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Review

Transcription regulation and chromatin structure in the pluripotent ground state☆

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ABSTRACT

The use of mouse embryonic stem cells (ESCs) has provided invaluable insights into transcription and epigenetic regulation of pluripotency and self-renewal. Many of these insights were gained in mouse ESCs that are derived and maintained using serum, either on feeder cells or supplemented with the cytokine leukemia inhibitory factor (LIF). These 'serum' ESCs are in a metastable state characterized by the expression of many lineage-specifying genes. The use of two small-molecule kinase inhibitors (2i), targeting mitogen-activated protein kinase (MEK) and glycogen synthase kinase-3 (GSK3), has enabled derivation of mouse ESCs in defined serum-free conditions. These '2i' ESCs are more homogeneous in morphology and gene expression than serum ESCs, and are postulated to represent the ground state of pluripotency. Recent studies have shown that the epigenome and transcriptome of 2i and serum ESCs are markedly different, suggesting that these ESCs represent two distinct states of pluripotency regulated by different factors and pathways. There is growing evidence that the 2i ESCs closely parallel the early blastocyst cells of the inner cell mass (ICM) or even earlier stages, while serum cells possibly reflect later stages. In this review, we will focus on the difference in chromatin structure, transcription regulation and cell cycle regulation between ground state pluripotent 2i ESCs and serum ESCs, and compare to corresponding data in embryos if available. This article is part of a Special Issue entitled: Chromatin and epigenetic regulation of animal development, edited by Dr. Peter Verrijzer and Dr. Elissa Lei.

1. Introduction

Embryonic stem cells (ESCs) are *in vitro* cultured cells that are derived from the Inner Cell Mass (ICM), the part of the early embryo that will give rise to the fetus. As such, ESCs have the potential to differentiate into all somatic cell lineages and into germ-line cells, referred to as pluripotency, and have the capacity to propagate indefinitely *in vitro* without losing cell fate [1,2]. Because of these unique properties, ESCs are an invaluable tool for both developmental studies and regenerative medicine, as limitless numbers can be produced for research or for clinical use.

Mouse ESCs were originally established and maintained on feeder cells in the presence of serum, and are present as homogenous colonies of small, tightly packed cells [1,2]. It was quickly recognized that the feeder cells could be replaced by a cytokine, leukemia inhibitory factor (LIF), although without feeders the ESCs are more flattened and heterogeneous in terms of morphology, and were reported to be more prone to aneuploidy [3–6]. In this review, ESCs grown in presence of serum (either on feeders or with LIF) will be referred to as 'conventional' or 'serum' ESCs. Although the use of conventional ESCs has yielded invaluable insights into embryonic development and pluripotency, they also have properties that makes them less attractive as a model system: (i) Conventional ESC do not faithfully reflect ICM cells: genome-wide transcriptome analyses revealed major changes occurring in the epigenome and transcriptome during outgrowth of the ICM to ESCs in the presence serum [7]. Based on activation of the germ-cell specification factor Blimp1, explanted blastocysts in serum are postulated to transiently activate a transcriptional program specific for primordial germ cells (PGC) during ESC derivation [8]; (ii) Once established, serum ESC cultures clearly display a wide variety of morphologies (Fig. 1). Within the serum population, ESCs show differential expression of pluripotency regulators and lineage specifying factors, and hence have different developmental/differentiation potential [9–15]; (iii) The variability and undefined factor composition of serum batches causes fluctuations among ESC cultures.

Recent developments have enabled the derivation of ESCs in defined serum-free medium supplemented with two small-molecule kinase inhibitors (2i) [16]: PD0325901 blocks differentiation *via* the MAP kinase pathway and CHIR99021 enhances self-renewal of ESCs by activating Wnt signaling [16,17]. These ESCs are postulated to represent the ground state of pluripotency, and will be further referred to as '2i' ESCs or 'ground state' pluripotent ESCs. Blimp1 is not activated during

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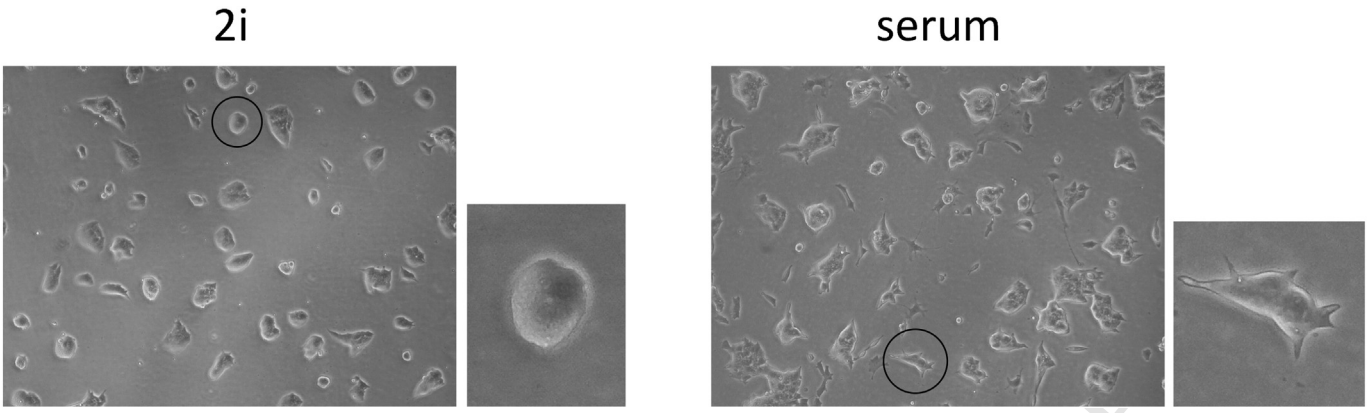

Fig. 1. Morphology of mouse ES cells maintained in culture media containing 2i + LIF (left) or serum + LIF (right).

ESC derivation in 2i [8]. Derivation of ESCs in 2i might therefore bypass the activation of a germ cell-like program and, as opposed to serum, stabilize the self-renewing early blastocyst state. As compared to conventional ESCs, 2i ESCs are much more homogeneous in morphology (Fig. 1), show higher clonogenicity (≥50%) and have been shown to be superior for various applications such as the generation of transgenic mice [18]. In contrast to serum and LIF, in which ESCs can only be derived from a minority of mouse strains, the use of 2i has enabled derivation of ESCs from all mouse strains tested and from rats [18–24]. Also, other small-molecule kinase inhibitors have now been described that maintain pluripotency and self-renewal capacity of mouse ESCs and are useful alternatives to the original 2i, like an Src kinase inhibitor [25]. However, these cells have not been further characterized thus far. Derivation of human ground state ES or iPS cells using 2i or similar conditions are ongoing, and have been successful if combined with forskolin and LIF or with continued overexpression of pluripotency-inducing factors [26,27].

Ground state and conventional ESCs are both functionally pluripotent, *i.e.* they contribute to differentiated derivatives of all three germ layers in chimeric embryos. However, there is growing evidence that next to the signaling also the epigenetic make-up of these ESCs is distinct, suggesting that 2i and serum ESCs represent two different states of pluripotency. Furthermore, it has been hypothesized that 2i ESCs reflect earlier embryonic stages as compared to serum ESCs. In this review, we focus on the difference in signaling pathways, transcriptional regulatory networks, chromatin structure and cell cycle regulation between these two different pluripotent states.

2. Signal transduction in 2i and serum ESCs

2.1. Signaling in serum ESCs

In the absence of any supplements added to the culture medium, ESCs tend to lose pluripotency and self-renewing capacity. This mainly occurs due to FGF4 secreted by the ESCs. FGF4 binds the FGF receptor on the cellular membrane inducing the MAP kinase pathway, which results in differentiation of the ESCs [28,29]. Therefore, independent of the culturing method, maintenance of ESCs requires supplements to override or inhibit the FGF4-mediated differentiation. In conventional ESCs, two signaling molecules are used: LIF (either as supplement or provided by feeder cells) inhibits endodermal and mesodermal differentiation, while Bone Morphogenetic Protein 4 (BMP4; either as a supplement or as part of the serum) blocks neural differentiation [30,31].

Mechanistically, LIF acts by binding to the gp130 receptor on the cell surface, which activates a range of intracellular signaling transduction pathways including JAK/STAT3, PI3K/AKT and SHP2/ERK/MAPK (Fig. 2; reviewed in Hirai et al. [32]). STAT3, the critical downstream effector, is phosphorylated in response to activation of these pathways and forms homo- or heterodimers that translocate from the cytoplasm to the nucleus where it binds to the DNA in a sequence-specific manner [31,33]. It was recently shown that Tfcp2l1 is the critical target of STAT3, and constitutive Tfcp2l1 expression substituted for LIF or Stat3 in sustaining self-renewal and pluripotency [34,35]. BMP4, the other supplement in conventional ESC cultures, binds to the membrane proteins BMPR1 and -R2. This activates the ERK/MAPK- as well as the

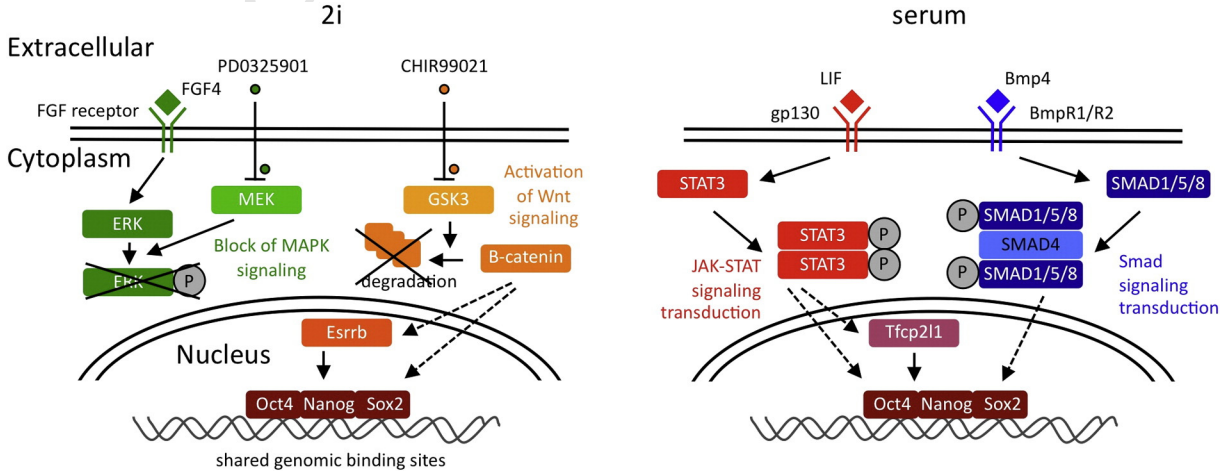

Fig. 2. Signaling transduction pathways affected in either 2i (left) or serum (BMP4) + LIF (right).

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