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SURVEY

ON THE ORIGIN OF PLURIPOTENT EMBRYONIC STEM CELLS IN THE MOUSE

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Abstract. Understanding the nature of embryonic stem (ES) cells is of great importance, because it may help us to identify the core regulatory gene networks which are essential for the long-term maintenance of the pluripotent state. For a long time developmental biologists discussed the *in vivo* equivalent to the ES cells. In mouse, remarkable similarity of ES cells to primordium germ (PG) cells was noted by several authors. Nonetheless a firm link between PG and ES cells generation has not been demonstrated. Several lines of evidence suggest that both PG and ES cells have in common many molecular markers of pluripotency and, under appropriate culture conditions, PG and ES cells are interconvertible. During development of mouse embryo there is a window of opportunity when a population of pluripotent cells may give rise to ES cells under appropriate culture conditions. Here, we review the current data concerning the origin of ES cells during the early development in the mouse.

Keywords: embryonic stem cells, epiblast, germ line, pluripotency, primordial germ cells.

1. Introduction

Pluripotent stem cell biology now is a vigorously growing research area. Embryonic stem (ES) cells are considered as the golden standard of genuine pluripotency, self-renewal, and differentiation. Formation and differentiation of a pluripotent cell population is central to mammalian development. Derivation of ES cells has given an impetus to the study of early stages of embryonic development itself. Elucidating the origin of ES cells is of importance, because it may help us to identify expression patterns of critical transcription factors that are essential for the long-term maintenance of the pluripotent state. ES cells have been used to study the mechanisms of cell differentiation *in vitro*. However, refined analysis is needed to demonstrate how accurately ES cell differentiation reflects events that normally occur *in vivo*. Human ES cells offer insights into early developmental events that cannot be studied directly in the intact human embryo. The ability to restore pluripotency to somatic cells through the ectopic

co-expression of reprogramming factors has created new powerful opportunities for modelling human diseases and offers hope for personalized regenerative cell therapies. The ES cells immortality, rapid growth *in vitro*, and the capacity to generate all cell lineages of the developing and adult organism make them the most ideal candidate for regenerative medicine. The biomedical impact of cultured pluripotent ES cells requires elucidation of their precise nature and relationship to pluripotent cells in organisms.

2. Preimplantation development of the mouse embryo

In mammals, the period of preimplantation development extends from egg fertilization to the implantation of the blastocyst. The fertilized egg first undergoes a series of early cleavage divisions, producing equipotent blastomeres, which get smaller and smaller without changing the overall size of the embryo. In the mouse, the first cleavage occurs about 24 hours after fertilization and the second and the third cleavages follow at intervals of about 12 hours. Differentiation of blastomeres begins at the eight-cell stage with the onset of polarization of the outside cells. During the 8 – 16 cell stage transition the blastomeres become tightly attached and the embryo adopts a spherical shape. This process is known as compaction. During the first differentiation event, blastomeres segregate into two cell lineages: the outside polar cells develop into an epithelial layer, the trophoblast, whereas the inner cells remain apolar and produce a clump of cells known as inner cell mass (ICM) [31]. The embryo is called a morula from compaction until about the 32-cell stage. During this period a fluid-filled blastocoel begins to form in the interior. The cavity expands the embryo into a blastocyst. At around the 64 cell stage embryo about one-quarter of the cells are found in the ICM and three-quarters in trophectoderm. Until full spatial segregation of trophoblast and ICM, cells can be relocated and will acquire the characteristics of cells in the new position [84].

From embryonic day 3.5 to 4.5 the ICM cells allocate to either epiblast or hypoblast (primitive endoderm). Hypoblast cells form a layer on the blastocoelic surface of the ICM and then contribute to the extraembryonic tissues. Pluripotent cells segregate to the other subcompartment, the epiblast. After implantation the epiblast progressively differentiates into definitive mesoderm, endoderm, and ectoderm and germ line, and these cells also form the amnion ectoderm and all the extraembryonic mesoderm [25]. The cells of epiblast proliferate with cell cycles as short as 5 hours and expand from about 25 cells at E 4.5 to 660 cells by E 6.5 [79]. At E 6.5, a population of cells situated posterior to the primitive streak in the extraembryonic mesoderm can be identified as precursors of primordial germ cells [39].

In an embryological context, the mammalian zygote and blastomeres are often thought to be totipotent, because they can give rise to an entire organism. However, the mammalian egg is a highly specialized and restricted cell. It is programmed to undergo a stereotyped process of cleavage divisions that accomplish the oocyte-to-embryo transition. The zygote itself does not have the ability to differentiate into all cell types. In fact, the mammalian zygote follows a

determined program of restricted differentiation. Dissociated blastomeres also divide and differentiate on schedule to form trophoblast vesicles or microblastocysts with as few as 2 cells [51, 84]. Thus, the egg and blastomeres produce directly only two cell types, the trophoblast and the ICM. Developmental potential is unlocked through formation of the pluripotent epiblast cells which acquire the capacity to generate other cell types and to do so in a flexible manner [23, 51]. This property of pluripotency naturally occurs in the epiblast, a transient tissue that persists for only a few days. *In vitro*, however, pluripotency can be maintained indefinitely through derivation of stem cell lines.

3. Embryonic stem cells

ES cells are permanent pluripotent stem cell lines established *in vitro* from pre-implantation embryos. The first pluripotent ES cell lines were isolated directly from *in vitro* culture of epiblast of delayed-implantation blastocysts [21] or from isolated ICM of late blastocysts cultured in medium conditioned by an established teratocarcinoma stem cell line [43]. During delayed implantation, the blastocyst is metabolically dormant and can maintain this state for days or even weeks. Careful study of mouse embryos conclusively showed that ES cells originate from the early epiblast after its segregation from the hypoblast. Isolated epiblst from the mouse gives rise to ES cell lines at a higher frequency than does isolated ICM. Moreover, the culture of epiblast from delayed-implanting blstocysts allows the isolation of ES cell lines from mouse strains that have been previously refractory to ES cell isolation. ES cell lines can be derived from single isolated epiblast cells what is not possible with ICM cells [24].

For many years pluripotent mouse ES cells were maintained in culture by using various empirical combinations of feeder cells, conditioned media, cytokines, hormones and serum, in particular leukemia inhibitory factor (LIF) and bone morphogenetic protein 4 (BMP4). The self-renewal of ES cells depends on the effect of LIF cytokine, which acts via the gp130 receptor [97, 100]. The gp130 signaling is essential for prolongation of epiblast lifespan during diapause, when cells can be maintained in the naïve state if embryogenesis is halted prior to implantation, what is commonly required of mouse embryos in the wild. The main function of gp130 pathway in the diapause cells is to suppress apoptosis and inappropriate differentiation [24, 49, 51]. It is remarkable that ES cells were first derived from blastocysts in diapause, and this generally appears to enhance the efficiency of ES cells generation [21]. The gp130 signaling proved to be the key to ES cells self-renewal and sustaining pluripotency *ex vivo*.

Then, it was found that ES cells have an innate program for self-replication that does not require extrinsic stimuli for the maintenance of pluripotency. To maintain self-renewal of ES cells and suppress residual differentiation it is sufficient to eliminate the differentiation-inducing signaling produced by mitogen-activated protein kinase and glycogen synthase kinase 3 [98]. Neutralizing this autoinductive pathway can preserve an uncommitted ground state. Oct-4 and SOX2 direct production of a destabilizing signal fibroblast growth factor 4 (FGF4), which drives ES cells toward differentiation.

The FGF4/Erk signal does not specify lineage but renders ES cells responsive to further inductive signals. Consequently, ES cells and epiblast cells lacking FGF4 or deficient in downstream mitogen-activated protein kinase Erk1/2 signaling exhibit a general impairment of commitment. Inhibition of Erk activity promotes ES cell self-renewal [7] and also improves the efficiency of ES cell derivation from embryos of non-129 strains of mice [6].

In culture, ES cells grow in colonies as round shaped tightly packed cells with smooth membrane surface, rounded nuclei and scarce cytoplasm containing a few organelles and some amount of fat vacuoles and pools of glycogen particles. ES cells are characterized by rapid cell cycling, short gap phases and an unusually high proportion of the S-phase (~65%) when compared with the G1 phase (~15%). Cell cycle time in rodent ES cells was estimated at 8-10h and is assumed to be similar to that of peri-implantation embryos [81]. Remarkably, ES cells are small in size when compared with somatic cells, a feature that is often attributed to a shortened period of growth in the truncated G1 phase [76]. Upon differentiation, the cell cycle is restructured such that approximately 40% of an asynchronously dividing population of cells is found in the G1 phase [90].

In cycling ES cells, p53 checkpoint pathways are compromised. The p53-mediated checkpoint controls cell functioning when cell differentiation occurs [1]. In contrast to other primary cultures, ES cell cultures show no evidence of crisis, whereas primary somatic cells lose their proliferative potential after a limited number of cell divisions.

ES cells can multiply in the absence of serum and are not subject to contact inhibition or anchorage dependence. These features are typical of transformed cells and, indeed, ES cells are tumorigenic. While they participate in embryogenesis when introduced into the blastocyst, they produce teratomas when injected into adult mice. Thus ES cells can be considered as conditional tumor cells [7]**.**

Remarkably, the state of ES cells is not identical to the state of immortalized adult cells [36]. There is no known means of inducing cell-cycle arrest and quiescence in ES cells, because suppression of proliferation results in differentiation of ES cells. It was suggested that ES cells continue to self-renew because of a self-organizing network of transcription factors that control epigenetic processes, prevent differentiation and promote proliferation of ES cells [55]. A cardinal feature of all authentic ES cells is the ability to self-renew that is to divide symmetrically without differentiation and to produce identical progeny. It was shown that clonally derived ES cell lines demonstrate pluripotency which is maintained during an extended period of culture.

In culture, ES cells are exposed to numerous foreign signals to which cells are never exposed *in vivo*. It seems that cells identical to ES cells never exist *in vivo*. Rather, ES cells, captured in pluripotent state, may be considered, in a sense, a tissue culture artifact, which arises through selection and adaptation of cells to the culture environment [6, 24, 97]. However, the ability of ES cells to re-enter embryonic development and contribute to the formation of chimaeras indicates that any adaptation to culture conditions is fully and rapidly reversible. When

introduced into blastocyst, ES cells intimately associate with ICM, proliferate and colonize all the tissues of the host [3].

In ES cultures, phenotypically "undifferentiated" cells may consist of a heterogeneous population of functionally distinct cell types. ES cells have an innate programme for self-replication that does not require extrinsic instruction [98]. Mouse ES cells grown in serum exhibit greater heterogeneity in morphology and expression of pluripotency factors than ES cells cultured in defined medium with inhibitors of two kinases (Mek and GSK3), nonetheless both kinds of ES cells have similar differentiation potential [41]. Spontaneous differentiation occurs continually even in the presence of high concentrations of LIF, and the loss of pluripotency might occur well before any discernible change in cell morphology [8, 78]. Hence, pluripotent stem cells may constitute only a minor proportion of the total population of morphologically undifferentiated cells in ES cell cultures. In culture, ES cells differentiate to the derivatives of all three germ layers and mimic some aspects of organogenesis when grown as aggregates in suspension to form embryoid bodies. ES cells may be induced to differentiate into over 200 cell types in response to developmental cues. However, it is not always clear how accurately ES cell differentiation reflects events that normally occur *in vivo*. Importantly, differentiation of ES cells into embryoid bodies or teratomas is spontaneous and uncontrolled. In culture, the first step toward achieving the directed differentiation of ES cells is the use of different growth factors [40, 71]. However, none of the examined factors exclusively directs differentiation of ES cells to only one cell type, but rather alters the relative proportions of a specific cell type. Therefore, to obtain a uniform population of differentiated ES cells the selection of specific cells from a heterogeneous cell population is needed.

4. The pluripotent state of ES cells

Early epiblast cells and ES cells represent pluripotent state. The epiblast is a transient tissue maintained for only a short time interval, however in the wild, mouse embryos commonly require prolongation of epiblast lifespan during diapause when cells can be maintained in the naïve state by means of gp130 signalling [49]. In vitro pluripotent cells can be maintained indefinitely through derivation of stem cell lines. *In vitro* pluripotency can be maintained indefinitely through derivation of stem cell lines. The pluripotency of ES cells is evident from the following main features. *In vitro*, under two-dimensional (substrate-attached) culture conditions ES cells can differentiate into a large variety of cell types. In three-dimensional suspension culture ES cells form highly organized cystic embryoid bodies comprising the cells of three embryonic germ layers. When subcutaneously injected into host animals, pluripotent ES cells form benign teratomas. The definitive test for pluripotency is the generation of germline-competent chimaeras.

Nichols and Smith suggested that early epiblast cells and ES cells may represent a naïve ground state without any prespecification of lineage choice, whereas later epiblasts cells may be primed in favor of particular embryonic lineages [50, 51, 95]. Pluripotent cells in the naïve state are intrinsically self-

maintaining if protected from inductive differentiation stimuli. ES cells are the most pluripotent, but not totipotent: in mouse chimeras, they do not contribute to extra-embryonic cell types of the trophectoderm and primitive endoderm lineages.

In ES cells, the pluripotent state is mainly regulated by the core set of transcription factors Oct-4, NANOG and SOX2 [101]. Oct-4 transcription factor is thought to be central among this group of transcription factors which is essential for the establishment and maintenance of the pluripotent state. *In vitro* expression of *Oct-4* was demonstrated in undifferentiated embryonal carcinoma (EC) cells, ES cells, and embryonic germ (EG) cells. A large proportion of pluripotent cell-specific genes appear to be downstream targets of Oct-4, which can activate the expression of their target genes through binding an octameric sequence motif [4, 67, 82, 96].

The fate of ES cells depends on the precise expression level of *Oct-4*. A critical amount of Oct-4 is required to sustain stem-cell self-renewal, and up or down regulation of *Oct-4* induces divergent developmental programs. Nichols and colleagues demonstrated that Oct-4-deficient embryos developed to the blastocyst stage, but the ICM cells were not pluripotent. A less than twofold increase in *Oct-4* expression causes differentiation into primitive endoderm and mesoderm. In contrast, repression of *Oct-4* induces loss of pluripotency and dedifferentiation to trophectoderm [53, 56].

NANOG is considered a core element of the pluripotent transcriptional network. Mouse ES cells require the expression of both NANOG and Oct-4 for self-renewal and maintaining a robust pluripotent state. Overexpression of NANOG renders ES cells self-renewal constitutive without requirement for LIF and BMP. NANOG-null ES cells are highly prone to differentiation, but nonetheless can sustain self-renewal. NANOG is dispensible for expression of somatic pluripotency but is specifically required for the formation of germ cells [10, 11, 74, 75].

Krüppel factors were demonstrated to sustain ES cell self-renewal. Oct-4 primarily induces Klf2 while LIF/Stat3 selectively enhances Klf4 expression. Thus, Oct-4 and Stat3 direct expression of Klf transcriptional regulators that additively reinforce ground-state pluripotency and ES cells self-renewal [29]. It is noteworthy that factors which are central to sustaining pluripotency in ES cells are also the critical factors that can collectively reprogram somatic cells to pluripotency. It seems that a hierarchy of transcription factors binding to ES cell enhancers occurs and the Oct/SOX motifs may be placed at the center of the pluripotency network [13].

Mouse ES cells require the expression of both NANOG and Oct-4 for self-renewal and maintaining a robust pluripotent state. Oct-4 and NANOG are identified as key regulators of pluripotency based on their relatively unique expression pattern in ES cells [11, 55, 74, 75, 77].

Cooperativity between Oct-4 and SOX2 was first described based on their functional binding to an FGF4 enhancer element. SOX2 is the best-characterized partner of Oct-4. It was proposed that the function of SOX2 may be in activation of Oct-4 [44]. Oct-4 and SOX2 form heterodimers through the POU and SOX

domains to bind to the promoters or enhancers of their target genes to activate or suppress their expression [64].

It was suggested that ES cells are the *in vitro* counterpart of the transient naïve pluripotent cell population. Similarly to the naïve epiblast cells, ES cells coexpress the pluripotency markers Oct-4, SOX2, NANOG, Rex1, Klf4, Klf2, Esrrb, Tbx3, and Tfcp2l1, have two active X chromosomes in the case of female cells, and can give rise to fully ES cell-derived mice [51]. There is similarity in chromatin organization between ES cells and pluripotent cells *in vivo*. Genome-wide gene expression using microarray analysis has revealed that a variety of normally silent repeat regions are expressed at low levels in ES cells The leaky expression of a large number of genes in ES cells is likely to be the result of both genetic and epigenetic mechanisms and processes. Through epigenetic processes, the pluripotent epigenome keeps the chromatin structure open to allow for rapid genetic regulation [9, 19, 66, 85]. ES cells possess globally relaxed transcriptionally active chromatin structure, which correlates with the fact that nuclei of ES cells are about double the volume of those in differentiated cells.

The core transcription factors together positively regulate their own promoters forming an interconnected autoregulatory loop necessary to maintain pluripotent cell state. Now it is thought that pluripotent ES cells reside in a particular functional state that is governed by connected protein and transcriptional networks which are intertwined with factors that affect chromatin structure and function. The unique identity of ES cells is governed by the core factors that establish autoregulatory loops that help maintain their own expression, activate transcription of a large fraction of the active genes, and contribute to the balanced state of lineage-specific genes. The autoregulatory networks of transcription factors play a central role in ES cells rapid cycling and preventing cellular senescence and differentiation [4, 55, 59, 101].

5. Primordial germ cells

PG cells are the embryonic precursors of the gametes of the adult organism and represent a part of the germline, the unique cell lineage, which transmit genetic information from one generation to the next. Strictly speaking, germ cells are referred to as primordial until they enter the genital ridge. PG cells are not lineage restricted while in the epiblast. Specification of PG cells occurs early during gastrulation under the influence of mesodermal induction signaling.

In mammals, the germ-line cycle consists of the fertilized egg, all blastomeres of the cleavage stages and the morula, cells of the ICM of the blastocyst, epiblast cells, germ cells, and gametes. The competence for pluripotency is maintained throughout the germ-line cycle. However, at different stages the cells have different functional properties. In mammals, cells of the early embryo are incorporated in the germ-line cycle that is exited upon somatic differentiation. In the mouse embryo, cells of the inner cell mass, specifically the pre-implantation epiblast, can contribute to chimaeras including the germline, following blastocyst injection [26]. Such proven capacity to reintegrate into the

embryo and contribute functionally into development of all somatic lineages and the germline are attributes associated with the term naïve pluripotency [50].

In mice, PG cells arise from a founder population in the E6.0-6.5 proximal epiblast adjacent to the extra-embryonic ectoderm. These founder cells then pass through the primitive streak and give rise to several extraembryonic mesodermal lineages and to germ cells. In mice, PG cell lineage is set aside as early as 7 dpc embryo, when a small number of PG cells are identified at the caudal end of the primitive streak in the extraembryonic mesoderm [28, 39].

Investigation of allocation and differentiation of mouse epiblast cells showed that the developmental fate of these cells is determined by their position in the epiblast. Epiblast cells from distal epiblast that are fated to become neuroectoderm can give rise to PG cells when they were transplanted to the proximal region of the epiblast. On the contrary, proximal epiblast cells transplanted to the distal region of the embryo do not form PG cells. Therefore, the germ line in the mouse is unlikely to be derived from a predetermined progenitor population, but may be specified as a result of tissue interactions that take place in the proximal epiblast. The entire PG cell population is derived from a finite number of progenitor cells and there is no further cellular recruitment to the germ line after gastrulation [39, 83, 87].

It was demonstrated that founder PG cells, similarly to their somatic neighbors, express the typical mesodermal markers Brachyury and Fgf8, suggesting that the PG cell fate is induced in a population of cells originally destined for a somatic mesodermal fate. In mice, the most proximal epiblast cells maintain direct contact with the extraembryonic ectoderm cells from E5.0 to E6.5, and receive a BMP4 signal from these cells to adopt the germ cell fate. Specification of PG cells occurs early in the proximal epiblast during gastrulation under the influence of Bmp signals. Bmp4 and Bmp8b emitted from the extraembryonic ectoderm, as well as the signal transducers known as Smads (Smad1, 4, and 5), are critical for the generation of PG cells. In addition, Bmp2 produced by anterior visceral endoderm seems to augment the role of Bmp4 to ensure the generation of sufficient numbers of PG cells. Germ cell competent cells express *Fragilis* and initially progress towards a somatic mesodermal fate. However, a subset of these cells, the future PG cells, which shows rapid upregulation of *Fragilis*, subsequently express *Stella*, a gene exclusively detected in lineage-restricted germ cell. In *Stella*-positive cells transcriptional repression of a number of genes occurs, including *Hoxb1* and *Hoxa1,* which are highly up-regulated in somatic mesodermal neighbors. This repression may be a key event associated with germ cell specification. It seems that repression of a somatic program represented by the *Hox* genes reveals one of the mechanisms by which the PG cells escape from a somatic fate and retain their pluripotency. Around E6.25, the cells start to express a set of transcription factors involved in PG cell specification, such as Blimp1/Prdm1, Prdm14 and Tfap2c. Expression of *Blimp1* is first induced in a few of the posterior proximal epiblast cells at E6.25 just prior to the onset of gastrulation. Blimp1-positive cells increase in number and form a cluster of around 20 cells at E6.75. Blimp1-positive cells at this early stage contribute almost invariably to Stella-positive PG cells. In *Blimp1* mutants, PG

cells specification seems to be blocked at a very early stage indicating that *Blimp1* is a master regulator for PG cell specification. It was proposed that *Blimp1* is not a single initiator but a dominant coordinator of the transcriptional program for the establishment of the germ cell fate in mice. Genetic analysis showed that Blimp1- and Prdm14-deficient PG cells fail to repress somatic cell genes, thereby resulting in severe disruption of PG cell development at the early stage [37, 38, 54, 57, 58, 69, 70, 92, 94, 99].

The first marker for the identification of pluripotent cells is the expression of *Oct-4* in the mammalian embryo. The activity of Oct-4 is indispensable for the formation of the pluripotent founder cell population of germ line cycle. Initial specification of pluripotent cells *in vivo* requires *Oct-4* gene expression in all blastomeres and abundant expression in all cells throughout the morula stage of the developing mouse embryo. Subsequently, its expression becomes restricted to the ICM of the blastocyst, and is downregulated in the trophectoderm and the primitive endoderm. Later in development, *Oct-4* expression is maintained in the embryonic ectoderm at the egg-cylinder stage. After this stage, *Oct-4* expression finally becomes restricted to PG cells. At maturity, *Oct-4* expression becomes confined exclusively to the developing germ cells [5, 53, 61, 62, 91, 96]. In the complete absence of Oct-4, ICM cells proceed to the mid-blastocyst stage, but these cells are not pluripotent. Down-regulation of Oct-4 expression prevents the formation of pluripotent cell lines from preimplantation embryos. Thus, Oct-4 may be an essential determinant of the germ line. Loss of Oct-4 function leads to apoptosis of PG cells rather than to differentiation into a trophectodermal lineage, as has been described for Oct-4-deficient ICM cells. These results suggest that a function of Oct-4 consists in maintaining viability of mammalian germline [33, 63].

Oct-4 expression in the germline cells is regulated separately from expression in epiblast cells. *Oct-4* gene activity in cells of the germ cell lineage is driven by distal enhancer and by proximal enhancer in the epiblast. In the small group of cells that are committed to become PG cells the shift in *Oct-4* gene activity from the proximal enhancer to the distal enhancer occurs. This shift coincides with the allocation of PG cells to the extraembryonic mesoderm [53, 96].

NANOG is considered a core element of the pluripotent transcriptional network. In cultured ES cells NANOG stabilizes pluripotent state by preventing alternative gene expression states. NANOG is dispensible for expression of somatic pluripotency but is specifically required for formation of germ cells [11].

6. Discussion

Across metazoan phylogeny, two types of germline determination are recognized. Animals with germ line determination by preformation have a continuous germline, while animals with germ line determination by epigenesis have a discontinuous germline, with somatic cells intercalated. The process of germ cell specification by epigenesis proceeds via *de novo* appearance of germ cells from other embryonic cells at some point in development through the

extrinsic signals that segregate a population of precursors of PG cells from a pluripotent group of cells [22].

August Weismann in his Germ Plasm Theory postulated that the germline, which is both totipotent and immortal, is defined by specific substances, which he termed 'determinants' [89]. Mammals lack distinctive visible components in the oocyte that could account for 'determinants', and attempts to define mammalian germline determinants have failed [18]. The lack of obvious localized cytoplasmic germ determinants in the eggs of Urodele amphibians and mammals, and the relatively late induction of germ cell lineages in these animals, suggest that soma and germ are indistinguishable during early development of these animals and that distinction between germ and soma is recovered through positional information during gastrulation rather than through the function of classical determinants. Embryonic segregation of the germline is widely used by both ecdysozoans (such as flies and nematodes) and by chordates (which include ascidians, fish, amphibians and mammals) [22].

Juliano and colleagues [32] suggested that in some animals like sea urchins, sponges, and cnidarians an overlapping set of genes, including *vasa*, *nanos* and *piwi*, traditionally classified as 'germ-line genes' have a broad role in establishing and maintaining multipotent precursors and in the germline. These authors propose that PG cells and multipotent cells both have a highly conserved germline multipotency program and suggest that PG cells and multipotent somatic progenitor cells appear to be sister cell types, each realizing their developmental potential differently, but still closely linked by a common regulatory program. It seems reasonable to suggest that pluripotent somatic stem cells, germline cells, and ES cells share a core regulatory gene networks which operate in a similar manner across Metazoa.

Based on the studies of molecular germ plasm components, Jordi Solana [80] introduced the concept of primordial stem (PriS) cells, which in mammalian embryogenesis corresponds to ICM and epiblast cells. In the animals with discontinuous germ line PriS cells are intercalated between the zygote and germ cells. The PriS cell hypothesis states that even though the continuity of the germline seems to be interrupted in animals with epigenesis there is still a continuity of the germ plasm components that flow from zygote to PriS cells and then to germ cells. Therefore, if PriS cells are included in the germline definition, then the continuity of the germline is not interrupted either. Thus, PriS cells may be evolutionary conserved stem cells that carry germ plasm molecular components. For instance, freshwater planarians show remarkable ability to regenerate complete animals from tiny body fragments. Planarian adult pluripotent stem cells (neoblasts) give rise to not only all types of somatic cells, but also germline cells. One of the molecular features of neoblasts is the expression of *vasa* and *piwi* family genes. It was suggested that the expression of germline specific genes by somatic stem cells might be a way to achieve asexual reproduction [73].

Several lines of evidence suggest similarity of PG and ES cells in the mouse. Nonetheless a firm link between PG cells and ES cells generation has not been demonstrated. The idea that ES cells represent an *in vitro* equivalent to the

ICM was firmly rooted in the literature. Historically however, the culture conditions that were established to support mouse ES cells, including the use of feeder cell layers, were essentially those first developed for the cultivation of EC cells (derived from teratocarcinomas, a subset of germ cell tumours) that does a hint at a relationship between ES cells and germ cells. It was shown that malignant teratocarcinomas arise from germinal cells as well as embryonal somatic cells [47]. First pluripotent stem cell lines were originally derived from mouse testicular teratocarcinomas. The germ cell tumors contain multiple differentiated tissues and undifferentiated EC stem cells, which when injected into mouse blastocysts can contribute to either the normal tissues of the resulting chimera or, in some cases, to tumors [12, 42, 68].

In mouse, remarkable similarity of ES cells to EG cells and EC cells was noted by several authors [24, 45, 52, 93, 102]. ES cells consistently produce teratomas or teratocarcinomas when grafted ectopically and occasionally give rise to tumors in chimeras. This fact gives support for the view that ES cells resemble euploid EC cells. During teratocarcinogenesis, PG cells can give rise to EC cells, while in vitro PG cells under appropriate culture conditions give rise to EG cells. Once established, EG cells are indistinguishable from ES cells functionally and at the molecular level apart from a variable degree of imprint erasure [72]. These facts suggest that, although germ cells are committed cells with a limited developmental potential, they can cross the developmental barrier to become ES cell-like cells. The resemblance of EG cells to ES cells prompted the suggestion that ES cells might arise from epiblast cells that are already predisposed to a PG cell fate [24, 102].

Mouse ES cells *in vivo* can contribute to all the lineages of chimeric embryos including the germline. And ES cells have the capacity to differentiate into PG cells *in vitro* [17, 20, 86]. EC cells derived from teratocarcinomas were considered as malignant counterpart of ES cells [2]. These experiments suggest that transformation of ES cells to EC cells and to PG cells is associated with epigenetic changes. Conversion between the various pluripotent cell types can be induced by manipulating culture conditions, through adding or withdrawing certain cytokines or growth factors and through changing the transcriptional activity of genes. It was suggested that pluripotent state is a continuum of states. Maintenance of pluripotency, viability and differentiation of ES cells are regulated by extrinsic signals [8, 60].

Sharova and colleagues examined global gene expression patterns of multiple ES cell lines and EG cell lines. All pluripotent cell lines showed similar gene expression profiling. Differences between pluripotent lines derived from different sources (ES cells vs. EG cells) were smaller than differences between ES cell lines derived from different mouse strains (129 vs. C57BL/6) [72]. Genome-wide comparisons of the gene expression profiles of freshly isolated PG cells and ES cells showed that both cell populations share expression of many pluripotency-associated genes. Micro-array data were classified into two groups: one consisting of all the ES cells and most of EG cells, and the other group containing PG cells samples [48]. Xu and colleagues [93] systematically analyzed the RNA and protein expression of germ-cell markers and demonstrated that germ-cell marker

genes are expressed in all mouse pluripotent cell types and emerge early during induced pluripotency. Analysis of gene expression in the process of iPS cells generation revealed that the expression of germ-cell markers precedes pluripotency markers. These authors demonstrated the parallel maintenance and independence of pluripotent and germ cell networks in ES cells.

ES cells express several PG cell markers [27, 48] that indirectly points to a common origin of these cells. Differentiation of germ cells from human ES cells *in vitro* was accompanied by expression of *Vasa* and other germ cell specific genes [16]. Mouse ES cells in culture were able to generate PG cells that had the capacity to form sperm [2]. A characteristic gene expression program appending genome-wide epigenetic change is observed in epiblast cells heading to PG cells. First, they express somatic mesodermal genes such as *T, Hoxa1 and Hoxb1*. However, around E6.25, the cells start to express a set of transcription factors involved in PG cell specification, such as Blimp1/Prdm1, Prdm14 and Tfap2c. In germ cell development, Blimp1 is a master regulator for PG cell specification. Genetic analysis showed that Blimp1-deficient and Prdm14-deficient PG cells fail to repress somatic cell genes, thereby resulting in severe disruption of PG cell development at the early stage. It was shown that transcriptional repressor Blimp1 has a critical role in the foundation of the mouse germ cell lineage. Blimp1-positive cells, which originate from the proximal posterior epiblast cells, are the primordial germ cell precursors [37, 58, 88].The fate-mapping experiments revealed that ES cells commonly arise from Blimp1-positive precursors. ES cells derivation efficiency can be improved by prescreening for Blimp1-positive cells [15]. Prdm14 ensures naïve pluripotency through antagonizing activation of the FGFR signaling and repressing expression of *de novo* DNA methyltransferases [95].

Several observations demonstrated that pluripotent mammalian stem cells can exist *in vitro* in several distinct states, which are defined, in part, by culture growth factor environment and cell-cell interactions. Mouse ES cells in culture were able to generate PG cells that had the capacity to form sperm [86]. Primordial germ cells from early somite-stage embryos or isolated from genital ridges can convert in culture, without genetic manipulation, into pluripotent stem cells known as EG cells [46, 65]. Kimura and colleagues [35] found that PG cell-like cells were efficiently induced from mouse ES cells by inhibition of ERK signaling, which upregulated germ marker genes but downregulated mesodermal genes. It was shown that mouse ES cells, PG, and EG cells are interconvertible in response to altered culture conditions [14, 30, 34]. Allocation of epiblast cells to the ectodermal and germ cell lineages may be subject to local tissue interactions and the restriction of morphogenetic tissue movement of different epiblast cell populations during gastrulation. This plasticity of cell fate suggests that the epiblast cells are not irreversibly allocated to any specific lineages, including the germ line [83]. This data suggest that under appropriate culture conditions PG and ES cells are interconvertible**.**

7. Conclusion

Formation of a pluripotent cell population during embryogenesis is central to mammalian development. The major property of pluripotent cells is the capacity of individual cell to initiate all cell lineages of the adult organism in the embryo or cell culture environment. An important aspect of pluripotent cells is the ability to generate germ cells. Pluripotency is generated naturally during mammalian development through formation of the epiblast, the founder tissue of the embryo proper. Pluripotency is a blank state in which all differentiation options are accessible: pluripotent cells have no predetermined program of lineage choice. Early epiblast cells and ES cells may represent a naïve pluripotent state when the genome has an unusual open conformation and possesses a minimum of repressive epigenetic marks.

Convincing data show a remarkable resemblance of ES cells to PG cells. Both cell types are interconvertable and have similar chromatin organization and many common molecular makers of pluripotency. The prevalent data gives ground to surmise that murine ES cells can be derived via capturing *in vitro* the state of naïve pluripotency, which arises in the early epiblast. During development of mouse embryo there is a window of opportunity when a population of naïve pluripotent cells may give rise to ES cells depending on culture conditions. It is reasonable to suggest that ES cells originate from a small group of early epiblast cells which represent a population of pluripotent founder cells before the allocation of germ and soma lineages. It can be assumed that this group of founder cells is similar to the hypothetical PriS cells that Solana [80] included in the germ line.

Conflict of interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

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