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Review Article

The Life Story of TGFβs superfamily: from the beginning to the end

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Abstract: TGFβ-superfamily consists a plethora of extracellular growth factors, modulating developmental procedures and homeostasis in vertebrates and invertebrates. TGFβ-superfamily ligands, synthesized as the large inactive precursors, transform into active ligands following by their interaction with extracellular proteolytic enzymes. Principally, TGFβs ligation to their responsive receptors can trigger two distinct transduction cascades, including 1- SMAD dependent or canonical pathway and 2- SMAD independent or non-canonical ones. R-SMADs are substrates for the type I receptors, as their GS domains act as a docking site for R-SMADs. In the canocical pathway, upon phosphorylation of SSXS of MH2, two phosphorylated-SMADs (P-SMADs) in accordance with receptor tetra-dimerization, homo or heterodimerize and then form a trimer complex by SMAD4. The trimers translocate to the nucleus, where in association with other transcription factors (activators and repressors) modulate their target genes expression. The purpose of this review is to provide a comprehensive information about these cascades and their downstream effectors with an emphasis on the canonical one.

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Introduction

Transforming Growth Factor β (TGFβ) superfamily consists a broad spectrum of extracellular growth factors, regulating development and homeostasis processes in vertebrates and invertebrates. Its highly conserved members have been identified in sea urchins, nematodes, flies and vertebrates (Raftery and Sutherland, 1999; ten Dijke and Hill, 2004; Cavaleri and Schöler, 2009; Wu and Hill, 2009; Pauklin and Vallier, 2015). The common feature of TGFβ-superfamily members is a conserved cysteine knot structure (Liang and Rubinstein, 2003; Gordon and Blobe, 2008). This superfamily, containing over 40 ligands, is divided into a number of families, including TGFβs (1-3), ACTIVIN (A & B), BMPs (1-20), GDFs, LEFTY (1-2), NODAL and INHIBIN (Reddi, 1997; Sutherland, 1999; Kramer, 2002; Liang and Rubinstein, 2003; Ober et al., 2003; Field et al., 2003a, b; ten Dijke and Hill, 2004; James et al., 2005; Kondo, 2007; Itoh and ten Dijke, 2007; Gordon and Blobe, 2008; Perrett, 2008; Cavaleri and Schöler, 2009; Raftery and Schier, 2009; Seuntjens et al., 2009; Chng et al., 2011; Lonardo et al., 2011; Oshimori and Fuchs, 2012; Quail et al., 2013; Itoh et al., 2014; Vallier, 2016; Fathi et al., 2017) . Some of these factors are synthesized by a wide variety of cells, whereas others are produced by a specific cell type. Furthermore, in contrast with some TGFβs, which are active for a short period, others are transcribed throughout the life (Kramer, 2002). TGFβ-superfamily members participate in a plethora of pleiotropic functions divided into the cellular and physiological works. The cellular functions managed by TGFβs are as follows: (1) pluripotency maintenance of stem cells, (2) proliferation (growth), (3) changes in cell shape (migration and adhesion), (4) apoptosis and (5) differentiation. The physiological processes controlled by TGFβs encompass: (1) early axial patterning, (2) inductive interactions during organogenesis, (3) wound healing and (4) tissue homeostasis. According to their wide roles, abnormal expression of TGFβs provokes cancer stem cells

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(CSC), neoplasia, developmental disorders and vascular disease (Raftery and Sutherland, 1999; ten Dijke and Hill, 2004; James et al., 2005; Itoh and ten Dijke, 2007; Gordon and Blobe, 2008; Schier, 2009; Lonardo et al., 2011; Donahue and Dawson, 2011; Oshimori and Fuchs, 2012; Beyer et al., 2013; Quail et al., 2013; Itoh et al., 2014; Pauklin and Vallier, 2015; Vallier, 2016).

Synthesis and primary process of TGFβs: TGFβ-superfamily ligands are synthesized as the large inactive precursors, consisting four main domains (in an amino to carboxyl terminal direction), including (1) The signal or leader peptide, (2) the latency associated peptide (LAP) or pro-domain, (3) a fURIN convertases cleavage sequence and (4) the biologically active mature domain. The LAP involves in folding, stability and dimerization of TGFβs in the intracellular space (Raftery and Sutherland, 1999; Kramer, 2002; ten Dijke and Hill, 2004; Jing et al., 2006; Itoh and ten Dijke, 2007; Gordon and Blobe, 2008; Schier, 2009; Seuntjens et al., 2009; Chng et al., 2011; Oshimori and Fuchs, 2012; Beyer et al., 2013; Pauklin and Vallier, 2015; Vallier, 2016). After their entrance in extracellular space, the TGFβ precursors are transformed into active ligands following the LAP separation by (1) extracellular proteolytic enzymes, including plasmin, Matrix Metallo-Proteinases (MMP2/9), Cathepsin-D and Thrombospondin or (2) Integrin αVβ6 and αVβ8 and or 3- FURIN convertases (FURIN (Spc1) and PACE4 (Spc4)) (Raftery and Sutherland, 1999; Kramer, 2002; Tam et al., 2003; Jing et al., 2006; Gordon and Blobe, 2008; Seuntjens et al., 2009; Chng et al., 2011; Oshimori and Fuchs, 2012; Beyer et al., 2013; Sun et al., 2014;). However, the BMPs do not lose their LAP conferring them stability in extracellular space as well. Therefore, their modulation merely depends on the antagonists compared to other TGFβs. BMPs antagonists regulate BMPs activity by preventing their interaction with receptors. Based on the size of their cysteine knot, BMPs antagonists are clustered into (1) CAN, (2) Twisted gastrulation (tsg) and (3) Chordin/Noggin families (Kramer, 2002; Minchiotti et al., 2002; Field et al., 2003a, b; Gordon and Blobe, 2008; Perrett, 2008; Wu and Hill, 2009; Chng et al., 2011; Beyer et al., 2013; Pauklin and Vallier, 2015; Vallier, 2016) .

Canonical and non-canonical TGFβ pathways: TGFβs can trigger two distinct and independent pathways including (Kodjabachian et al., 1999; Munoz-Sanjuan and A, 2001; Stemple, 2001; Whitman, 2001; Kramer, 2002; Liang and Rubinstein, 2003; ten Dijke and Hill, 2004; James et al., 2005; Sun et al., 2006; Itoh and ten Dijke, 2007; Kondo, 2007; Blobe, 2008; Jia et al., 2008; Gordon and Xu et al., 2008; Cavaleri and Schöler, 2009; Chan et al., 2009; De Robertis, 2009; Harvey and Smith, 2009; Schier, 2009; Seuntjens et al., 2009; Wu and Hill, 2009; Zhendong, 2009; Chng et al., 2011; Oshimori and Fuchs, 2012; Beyer et al., 2013; Ramel and Hill, 2013; Stewart et al., 2014; Itoh et al., 2014; Pauklin and Vallier, 2015; Fathi et al., 2017): (1) SMAD dependent or canonical transduction cascade and (2) SMAD independent or non-canonical signaling pathways. The second one, itself, encompasses (1) MAPK pathway (e.g. MAPK/ERK, P38 and JNK), (2) PI3K/AKT pathway and (3) NF-kB pathway (Fig. 1).

Non-canonical TGFβs pathways: In MAPK pathway, ligand binding phosphorylates TRAF6 (E3 ligase TNF receptor-associated factor 6) that in turn induces the activation of TAK1. The activated TAK1 then transmits the signal by activating MEK that phosphorylates P38/JNK/ERK1/2. These transcription factors translocate to the nucleus to transcribe genes modulating proliferation (Carmany-Rampey and Moens, 2006; Gordon and Blobe, 2008; Perrett, 2008; Seuntjens et al., 2009; Wu and Hill, 2009; Oshimori and Fuchs, 2012; Quail et al., 2013; Vallier, 2016).

TGFβ type I receptors can also enable the scaffold protein (shcA) to bind with GRB2-SOS to activate RAS-ERK1/2 signaling pathway. Furthermore, the activated type II receptors can also triggered PAR6 that consequently induces tight junction destruction and epithelial mesenchymal transition (EMT) (Carmany-Rampey and Moens, 2006; Gordon and Blobe, 2008; Perrett, 2008; Seuntjens et al., 2009; Wu and Hill, 2009; Oshimori and Fuchs, 2012; Quail et

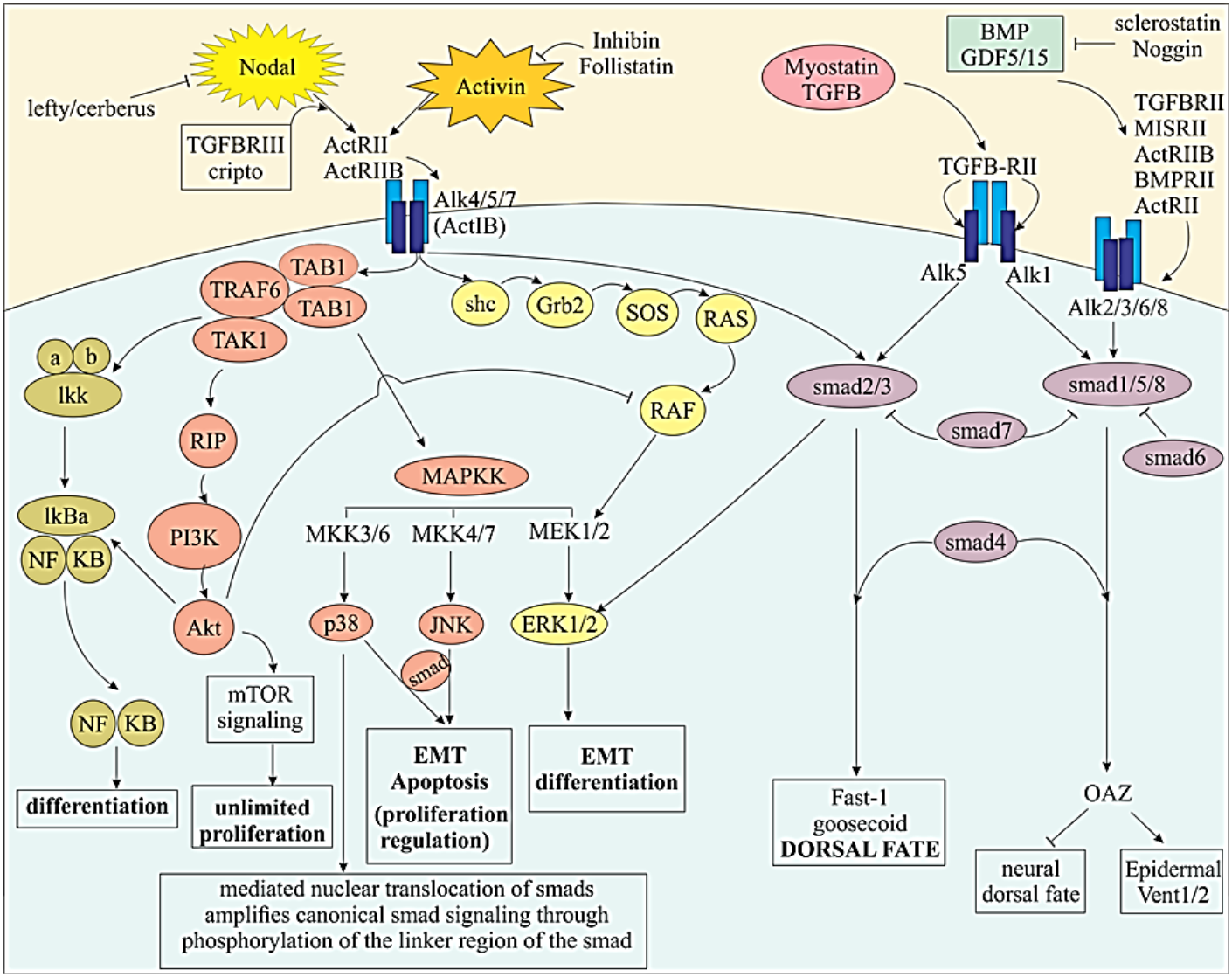

Figure 1. Canonical and non-Canonical TGFβ transduction cascades.

al., 2013; Vallier, 2016).

Canonical TGFβs pathways

1. Introduction of the components, involved in the canonical TGFβs signaling cascades

TGFβs superfamily receptors: TGFβs recruit serine/threonine protein kinase transmembrane receptors to relay the signal into their downstream effectors. Based on the structural and functional characteristics, the receptors are subdivided into two subfamilies including the type I and type II receptors. There are seven type I (ACTIVIN-receptor Like Kinase; ALK1-7) and five type II receptors in the human genome. These glycoprotein receptors have a single transmembrane span and an intrinsic serine/threonine kinase domain in their C-terminal segment. However their main difference refers to the GS domain of type I receptor which is associated by immunophiline FKBP1A (Raftery and Sutherland, 1999; Lele et al., 2001; Whitman, 2001; Kramer, 2002; Ober et al., 2003; Field et al., 2003a; Gordon and Blobe, 2008; Seuntjens et al., 2009; Wu and Hill, 2009; Chng et al., 2011; Oshimori and Fuchs, 2012; Beyer et al., 2013; Pauklin and Vallier, 2015).

Following Ligand binding, TGFβ type II receptor, as a constitutively active kinase, hetero-dimerize with an appropriate type I receptor. In fact, the active receptor complex includes two type I and two type II receptors (tetra-dimerization) due to its recruitment by a dimeric ligand. Upon ablation of the immunophilin FKBP1A/FKBP12, the GS domain is phosphorylated

by the type II receptor. The phosphorylated GS domain acts as a docking site for receptor-regulated SMADs (R-SMADs), the unique substrates of type I receptors (Raftery and Sutherland, 1999; Kramer, 2002; Gordon and Blobe, 2008; Perrett, 2008; Xu et al., 2008; Schier, 2009; Seuntjens et al., 2009; Wu and Hill, 2009; Chng et al., 2011; Oshimori and Fuchs, 2012; Beyer et al., 2013; Pauklin and Vallier, 2015; Vallier, 2016).

In addition to the main receptors, there are three accessory or co-receptors, including; (1) TGFβR3 (known as β-glican), (2) ENDOGLIN (co-receptor in BMPs signaling) and (3) TDGF1 (namely crypto). Crypto belongs to the EGF-CFC family (Epidermal Growth Factor- Crypto, FRL1, and Cryptic). EGF-CFC family includes Crypto and Cryptic in mice, h-CRIPTO and h-CRIPTIC in human, Crypto in chick, one-eyed pinhead (oep) in zebrafish and FRL-1 in frog (*Xenopus laveis*). Members of this family are anchored in the lipid membrane by peptidoglycans. They can be detached from the membrane as a soluble molecule, in turn; competent cells (even oep mutant one) will be able to respond properly to Nodal signaling. The co-receptors merely reinforce the signal transduction because their intracellular domain are devoid of any sequence motif involving in signal transduction (Mullins, 1998; Kodjabachian et al., 1999; Raftery and Sutherland, 1999; Shen and Schier, 2000; Chen and Schier, 2001; Schier, 2001; Whitman, 2001; Minchiotti et al., 2002; Kramer, 2002; Liang and Rubinstein, 2003; Tam et al., 2003; ten Dijke and Hill, 2004; Bartscherer and Boutros, 2008; Gordon and Blobe, 2008; Xu et al., 2008; Schier, 2009; Wu and Hill, 2009; Chng et al., 2011; Lonardo et al., 2011; Beyer et al., 2013; Nozawa et al., 2013; Pauklin and Vallier, 2015; Tuazon and Mullins, 2015; Vallier, 2016).

SMADs: Mad (mothers against Dpp (BMP orthologue)) is known as the SMAD orthologue in *Drosophila Melanogaster*. It transduces the Dpp signal from the receptor to the nucleus (Raftery and Sutherland, 1999; Neave et al., 1997; Reddi, 1997; Kramer, 2002; Kondo, 2007; Wu and Hill, 2009; Quail et al., 2013). SMADs, a family of conserved transcription factors, are clustered into third class according to their phylogenetic relationships and functional evaluations: (1) R-SMADs (Receptor-regulated SMAD: SMAD1/2/3/5/8), (2) Co-SMAD (Common-mediator SMAD: SMAD4) and (3) I-SMADs (Inhibitory SMADs: SMAD6/7). In general, R-SMADs have a central role in the canonical transduction cascade and SMAD4 enhances the signaling triggered by TGFβs. However, SMAD6/7 down regulate the signal transduction.

R-SMADs and SMAD4 show sequence homology in two unrelated regions, including the N-terminal Mad Homology (MH1) domain to bind with DNA and the C-terminal MH2 domain to bind with other transcription factors. These domains are separated by a poorly conserved prolin-rich linker region. Furthermore, R-SMADs contain a SSXS motif in their MH2 domain to bind with the type I receptors. In this regard, R-SMADs accession to type I receptors is facilitated by auxiliary proteins such as SMAD Anchors for Receptor Activation (SARA). In addition, R-SMADs encompass a PPXY motif in their linker region that can be phosphorylated by ERK, GSK3 as well as CDK8/9; thereby creating a docking site for WW domain containing proteins. Therefore HECTE3 ligases as the WW-domain containing proteins (including SMURF1/2, ECTODERMIN (E3 ubiquitin ligase), WWP2 and NEDD4L) marked R-SMADs for proteasome degradation (Raftery and Sutherland, 1999; Kramer, 2002; Liang and Rubinstein, 2003; Field et al., 2003a, b; Ober et al., 2003; ten Dijke and Hill, 2004; Kondo, 2007; Gordon and Blobe, 2008; Xu et al., 2008; Cavaleri and Schöler, 2009; Schier, 2009; Seuntjens et al., 2009; Wu and Hill, 2009; Chng et al., 2011; Oshimori and Fuchs, 2012; Quail et al., 2013; Beyer et al., 2013; Itoh et al., 2014; Pauklin and Vallier, 2015). R-SMADs linker region provide a platform to converge TGFβ signaling pathway and other biological transduction cascades like Wnt, FGF and IGF. Other WW domain containing proteins such as YAP1 transcription factor and SIN1 coactivator help to SMADs maintenance in the nucleus.

Latent conformation of R-SMADs and SMAD4 is due to a reciprocal intramolecular interaction between

their MH1 and MH2 domains during the absence of signaling factors. SMADs phosphorylation precludes this auto-inhibition and makes their MH1 and MH2 domains available to interact with DNA and other transcription factors respectively (Raftery and Sutherland, 1999; Kramer, 2002; Field et al., 2003a, b; Liang and Rubinstein, 2003; Ober et al., 2003; ten Dijke and Hill, 2004; Kondo, 2007; Gordon and Blobe, 2008; Xu et al., 2008; Cavaleri and Schöler, 2009; Schier, 2009; Seuntjens et al., 2009; Wu and Hill, 2009; Chng et al., 2011; Oshimori and Fuchs, 2012; Beyer et al., 2013; Quail et al., 2013; Itoh et al., 2014; Pauklin and Vallier, 2015).

I-SMADs exclusively retain the MH2 domain without its SSXS motif. They alleviate phosphorylation of R-SMADs by type I receptors. Principally I-SMADs terminate the TGFβs signaling pathway in several different ways. First, I-SMADs mark the type I receptors or R-SMADs by recruiting E3-ubiquitinligases known as SMURF1/2 (SMAD Ubiquitination Regulatory Factors). In this regard, SMAD7 and SMAD6 suppress all R-SMADs and SMAD1/5/8 respectively. Second, SMAD7 inactivates type I receptors by recruiting GADD34 complex and catalytic subdomain of protein phosphatase I (Raftery and Sutherland, 1999; Kramer, 2002; ten Dijke and Hill, 2004; James et al., 2005; Mizoguchi et al., 2006; Bennett et al. 2007; Gordon and Blobe, 2008; Schier, 2009; Seuntjens et al., 2009; Wu and Hill, 2009; Beyer et al., 2013; Itoh et al., 2014; Vallier, 2016; Fathi et al., 2017).

The canonical TGFβs signaling pathway: R-SMADs are substrates for the type I receptors, as their GS domains act as a docking site for R-SMADs. Upon phosphorylation of SSXS sequence of MH2, two phosphorylated-SMADs (P-SMADs) in accordance with receptor tetra-dimerization, homo or heterodimerize and then form a trimer complex by SMAD4. The trimers translocate to the nucleus, where in association with other transcription factors (activators and repressors) modulate their target genes expression (Raftery and Sutherland, 1999; Munoz-Sanjuan and A, 2001; Kramer, 2002; ten Dijke and Hill, 2004; Itoh and ten Dijke, 2007; Gordon and Blobe, 2008; Xu et al., 2008; Cavaleri and Schöler, 2009; Schier, 2009; Wu and Hill, 2009; Oshimori and Fuchs, 2012; Beyer et al., 2013; Itoh et al., 2014; Pauklin and Vallier, 2015).

Functional analysis of SMAD proteins suggests that, the canonical or SMAD dependent TGFβ signaling is clustered into two distinct branches with respect to the R-SMADs. In this regard, BMPs and GDFs exclusively transmit signal through ALK1/2/3/6/8 receptors and SMAD1/5/8 whilst TGFβs, ACTIVIN and NODAL (Activin-like factors) transmit signal through ALK4/5/7 and SMAD2/3. However, TGFβs itself robustly activate both SMAD1/5/8 and SMAD2/3 in many cell types and GDF8/9/11 can also transduce signal through ALK4/5/7. Therefore, the above-mentioned category is almost over simplification. Additionally, due to high affinity between the type II receptors and all TGFβ members except BMPs, the ligand-receptor interaction is highly cooperative and upon their binding, both type of receptors in direct association transduce the signal into SMADs. While in the case of BMPs, the type II receptors indirectly binds to the type I by recruiting the BMP ligands (Kodjabachian et al., 1999; Munoz-Sanjuan and A, 2001; Stemple, 2001; Whitman, 2001; Kramer, 2002; Liang and Rubinstein, 2003; ten Dijke and Hill, 2004; James et al., 2005; Sun et al., 2006; Itoh and ten Dijke, 2007; Kondo, 2007; Gordon and Blobe, 2008; Jia et al., 2008; Xu et al., 2008; De Robertis, 2009; Cavaleri and Schöler, 2009; Chan et al., 2009; Harvey and Smith, 2009; Schier, 2009; Seuntjens et al., 2009; Wu and Hill, 2009; Zhendong, 2009; Chng et al., 2011; Oshimori and Fuchs, 2012; Beyer et al., 2013; Ramel and Hill, 2013; Itoh et al., 2014; Stewart et al., 2014; Pauklin and Vallier, 2015; Fathi et al., 2017).

SMAD2/3 binds to 5'-AGAC-3' or its complement GTCT known as SMAD binding elements (SBE), whereas, SMAD1/5/8 preferentially binds to GGCGCC or GGAGCC namely BMP response elements (BRE). Except the SMAD2, all R-SMADs and SMAD4 can directly bind to DNA with low affinity and specificity. Hence, to achieve high affinity and selectivity, SMAD proteins must thus interact

with other transcription factors (Raftery and Sutherland, 1999; Munoz-Sanjuan and A, 2001; Kramer, 2002; ten Dijke and Hill, 2004; Itoh and ten Dijke, 2007; Gordon and Blobe, 2008; Xu et al., 2008; Cavaleri and Schöler, 2009; Schier, 2009; Wu and Hill, 2009; Oshimori and Fuchs, 2012; Beyer et al., 2013; Itoh et al., 2014; Pauklin and Vallier, 2015).

The Regulator factors of TGFβ signaling: The modulator factors of TGFβ signaling divide into two main parts. The first one is extracellular factors, including (A) antagonist agents, (B) agonist agents like co-receptors and diffusible ligand binding proteins, which promote the ligand accessibility, (C) Processing enzymes like FURIN and (D) secreted protein acidic rich in cysteine (SPARC). In fact, TGFβs increase the SPARC transcription to intensify TGFβs signaling, because acidic condition promotes TGFβs transformation into their mature ligands (Gordon and Blobe, 2008; Schier, 2009; Seuntjens et al., 2009; Wu and Hill, 2009; Beyer et al., 2013; Pauklin and Vallier, 2015; Vallier, 2016). The second one encompasses intracellular factors such as (A) Smad6/7, (B) the shuttling system of SMADs (Ran/GTPase export/import system), (C) proteins involved in receptor trafficking, (D) SARA, (E) Erb2/Her2 that sequester SMAD2/3 away from SMAD4, (F) transcription factors, coactivators and corepressors and (G) miRNAs (Gordon and Blobe, 2008; Schier, 2009; Seuntjens et al., 2009; Wu and Hill, 2009; Oshimori and Fuchs, 2012; Beyer et al., 2013; Pauklin and Vallier, 2015; Vallier, 2016). Additionally, TRIM33/ECTODERMIN alleviates TGFβs signaling through mono-ubiquitination of SMAD4. Whereas SMAD4 de-ubiquitination by FAM/USP9X attenuates the effect of TRIM33/ECTODERMIN (Schier, 2009; Seuntjens et al., 2009; Oshimori and Fuchs, 2012; Beyer et al., 2013; Pauklin and Vallier, 2015; Vallier, 2016).

During unstimulated condition, SMADs interaction with Ran GTPase export/import system creates a highly dynamic equilibrium in which un-phosphorylated SMADs continuously shuttle between the cytoplasm and nucleus. Upon SMADs phosphorylation and trimer complex formation by means of SMAD4, they are accumulated in the nucleus. This accumulation is due to their higher import rate in comparison with monomeric un-phosphorylated SMADs. In addition, downstream effectors of the Hippo pathway e.g. YAP and TAZ transcription factors maintain the trimer complex in the nucleus (Schier, 2009; Seuntjens et al., 2009; Wu and Hill, 2009; Pan, 2010; Beyer et al., 2013; Pauklin and Vallier, 2015; Vallier, 2016).

To turn off the SMAD signaling, protein phosphatases (e.g. PPM1A, pyruvate dehydrogenase phosphatases (PDP) and small C-terminal phosphatase (SCPs1, 2 & 3)) dephosphorylate R-SMADs, thereby trimer complexes disruption. Dephosphorylated SMADs is then recognized by RANBP3 and exported from the nucleus (Raftery and Sutherland, 1999; ten Dijke and Hill, 2004; Gordon and Blobe, 2008; Schier, 2009; Seuntjens et al., 2009; Wu and Hill, 2009; Pan, 2010; Beyer et al., 2013; Pauklin and Vallier, 2015; Vallier, 2016).

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