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Review

Roles of TGF-β family signals in the fate determination of pluripotent stem cells

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ABSTRACT

Members of the transforming growth factor-β (TGF-β) family have been implicated in embryogenesis as well as in the determination of the cell fates of mouse and human embryonic stem (ES) cells, which are characterized by their self-renewal and pluripotency. The cellular responses to TGF-β family signals are divergent depending on the cellular context and local environment. TGF-β family signals play critical roles both in the maintenance of the pluripotent state of ES cells by inducing the expression of Nanog, Oct4, and Sox2, and in their differentiation into various cell types by regulating the expression of master regulatory genes. Moreover, multiple lines of evidence have suggested the importance of TGF-β family signals in establishing induced pluripotent stem (iPS) cells. Since ES and iPS cells have great potential for applications in regenerative medicine, it is critical to figure out the mechanisms underlying their self-renewal, pluripotency, and differentiation. Here, we discuss the roles of TGF-β family ligands and their downstream signaling molecules, Smad proteins, in the maintenance of the pluripotency and lineage specification of mouse and human ES and iPS cells.

Contents

Abbreviations: ALK, activin receptor-like kinase; BMPs, bone morphogenetic proteins; Bra, Brachyury; Co-Smads, common-mediator Smads; Dusp9, dual specificity phosphatase 9; EBs, embryonic bodies; EMT, epithelial to mesenchymal transition; ES, embryonic stem; FGF, fibroblast growth factor; Flk$^+$, fetal liver kinase positive; GDFs, growth differentiation factors; hES, human embryonic stem; ICM, inner cell mass; Id, inhibition of differentiation; IGF, insulin-like growth factor; iPS, induced pluripotent stem; I-Smads, inhibitory Smads; ITD-1, inducer of Type II receptor degradation-1; LIF, leukemia inhibitory factor; MAPK, mitogen activated protein kinase; MEFs, mouse embryonic fibroblasts; mEpiSCs, mouse epiblast stem cells; mES, mouseembryonic stem; MET, mesenchymal to epithelial transition; Mvh, mouse vasa homolog; PGCs, primordial germ cells; PI3K, phosphatidylinositol-3 kinase; PS, primitive streak; R-Smads, receptor-regulated Smads; SBE, Smad binding element; TβR, TGF-β receptor; Tet1, Ten-eleven translocation 1; TGF-β, transforming growth factor-β; VEGF, vascular endothelial growth factor; Zeb2, zinc finger E-box binding homeobox 2.

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1. Introduction

The complex architecture of our body originates from one fertilized egg, which proliferates and differentiates to give rise to various types of cells in multiple organs. In adults, the homeostasis of most organs is maintained by a continuous supply of newly generated, functionally differentiated cells. Stem cells have been implicated in the development and maintenance of our bodies. These cells are unspecialized and have remarkable potential to develop into many different cell types during life. A noteworthy character is their ability to self-renew. Unlike somatic cells, such as blood or muscle cells, stem cells can divide symmetrically to replicate themselves many times or proliferate asymmetrically to generate differentiated cell types for long periods. There are various types of stem cells in mammals including embryonic stem (ES) cells and adult (somatic) stem cells. The proliferation and differentiation of stem cells are regulated by multiple types of signaling cascades, including those mediated by the members of the transforming growth factor (TGF)-β family. The roles of TGF-β family signals in somatic stem cells have been described [1]; therefore, in this review, we focus on their roles in the maintenance of pluripotency of ES cells and their differentiation into multiple lineages.

2. TGF-β family signaling

The TGF-β family has over 30 members, including TGF-βs, activins, Nodal, bone morphogenetic proteins (BMPs), and growth differentiation factors (GDFs) [2]. They are multifunctional cytokines, and are involved in the morphogenesis of many organs and homeostasis of adult tissues [3,4]. When TGF-β family signaling is disrupted, developmental defects or diseases are observed [5]. In addition, TGF-β family members sustain the pluripotent state of human embryonic stem (ES) cells, and contribute to the germ layer commitment of stem cells to mature organs [6].

TGF-β family members transduce their signals to the nucleus by the formation of heteromeric receptor complexes of specific type I and type II serine/threonine kinase receptors known as TGF-β type I (TβRI) and type II receptors (TβRII), respectively (Fig. 1). TβRI, also termed activin receptor-like kinase (ALK) 5, acts downstream of TβRII and phosphorylates receptor regulated Smads (R-Smads), which are gateways for canonical TGF-β signaling.

Smad proteins are classified into three subfamilies depending on their function and structure; R-Smads, common-mediator Smads (Co-Smads), and inhibitory Smads (I-Smads) (Fig. 1). Each R-Smad has an SXS motif at their extreme C-terminus, and both serine residues are direct targets of type I receptor kinases. Among R-Smads, Smad2 and Smad3 are phosphorylated by ALK4, TβRI (ALK5), and ALK7 and transduce canonical TGF-β and activin/Nodal signals, whereas ALK1, ALK2, ALK3, and ALK6 activate Smad1, Smad5, and Smad8 and transduce BMP signals. After ligand stimulation, R-Smads are phosphorylated and make a ternary complex with the Co-Smad, Smad4. Then the complex accumulates into the nucleus, where it regulates the expression of various TGF-β family target genes. The expression of I-Smads, *i.e.* Smad6 and Smad7, is induced by TGF-β family members. I-Smads prevent R-Smads from being phosphorylation by type I receptor kinases, thereby terminating TGF-β family signals. Smad7 has multiple inhibitory effects on both TGF-β and BMP signals, whereas Smad6 preferentially blocks BMP signaling.

The N-terminal domains of Co-Smad and R-Smad are able to bind to a DNA sequence termed 5′-AGAC-3′, known as the Smad binding element (SBE). Since Smads alone generally have a weak affinity to bind to DNA, other transcription factors are needed. Due to the differential expression of various binding partners in multiple cell types, Smads are able to regulate the transcription of many groups of target genes in different ways. In addition to transcriptional factors, Smads can associate with chromatin modifiers and other chromatin remodelers [7].

3. Pluripotent stem cells

ES cells are derived from the inner cell mass (ICM) of blastocysts at an early embryonic stage. ES cells are able to proliferate without differentiation since they are established. When the inner cells are isolated and cultured on feeder cells, the cells can grow while retaining their potency to form all three layers: the endoderm, mesoderm, and ectoderm [8]. Therefore, ES cells have been used in an *in vitro* model of early embryogenesis to investigate the detailed molecular mechanisms for developmental processes. When ES cells can be conventionally used, we can easily establish genetically modified or knockout mice using these cells, which will be useful tools for regenerative medicine. Meanwhile, adult stem cells exist in almost all tissues of the body after embryonic development. They are ready for emergencies such as disease or tissue injury as well as maintenance of tissue homeostasis.

ES cells can be generated from various species at different embryonic stages. Several groups have obtained ES cells from the mouse morula stage and these cells are already committed to blastomeres at the later stage of blastocysts [9]. Recently, an additional type of ES cells was established from the epiblast of mouse embryos, termed epiblast stem cells (mEpiSCs) [10,11]. The mEpiSCs are molecularly and epigenetically distinct from mES cells. Their characteristics are very similar to human ES (hES) cells, showing similar gene expression profiles and signaling responses, which can be observed in the mouse epiblast.

4. Role of TGF-β family signals in maintaining pluripotency

The maintenance of pluripotency in mES cells relies on a complex network of transcription factors [12], with Oct4, Nanog, and Sox2 playing a central role [13,14]. Oct4, a POU domain protein, is expressed in early mouse embryogenesis and germ cells [15]. Reduced expression of Oct4 leads ES cells to differentiate into trophoblasts, whereas overexpression of Oct4 triggers differentiation into endodermal and mesodermal cells. Therefore, adequate levels of Oct4 expression are needed for maintenance of the pluripotency of ES cells. Nanog, a homeobox-containing protein, is expressed specifically in early embryos and pluripotent stem cells, including mES and hES cells. Forced expression of Nanog can elicit self-renewal of ES cells without cytokine-induced activation of STAT3 [16], although impaired Nanog expression causes ES cell differentiation. Thus, Nanog is thought to stabilize inhibition of differentiation [13]. In contrast, Sox2, a SRY-related HMG box protein, is expressed in various phases of embryonic development. Sox2 cooperates with Oct4 to activate Oct-Sox-target genes. In addition, Oct4, Nanog, and Sox2 cooperatively regulate the expression of many genes which are responsible for self-renewal and pluripotency [17]. Due to their essential roles in early embryonic

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