattern of p(MI) ES cells after injection into mice was same as that of p(MII) ES cells. Of note, p(MI) ES cells have a higher potential for the derivation of histocompatible cells and tissues for therapeutic applications, because they harbor a much higher frequency of heterozygosity than p(MII) ES cells,

Derivation of p(hap) ES Cells

Haploid embryos can be obtained when unfertilized mature oocytes are activated parthenogenetically and extrusion of the second polar body is allowed to occur.

pES cells derived from such embryos are called p(hap) ES cells. In an earlier study published by Kaufman *et al.* [8], p(hap) ES cells became diploid during early passage. Diploid p(hap) ES cells contain a duplicated set of haploid chromosomes with 2n DNA content and are homozygous at all loci (Fig. 1C, Fig. 2C). Recently Leeb and Wutz [21] reported the creation of p(hap) ES cell lines that maintain haploidy with the addition of mitogen-activated protein kinase and glycogen synthase kinase 3 inhibitors into the ES cell maintenance media, or by using knockout serum replacement as a substitute for FBS. However, they also observed the rapid diploidization of p(hap) ES cells upon differentiation. It will be important to explore the detailed mechanisms underlying the diploidization in p(hap) ES cells to determine their potential utility in tissue replacement therapy.

Derivation of Human pES Cells

Derivation of parthenogenetic human (h) pES cell lines by chemical activation of oocytes was first reported by Revazova *et al.* [16]. Subsequently, two additional groups claimed derivation of hpES cell lines using different methodologies, namely, a combination of chemical and electrical activation of oocytes [22], and spontaneous activation of oocytes [23]. Subsequently, Revazova *et al.* isolated homozygous HLA donor-matched hpES cell lines from human parthenogenetic blastocysts created via chemical activation [17]. The human ES cell line (SCNT-hES-1) which was originally claimed to be derived by SCNT [24], is reported to be an inadvertently derived parthenogenetic line. All of these lines were derived by inhibiting the second polar body extrusion, resulting in *in vitro* development of pMII blastocysts, at approximate efficiencies of 60% [16], 24% [17], and 25% [22] by various methods, and 7% by spontaneous activation of the oocyte [23]. Furthermore, hpMII ES cell lines were derived at a frequency of 6/11 [16], 8/18 [17], 2/4 [22], and 1/1 [23] from whole blastocysts, indicating that parthenogenetic activation of oocytes can result in significant yields of hpES cells. Derived hpES cells were determined to be histocompatible with the oocyte donors at all loci tested; to exhibit morphological resemblance to hES cell colonies; to express SSEA-3/4 and Oct4 markers of pluripotent cells, as well as high levels of alkaline phosphatase and telomerase activity; and to be able to form teratomas when injected into mice. The highly efficient isolation of phES cells that are immunogenically matched to the donor provides a major advantage for tissue matching to the oocyte donor and her siblings. Furthermore, they could be harnessed for generating a phES cell bank that could serve as a source of tissue that can be applied to various regenerative therapies. On average, a single *in vitro* fertilization (IVF) cycle can yield approximately 20 oocytes. Successful activation of 45% of these and a blastocyst derivation efficiency of 60% would yield approximately 6 blastocysts for derivation of phES cell lines [16], with 68.1% efficiency for generating heterozygote donor MHC-matched cells. The ease with which parthenogenetically derived hpES cell lines can be derived underscores their potential clinical usefulness as a source of histocompatible/MHC matched cells for tissue replacement therapy in humans.

Conclusion

In this review, we summarized the derivation of MHC-matched parthenogenetic mouse and human ES cells from embryos that are activated parthenogenetically at various stages of meiosis. p(MI) ES cells show a much higher frequency of heterozygosity than p(MII) ES cells, suggesting that p(MI) ES cells may be a better source of MHC-matched cells or tissues. According to the HLA matching results reported by Nakajima *et al.* [25], if parthenogenetic human ES cell lines are established from 55 randomly selected oocyte donors, 80% of patients would be expected to find at least one donor with complete matching at all three HLA loci in the Japanese population. This estimate is higher than that of Taylor *et al.* [26], who used HLA data from cadaveric organ donors and kidney transplantation waiting lists in the United Kingdom. A likely reason for this difference is that the Japanese population shows relatively low ethnic diversity compared to that of the UK. The derivation of human pES cells may reduce the number of ES cell lines required for the matching of HLA loci, and human HLA-matched pES cells may have high potential for therapeutic use in regenerative medicine, if they are proven to be safe.

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