


DIFFERENTIATION OF GERM CELLS FROM STEM CELLS- A REVIEW

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ABSTRACT

Embryonic stem (ES) cells possess unlimited self-renewal capacity and developmental potential to differentiate into virtually any cell type of an organism. ES cells can differentiate under appropriate in vitro and in vivo conditions into different cell types. Embryonic stem cell-derived gametes could find several applications in animal breeding and assisted reproductive technology. One of the most common causes of male infertility is abnormal germ cell differentiation leading to azoospermia or oligospermia. In females, the most common cause of infertility involves ovulatory dysfunction. The emergence of techniques allowing the in vitro derivation of both germ cells and mature gametes will allow investigations into processes guiding germ cell formation and into academic and therapeutic uses for these fascinating cells. Producing germ cells in vitro would open important new avenues for regenerative medicine, and obtaining alternative sources of pluripotent stem cells is desirable. Advances in stem cell research have opened new perspectives for regenerative and reproductive medicine.

Key word: Embryonic stem cell, Differentiation, Germ cell, Regenerative, Infertility

The ability to generate functional haploid germ cells acts as a yardstick to measure reproductive performance of mammals. One of the most common causes of male infertility is abnormal germ cell differentiation leading to azoospermia or oligospermia. In females, the most common cause of infertility involves ovulatory dysfunction. Evaluating the molecular events involved in establishing the oocyte reserve and formation of spermatogonial stem cells is challenging because these events are completed before birth. Therefore, understanding early germ cell differentiation requires the establishment and validation of new and innovative laboratory models that begin at the embryonic stage. One of the most promising, yet still emerging technologies aimed at directly addressing germ cell differentiation involves the use of pluripotent stem cells to generate germ cells in vitro.

Stem cells (SC) are undifferentiated cells that have the potential to self-replicate and give rise to specialized cells. These include cells lost from normal turnover or from a disease in specific organs and tissues, reflecting on their possible use in future therapies. SCs can be obtained from the embryo at cleavage or blastocyst stages but also from extra-embryonic tissues such as the umbilical cord blood obtained at birth (McGuckin et al., 2005), the placenta (Miki et al., 2005) and the amniotic fluid (De Coppi et al., 2007).

In vivo development of primordial germ cells

Germ cells hold a unique place in the life cycle of mammal as they carry the genome onto the next generation (McLaren 2003). In mammals, gametes are derived from a small founder pluripotent population, known as primordial germ cells (PGCs). Germ cell specification in mammals takes place at the onset of gastrulation, after implantation of the embryo. In mouse, PGCs arise from the proximal epiblast, a region of the early mammalian embryo that also contributes to the first blood lineages of the embryonic yolk sac (Ginsburg et al., 1990). Bone morphogenic protein (BMP-4 and BMP-8b) a soluble

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growth factor belonging to the transforming growth factor super family, produced by extraembryonic ectoderm are required for the generation of PGCs from epiblast cells (Lawson, et al., 1999).

In vitro germ cells differentiation from ESCs

ESCs have been defined as pluripotent as they differentiate along somatic cell lineages but were not believed to form either trophoblast or germline cells. In the last few years it has become clear that murine ESCs can develop into PGCs in vitro (Hubner et al., 2003; Geijsen et al., 2004; Payer et al., 2006). Further culture may occasionally form early spermatids or oogonia, which form follicle or ovarian-like structures and oocytes (i.e. having morphological similarities to follicles and gametes from gonads) that subsequently develop to blastocyst-like structures, presumably due to parthenogenetic activation (Hubner et al., 2003; Geijsen et al., 2004; Lacham-Kaplan et al., 2006). Some biological functions of mESC-derived spermatids were tested in vitro using ESCs initially carrying an Oct 4 promoter-driven GFP reporter gene, which enabled the tracking of these cells (Hubner et al., 2003; Geijsen et al., 2004). When haploid spermatids generated from these GFP–ESCs were isolated by flow cytometry and injected intracytoplasmically into oocytes, the diploid chromosome complement was restored and green fluorescent blastocysts generated.

Toyooka et al., (2003) showed that Mvh-LacZ murine germ cells (transgene with germ cell-specific mouse vasahomolog and LacZ reporter) generated in vitro from mESCs could also develop to some extent in vivo. To achieve this, cells were aggregated with day 12.5–13.5 (d.p.c) gonadal tissue, which formed seminiferous tubules (but separate from host tubules) within 6–8 weeks when co-transplanted under the testis capsule. These tubules participated in spermatogenesis to produce elongated α-gal-stained spermatids. This demonstrated that such mvh-lacZ ESCs formed PGCs with developmental potential to integrate into a somatic epithelium and undergo meiosis to form early sperm cells.

There had remained considerable uncertainty as to the biological fidelity of these primordial germ cell, but Nayernia et al., (2006a, 2006b) recently produced viable transgenic offspring from sperm produced from mESCs using a novel two-stage culture system. First, mESCs were transfected with the reporter gene Stra8-EGFP, a retinoic acid (RA)-responsive gene expressed in pre-meiotic mouse germ cells and fused with enhanced green fluorescent protein gene construct. Following ESC culture, green cells expressing the gene and therefore presumably marking pre-meiotic germ cells were isolated by fluorescent activated flow cytometry and cultured initially in the presence of RA as a proliferative cell line. Subsequently, the cells were transfected with a second fusion reporter gene Prm1-dsred (protamine1 gene fused to red fluorescent protein gene construct) to identity post-meiotic sperm cells. After further RA induction for a few days, red post-meiotic sperm cells were isolated and microinjected into mouse eggs to generate viable offspring although these exhibited abnormalities and died prematurely (5 days to 5 months). While investigations with hESCs are more preliminary, it has been shown that spontaneous or induced differentiation in culture can generate cells displaying mRNA expression profiles and cell surface markers consistent with PGCs (Clark et al., 2004; Clark & Reijo Pera, 2006) and of subsequent meiosis.

Adult stem cell, like porcine skin stem cell can be differentiated into oocyte like structure (Dyce et al., 2006). But now days, induced pluripotent stem cell (iPS) can be differentiated in to germ cells. Human iPS cell can be differentiated into germ cell and its derivation is significantly improved by co-culture with human fetal gonadal cells (Park et al., 2009). Similarly, mouse iPS cells derived from adult hepatocytes were able to differentiate into presumptive germ cells marked by mouse vasa homolog (Mvh) expression in feeder-free or suspension cultures (Imamura et al., 2010).

Germ cell specific markers
a) B-lymphocyte induced maturation protein-1 (Blimp1)

Blimp1 is expressed at E6.25 in the first cell layer of the posterior proximal epiblast. Blimp1 is thought to be the earliest gene to identify PGCs in the embryo (Ohinata et al., 2005; Vincent et al., 2005). In PGCs, Blimp1 functions by forming a complex with the arginine methyltransferase Prmt5. This complex is believed to be important in germ cell specific epigenetic programming (Ancelin et al., 2006). Blimp1 expression in the embryo is detected
before gastrulation, at E6.25, in about four to eight cells located in a single layer in the most proximal epiblast cells (Table 1; Raz, 2005). Expression of Blimp1 persists in PGCs as they migrate towards the genital ridges (Chang and Calame, 2002).

b) Fragilis

Fragilis is a member of the interferon-induced transmembrane protein gene family (Saitou et al., 2002). Three of the members known as fragilis, fragilis2 and fragilis3, appear to be associated with germ cells, while the remaining two members, fragilis4 and fragilis5, are not detected within germ cells (Lange et al., 2003). Fragilis appears to be expressed in response to BMP4 signaling from the extra-embryonic ectoderm (Saitou et al., 2002). Fragilis expression occurs early in development, being detected throughout the epiblast in E6.0 embryos (Table 1). The expression of the marker becomes restricted at E6.5 to a cluster of cells at the posterior end of the embryo. The expression of fragilis in PGCs at this time is believed to be important for the migration of PGCs towards the genital ridges.

c) Stella

The Stella gene codes for a protein of 150 amino acids that is detected in both the nucleus and the cytoplasm of pre-implantation embryos in addition to the germ line (Saitou et al., 2002). The protein possesses a nuclear export signal, allowing it to shuttle between the nucleus and the cytoplasm. In addition, the Stella protein is highly basic, allowing it to bind to both RNA and DNA (Aravind and Koonin, 2000). Stella is inherited as a maternal factor and is thus detected in oocytes and in zygotes (Payer et al., 2003). At the early morula stage, the maternally inherited Stella is degraded and replaced by the onset of zygotic expression. Stella expression persists until the blastocyst stage after which it is down regulated and doesn’t appear again until approximately E7.0 in 36–43 cells showing high fragilis expression (Saitou et al., 2002). It continues to mark PGCs as they migrate through the hindgut at E8.5, and remains expressed in the germ line until about E13.5 in the female and E15.5 in the male (Table 1; Saitou et al., 2002).

d) Mouse vasa homologue (Mvh)

The mouse vasa homologue gene encodes an ATP-dependant RNA helicase of the DEADbox protein family (Hay et al., 1990). Mvh expression has not been detected in any tissue or embryonic cells and appears to be exclusive to germ cells (Toyooka et al., 2000). In PGCs, Mvh expression is first expressed post migration, when PGCs have colonised the genital ridges, from E10.5–E12.5 (Table 1). It appears that a germ-soma interaction is necessary for Mvh expression. Its expression continues in germ cells until the spermatogenic cell and maturing oocyte stages in adult mice (Toyooka et al., 2000). In the female, Mvh protein is located in the cytoplasm of developing oocytes. As the follicle matures, there is a decrease in Mvh until it is undetectable in mature oocytes (Toyooka et al., 2000). In the male, Mvh appears to be closely associated with chromatoid bodies of spermatocytes and spermatids.
**e) Deleted azoospermia like (Dazl)**

Dazl, a member of the DAZ family of proteins, is known to bind RNA and participate in translation of bound RNA by the recruitment of 80S ribosomes (Collier et al., 2005). Specific to the germ cell lineage, Dazl is first expressed in mice at E11.5 in post-migratory PGCs (Table 1; Seligman and Page, 1998). In males, Dazl is situated in the cytoplasm of spermatogonia, pre-leptotene and zygote spermatocytes, with the highest expression being found in pachytene spermatocytes (Ruggiu et al., 1997).

**f) Germ cell nuclear antigen (Gcna1)**

Germ cell nuclear antigen 1 is a nuclear antigen expressed in both male and female germ cells (Enders and May 1994). Gcna1 is first expressed in post-migratory PGCs (E10.5–E11.5), once they have entered the genital ridge (Table 1). Gcna1 continues to be present for the remaining embryonic period. In females, Gcna1 remains in oocytes until 14 days post-partum, where it is no longer detected (Enders and May 1994). In males, Gcna1 is detected in spermatogonia, leptotene and zygote spermatocytes and spermatids. However more mature sperm located in the vas deferens and epididymis are not positive for Gcna1 expression (Enders and May 1994). Gcna1 appears to be very specific to germ cells. It has not been detected in any somatic cells tested to date; however, it has been detected in embryonic carcinoma cells.

**Implication**

The emergence of techniques allowing the in vitro derivation of both germ cells and mature gametes will allow investigations into processes guiding germ cell formation and into academic and therapeutic uses for these fascinating cells. Embryonic stem cell–derived gametes could find several applications in animal breeding and assisted reproductive technology. Additionally, ESC-derived gametes and germ cells may provide an important culture system to examine the roles of key genes and complex signaling processes involved in genomic imprinting and reductive division during meiosis. Sperm derived from hESCs may also perhaps be used to treat male infertility. In addition, if male germ cells derived from ESCs can engraft in host testes and produce viable sperm; these techniques could provide insight into spermatogenesis and possibly provide new treatments for male infertility. The use of primordial germ cells (PGCs) and germline stem cells (GSCs) in transplantation studies to restore fertility has been initiated, with varied degrees of success (Parks et al., 2003; Nayernia et al., 2004). Indeed, past studies have shown that testis cell transplants can functionally engraft in the testes of infertile recipients (Brinster and Zimmermann, 1994; Brinster and Avarbock, 1994; Brinster, 2002.), and preliminary work with germ cells derived from mESCs suggests that these cells exhibit the same potential (Nayernia et al., 2006).

A limitless supply of human eggs derived from hESC lines could have a radical impact on medicine. An ability to efficiently develop ESC-derived oocytes for nuclear transfer studies would be a significant advance and may provide a limitless source of oocytes. If these oocytes provide all the cues necessary to allow reprogramming of donor nuclei and successfully develop to the blastocyst stage, patient-specific hESC lines resulting from nuclear transfer could be created, helping circumvent the major obstacle of donor oocyte availability in the construction of patient-specific hESC lines by nuclear transfer.

Further studies that incorporate the use of reporter constructs may also be required to enrich and isolate putative haploid gametes from differentiating hESC cultures. At this point in time hESCs definitely seem to support the early stages of germ lineage formation in cell culture. However, more work needs to be done to develop more specific culture conditions for the later stages of gamete formation, so as to generate functional haploid gametes.

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