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Histocompatible parthenogenetic embryonic stem cells as a potential source for regenerative medicine

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Abstract

Parthenogenesis is the process by which an oocyte develops into an embryo without fertilization. Parthenogenetic activation can be performed at various stages of meiosis, yielding embryos with a distinct genetic pattern of homozygosity and heterozygosity. The heterozygosity pattern specific to parthenogenetic embryonic stem (pES) cells derived from such embryos, can be predicted using genome-wide single nucleotide polymorphism (SNP) analysis to determine whether extrusion of the first or second polar body is prohibited. The heterozygous pES cells carrying the full complement of major histocompatibility complex (MHC) antigen matched to the oocyte donor, could therefore provide a potential source of MHC matched cells or tissue for cell replacement therapy. In this review, we summarized the mechanism of deriving heterozygous MHC-matched pES cells using a mouse and human models.

Introduction

Embryonic stem (ES) cells can be isolated from inner cell mass (ICM) of blastocyst stage preimplantation embryos. They can self renewal and retain the ability to differentiate into one or more specialized cell types in vitro and vivo. Because of their unique properties, ES cells hold great promise for cell replacement therapy and drug discovery. Creation of cell replacement products from ES cells that are genetically matched to the patients', however, is hampered by immunological barriers defined by major histocompatibility complex (MHC, Human leukocyte antigen: HLA in human) antigen. Parthenogenesis and somatic nuclear transfer (SCNT) are two methods so far for deriving ES cells which are genetically matched to the oocyte donor and somatic cell donor, respectively. Several studies demonstrated that autologous ES cells can be derived via SCNT in mouse [1-3], monkey [4,5] and human [6], albeit the technology used to obtain ES cell lines by SCNT remains largely inefficient.

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Therefore, parthenogenesis provides an additional means for deriving MHC matched ES cell lines.

Parthenogenesis is the event by which the oocyte develops 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) and together with inhibition of microfilament production, this results in prohibition of polar body extrusion and the oocyte now becomes diploid. Deriving parthenogenetic ES cell (pES) lines was first demonstrated in mouse in 1983 [7, 8] which was just 2 years 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 all three germ layers [11]. Mouse ES cells derived from fertilized embryos can develop into pups when they are injected into tetraploid blastocysts [12]. Although, recently Kono et al generated live-born pups from mouse parthenogenetic embryos by altering imprinted genes via nuclear transfer [13], pES cells can only develop to the early heart beating stage as a result of impaired paternal imprinted genes [14].

Parthenogenetic activation can be performed at meiosis I or meiosis II describing the distinct patterns of homozygosity and heterozygosity in 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 to determine whether the first or second polar body extrusion is prohibited. Heterozygous pES cells carry the full complement of MHC antigen of the oocyte donor could thus serve as a potential source of histocompatible cells or tissues for cell replacement therapy.

In this review, we summarize the mechanism for the distinct patterns of embryos activated parthenogenetically during various stage of meiosis and the creation of histocompatible mouse pES cells derived from such embryos. Human pES cell lines have been established [16, 17] and a similar mechanism has been observed in human pES cells [18]. Therefore we anticipate the future possibility of the use of human pES cells for histocompatible cells and tissue derivation for cell replacement therapy.

Derivation of p(MII) ES cells

Immature oocytes arrested at diplotene of the first meiosis carry 20 sets of two homologous chromosomes (i.e. bivalents). During the first meiosis, chromosome crossing over events occur at a distant from centromere, subsequently homologue segregation occurs and the paternal or maternal chromosomes segregate into the first polar body. Oocytes then begin to enter the second meiosis and arrest at metaphase of meiosis II until fertilization (MII oocytes). When MII oocytes are activated parthenogenetically and together with prevention of the second polar body extrusion by cytochalasin B, an inhibitor of microfilament production, the reconstructed embryos become diploid embryos and develop to the blastocyst. pES cells derived from these blastocysts – termed p(MII) ES cells, retain pericentric homozygosity with distal heterozygosity at loci (Fig 1A). Based on the total length of chromosome 17 (83.10 cM) [19], mouse H-2 MHC region are located on proximal

region of chromosome 17 (position 18.40-20.43 cM) [20]. Therefore, theoretically, the H-2 MHC region in p(MII) ES cells is homozygous at either paternal or maternal loci. We [15] collected unfertilized MII oocytes from C57BL/6 $\times$ CBA F1 mice and proceeded to activate parthenogenetic embryo development by preventing of second polar body extrusion. Of 72 p(MII) ES cell lines derived from those embryos, surprisingly 24 p(MII) ES cell lines (33%) were confirmed to have restored heterozygosity, by sequencing of SNPs within the H-2 loci. Moreover, we took 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 genotype analysis revealed that p(MII) ES cells showed a high degree of heterozygosity and that heterozygosity increased in proportion to the genetic distance from centromere (Fig 2A). Interestingly, p(MII) ES cells showed a much lower degree of homozygosity than we anticipated indicating that recombination event before meiosis II render the majority of loci in p(MII) ES cells heterozygous. When p(MII) ES cells are injected to immunodeficient mice, they were engrafted and formed teratomas. And when injected into C57BL/6 $\times$ CBA F1 mice, p(MII) ES cells were engrafted, although they were not engrafted to C57BL/6 and CBA inbred mice. Taken together, those p(MII) ES cells were completely matched to the oocyte donor.

Derivation of p(MI) ES cells

When extrusion of the first polar body is inhibited by treatment with cytochalasin D, after chromosome crossing events in meiosis I during oocyte maturation, parental chromosomes fail to segregate and oocytes carry two pairs of parental chromosomes at metaphase at meiosis II. These oocytes are then activated parthenogenetically, and induced to extrude the second polar body to become diploid. pES cells derived from embryos developed from such oocytes are termed p(MI) ES cells. Because parental chromosome pairs fail to segregate, p(MI) ES cells retain pericentromeric heterozygosity and distal homozygosity at loci (Fig 1B). We [15] confirmed that 21 p(MI) ES cell lines out of 23 p(MI) ES cell lines (91%) showed heterozygosity within H-2 MHC loci by SNP analysis. And 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 injecting to the mice was same as p(MII) ES cells. p(MI) ES cells harbor the much higher frequency of heterozygosity than p(MII) ES cells suggesting that p(MI) ES cells hold higher possibilities for deriving therapeutic histocompatible cells and tissues.

Derivation of p(hap) ES cells

Haploid embryos can be obtained when unfertilized mature oocytes are activated parthenogenetically along with the induction of second polar body extrusion. 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 become 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 which maintain haploidy by adding mitogen activated protein kinase and glycogen synthase kinase 3 inhibitors in ES cell maintenance media, or by using knockout serum replacement for the substitution of FBS, albeit they also observed the rapid

diploidization of p(hap) ES cells when differentiated. It is interesting to explore the detailed mechanisms for the occurrence of dioloidization in p(hap) ES cells.

Derivation of human pES cells

Derivation of human parthenogenetic (h)pES lines by chemical activation of oocytes was first reported by Revazova et al. [16]. Subsequently two more groups claimed derivation of hpES lines, [24, 25] by different methodologies including, a combination of chemical and electrical activation [24], and spontaneous activation [25] of oocytes. A year later the initial group isolated homozygous HLA donor matched hpES cell lines from human parthenogenetic blastocysts created via chemical activation [17]. Additionally the human ES line (SCNT-hES-1), claimed to be derived by SNCT, is reported to be an inadvertently derived parthenogenetic line. All 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% [24][26] by various methods, and 7% by spontaneous activation [25] of the oocyte. Furthermore, hpMII ES cell lines were derived at a frequency of 6/11 [16], 8/18 [17], 2/4 [24], and 1/1 [25] from whole blastocysts, indicating that parthenogenetic activation of oocytes can result in significant yields of hpES cells. Derived hpESCs were determined to be histocompatible with the oocyte donors at all loci tested, exhibited morphological resemblance to hESC colonies, and express SSEA-3/4 and Oct4 markers of pluripotent cells, as well as high levels of alkaline phosphatase and telomerase activity, and finally are able to form teratomas when injected into mice. Thus parthenogenetically derived human pES lines can be a potent source to generate histocompatible/MHC matched cells and tissues for replacement therapies in humans.

Conclusion

In this review, we summarize the derivation of MHC matched parthenogenetic mouse and human ES cells from embryos which are activated parthenogenetically at various stage of meiosis. p(MI) ES cells showed the much higher frequency of heterozygosity than p(MII) ES cells suggesting that p(MI) ES cells could be a better source of MHC matched cells or tissues. According to the HLA matching results reported by Nakajima et al. [22], if parthenogenetic human ES cell lines are established from 55 randomly selected oocyte donors, 80% of patients are expected to find at least one donor with complete matching at all three HLA loci in the Japanese population. This estimation holds better probability compared to Taylor et al. [23] in which they used HLA data from cadaveric organ donors and kidney transplantation waiting lists in United Kingdom because Japanese population shows relatively low ethnic diversity. Human pES cells could reduce the number of ES cell lines required for the matching of HLA loci. Human HLA matched pES cells may hold the therapeutic potential of regenerative medicine if they are proven to be safe and useful.

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Figure 1.

Diagram of chromosome dynamics of oocytes during parthenogenetic activation. (A) p(MII) embryos: MII arrested oocytes are activated in the presence of cytochalasin B (CB) to block the 2nd 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) to block the 1st 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. The homozygous embryos can be obtained when diploidization occurred during mitosis. hCG: Time frame after hCG administration. GV: Germinal Vesicle Oocyte, MI: MI stage oocyte. MII: MII stage oocyte.

Figure 2. Diagram 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 retain both parental genome. cen: centromere. tel: telomere