Regulatory Role and Mechanism of Transforming Growth Factor- 1/Smads Signaling Pathway in Mammalian Ovarian Development Postprint

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Abstract

The coordination among different signal transduction pathways ensures the normal functioning of the organism. Among numerous signaling pathways, the transforming growth factor (TGF) 1/Smads signal transduction pathway has increasingly attracted scholars' attention and has become a major hotspot in molecular and cell biology research. Previous studies have confirmed that the TGF- 1/Smads signal transduction pathway represents an important mechanism for regulating follicular development. This article reviews the TGF- 1/Smads signal transduction pathway and its role in mammalian ovarian follicle development, elaborating the regulatory functions and mechanisms of this pathway in mammalian ovarian development from the perspectives of TGF- receptors, Smads proteins, bone morphogenetic protein (BMP), thrombospondin-1 (THBS1), S-phase kinase-associated protein 1 (SKP1), and other regulatory mechanisms, aiming to draw attention to TGF- 1/Smads-mediated regulation of ovarian development and to provide references for the treatment of certain diseases occurring during ovarian development.

Full Text

Regulation and Mechanism of the Transforming Growth Factor- 1/Smads Signaling Pathway in Mammalian Ovarian Development

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Abstract: The coordinated interplay among different signal transduction pathways is essential for maintaining normal physiological functions. Among numerous signaling pathways, the transforming growth factor (TGF)- 1/Smads signaling pathway has attracted increasing attention from researchers and become a major focus in molecular and cell biology studies. Previous research has confirmed that the TGF- 1/Smads signaling pathway serves as a critical regulatory mechanism for follicular development. This review examines the regulatory roles and mechanisms of the TGF- 1/Smads signaling pathway in mammalian ovarian follicular development, discussing TGF- receptors, Smads proteins, bone morphogenetic protein (BMP), thrombospondin 1 (THB-S1), S-phase kinase-associated protein 1 (SKP1), and other regulatory mechanisms. The aim is to highlight the importance of TGF- 1/Smads in ovarian development and provide insights for treating diseases associated with ovarian development.

Keywords: TGF- 1/Smads; mammalian; ovary; BMP; THB-S1; SKP1

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The reproductive performance of female animals is a crucial factor affecting animal productivity, and is controlled by the coordinated regulation of multiple factors. The ovary is the reproductive organ responsible for oocyte production and ovulation. Abnormal ovarian development can lead to disorders such as impaired follicular development, ovarian dysfunction, and infertility in female animals. In female mice, these abnormalities manifest as delayed sexual maturity, irregular ovulation, and reduced ovulation rates [1]. The proper functioning of biological processes depends on the coordinated interaction among different signal transduction pathways, and follicular growth and development are regulated by multiple factors collectively. Recent studies have demonstrated that the transforming growth factor- (TGF-) superfamily plays important roles in regulating cell differentiation, proliferation and cycle, embryonic development, bone formation, wound healing, immunity, endocrine function, and tumor formation and progression [2-4]. As the most active, functionally diverse, and widely distributed member of the TGF- superfamily [5], TGF- 1 plays a significant role in regulating follicular development, signal transduction between oocytes and granulosa cells [6], as well as in signal transduction processes controlling cell growth, differentiation, migration, apoptosis, and extracellular matrix production [7]. Smads proteins are important intracellular TGF- signal transduction and regulatory molecules that can directly transmit TGF- signals from the cell membrane into the nucleus [8]. Studies have found that mutations in the genes encoding TGF- signaling pathway receptors and Smads proteins are associated with tumorigenesis [9]. Exploring signal transduction pathways has undoubtedly become one of the frontier areas in life sciences research; however, the elucidation of the molecular mechanisms underlying the TGF- signaling pathway has only just begun, with many questions remaining to be addressed. Actively investigating the regulatory mechanisms of the classical TGF- 1/Smads signaling pathway has become a top priority for re-understanding the TGF- superfamily.

1. TGF- 1/Smads Signaling Pathway and Ovarian Follicular Development

The ovary is the primary gonadal organ in female mammals, producing oocytes for reproduction and secreting estrogen to promote the development of secondary sexual characteristics. Normal ovarian function development in mammals is a complex and precise process coordinated by multiple factors, beginning during embryonic development. Throughout ovarian development, numerous hormones and cytokines regulate the process through paracrine/autocrine signaling between cells [6]. Extensive research has demonstrated that the TGF- 1/Smads signaling pathway plays a pivotal regulatory role in granulosa cells and follicular growth and development. Abnormal temporal or spatial expression or activation of any signaling molecule in this pathway can lead to abnormal ovarian development in animals and has been linked to human tumors, cardiovascular diseases, and autoimmune disorders [7-8]. In recent years, the role of TGF- superfamily members in animal reproduction has been extensively studied, revealing their critical importance throughout the development of the reproductive system. Experimental evidence has confirmed the presence of a complete TGF- 1/Smads signaling pathway in normal ovarian tissue [9].

The TGF- 1/Smads pathway is a branch of the TGF- signaling pathway and represents a key signaling pathway discovered in recent years that regulates follicular growth and development in higher animals. This signaling cascade consists of extracellular ligands, cell surface-specific receptors, and intracellular Smads signal transduction molecules, which form a tightly linked cascade that ultimately transmits extracellular signals into the nucleus to regulate target gene transcription and elicit biological effects. When TGF- 1 is present, activated superfamily ligand signaling molecules bind to the corresponding transforming growth factor- type II receptor (TGF- RII) on the cell membrane surface. TGF- RII undergoes autophosphorylation and then directly binds to or indirectly associates with transforming growth factor- type I receptor (TGF- RI) via -glycan to form a dimeric complex with kinase activity. The phosphorylated, kinase-active TGF- RI can activate receptor-regulated Smads proteins (R-Smads, Smad2/3) in the cytoplasm, transmitting the signal to the phosphorylated receptor. These then combine with common mediator Smads (Co-Smads, Smad4) to transport the TGF- signal from the cytoplasm to the nucleus, where they act on specific gene promoters. Together with various co-activators and co-repressors, they regulate target gene transcription, induce downstream gene expression, and trigger a series of biological effects. In the TGF- /Smads pathway, inhibitory Smads (I-Smads, Smad7) negatively regulate this signaling pathway by blocking the activation of R-Smads and Co-Smads or by competitively binding with activated receptors or receptor-activated Smads to form inactive complexes [10] (Figure 1 [Figure 1: see original paper]).

Figure 1. TGF- /Smads Signaling Pathway [10]

TGF- ligands: TGF- superfamily ligands; BMP ligands: bone morphogenetic protein ligands; Type II receptor: TGF- RII; Type I receptor: TGF- RI; TAK1: transforming growth factor- -activated kinase 1; p38: a member of mitogen-activated protein kinases (MAPKs) family; JNK: c-Jun N-terminal kinases; RhoA: a small molecule protein of Ras homologue family; Pi3K: phosphatidylinositol-3 kinase; Non-Smad pathway: Smad-independent pathway; ErK: extracellular signal-regulated kinase; Smad1/2/3/5/8: receptor-regulated Smads (R-Smads); Smad7: inhibitory Smad (I-Smads); Smad4: common mediator Smads (Co-Smads).

2.1. Regulation of TGF- Receptors on the TGF- 1/Smads Signaling Pathway

TGF- receptors are transmembrane proteins located on the cell surface. Currently, three main subtypes of TGF- superfamily receptors have been identified: TGF- RI, TGF- RII, and transforming growth factor- type III receptor (TGF- RIII), all of which contain extracellular, transmembrane, and intracellular domains [11]. TGF- RI possesses a highly conserved glycine (Gly) and serine (Ser) residue domain in its intracellular region, known as the GS domain, which plays a crucial role in TGF- RI kinase activation. In contrast, TGF- RII lacks a GS domain and binds to free TGF- through autophosphorylation of threonine (Thr) and Ser residues in its short intracellular tail [12]. TGF- RIII is a proteoglycan that does not directly participate in signal transduction and is therefore referred to as a co-receptor [13]. Phosphorylation of Ser/Thr residues in the GS region of TGF- RI is required for mediating TGF- signal transduction and determines the specificity of downstream intracellular signals. Current research has shown that both TGF- 1 and TGF- RI are expressed at different stages of ovarian development and participate in granulosa cell proliferation, oocyte maturation, and steroidogenesis through autocrine/paracrine mechanisms, playing a vital role in maintaining ovarian homeostasis [14]. Genetic knockout of TGF- RII leads to embryonic or neonatal lethality in mice [15], demonstrating that TGF- receptors are involved in various physiological activities in mammals.

2.2. Regulation of Smads Proteins on the TGF- 1/Smads Signaling Pathway

Smads proteins are important intracellular TGF- signal transduction and regulatory molecules that can transmit TGF- signals directly from the cell membrane into the nucleus [16]. Their inactivation or mutation can lead to tumorigenesis. Studies have identified Smad2 and Smad4 as tumor suppressor genes. Without Smad2, cells can escape TGF- -regulated growth inhibition and undergo malignant transformation; deficiency in Smad4 protein reduces DNA binding capacity, altering the expression of downstream target genes in ovarian cancer [8]. Smads proteins are key downstream signal transduction molecules in the TGF- signaling pathway that transmit signals from the cytoplasm to the nucleus. Upon activation, the receptor-regulated/common mediator Smads

(R-Smad/Co-Smad) heterodimer enters the nucleus and cooperates with other transcription factors to accumulate at the promoter regions of target genes, exerting positive or negative regulation of transcription [16]. Smad2/Smad3 are the first signaling molecules downstream of TGF- 1 [17] and can be recruited to TGF- RI for phosphorylation by the Smad anchor for receptor activation (SARA) to exert positive regulation [18]. In contrast, Smad7 can be presented by serine-threonine kinase receptor (STRAP) to bind with TGF- RI and form a complex that exerts negative regulation. Tomic et al. [19] demonstrated that female mice lacking the Smad3 gene exhibit disordered follicular development, increased granulosa cell apoptosis, and consequently increased follicular atresia. Additionally, Smad3 deficiency leads to abnormal estrous cycles in mice [20]. Stephanie et al. [21] found that ovarian-specific knockout of the Smad4 gene in mice causes premature ovarian failure, premature luteinization of granulosa cells, and reduced fertility. These findings suggest that Smads proteins play important roles in mammalian follicular development and granulosa cell proliferation and differentiation, which will undoubtedly become a future research hotspot.

2.3. Regulation of Bone Morphogenetic Protein (BMP) on the TGF- 1/Smads Signaling Pathway

BMP is a highly conserved glutamic acid-rich glycoprotein extracted from adult bone tissue. Except for BMP-1, all BMPs belong to the TGF- family [22] and play important roles in skeletal development and organ formation in vertebrates and invertebrates, while also regulating the growth and differentiation of various cell types [23]. Mature BMP proteins are homodimeric or heterodimeric complexes linked by disulfide bonds, released extracellularly to bind with corresponding receptors on target cell surfaces and exert their functions [22]. BMP receptors consist of a short extracellular region, a transmembrane region, and an intracellular region [22]. BMPs serve as upstream transforming factors in the TGF- signaling pathway. Studies have shown that BMPs play important roles in follicular growth and development, oocyte maturation, ovulation, and granulosa cell proliferation [24], and are also crucial in controlling and regulating ovarian physiological functions and folliculogenesis [25].

2.4. Regulation of Thrombospondin 1 (THB-S1) on the TGF- 1/Smads Signaling Pathway

THB-S1 is a matrix adhesive glycoprotein secreted by platelet -granules that is widely expressed in human tissues [26]. It regulates wound healing [27], cell adhesion, migration, proliferation, and differentiation, and induces platelet aggregation while inhibiting angiogenesis [26]. THB-S1 is a homotrimeric extracellular matrix glycoprotein composed of three identical peptide chains linked by disulfide bonds [28], with each peptide chain divided into six domains that bind to various extracellular matrix components and cell surface receptors to exert diverse biological functions [29]. THB-S1 is an important activator of TGF- that can alter TGF- conformation, exposing its binding sites for cell receptors and thereby activating the TGF- signaling pathway [30]. Studies have found that THB-S1 gene deficiency reduces litter size and causes reproductive dysfunction in mice [31]. THB-S1 is regulated by various reproductive hormones and participates in the biological processes of granulosa cell proliferation, differentiation, and follicular development [32-33].

2.5. Regulation of S-Phase Kinase-Associated Protein 1 (SKP1) on the TGF- 1/Smads Signaling Pathway

SKP1 is a multifunctional protein first discovered in yeast that participates in cell cycle regulation by encoding centromere-binding proteins and is also involved in the ubiquitin degradation of related substances [34]. As a downstream regulatory factor of the TGF- /Smads signaling pathway, SKP1 regulates mammalian folliculogenesis and ovulation [34]. SKP1 is a key scaffold protein in the SCF ubiquitin ligase complex (Skp1-Cullin-F-box protein), binding to different F-box proteins to mediate the ubiquitination and degradation of various cell cycle proteins [35], thereby promoting proper cell cycle progression [36]. Years of research have demonstrated that SKP1 is a multifunctional protein that plays key roles in many pathways. Studies have shown that SKP1 is an important factor in early mouse embryonic development, and its overexpression can hinder embryonic development [37]. Therefore, it is speculated that the SKP1 gene may be associated with porcine follicular development and atresia, thereby affecting ovulation rate and reproductive capacity in pigs, though this hypothesis requires further investigation.

2.6. Other Regulatory Mechanisms of the TGF- 1/Smads Pathway

Homeostasis in organisms is maintained through the interaction of numerous regulatory factors and multiple pathways. The TGF- 1/Smads signaling pathway communicates extensively with other signaling pathways. Epidermal growth factor (EGF), lipopolysaccharide, tumor necrosis factor (TNF), and interleukin-1 (IL-1) can all induce Smad7 production and cross-talk with the TGF- signaling pathway. The TGF- 1/Smads signaling pathway also interacts with downstream pathways mediated by enzyme receptors. The mitogen-activated protein kinase (MAPK) system exerts dual regulatory effects on R-Smads, both releasing the biological effects of TGF- 1/Smads signals and preventing nuclear accumulation of R-Smads. Additionally, TGF- signaling can interact with Wnt signaling, p38, and other pathways. This intricate "cross-talk" between pathways forms a complex regulatory network that not only effectively modulates the normal operation of the TGF- signaling pathway but also confers diverse biological effects upon TGF- [8].

Despite significant progress in TGF- signaling pathway research, many important questions remain unresolved. While TGF- 1 is recognized as the most potent fibrotic factor, the precise mechanisms by which it induces increased extracellular matrix synthesis require further investigation beyond current findings. The Smads protein family was discovered a decade ago, but relatively few studies have examined its role in ovarian and reproductive functions. How Smads proteins target and regulate various components and key links in the signaling pathway for clinical therapeutic purposes, and whether these Smads molecules can be applied to treat infertility, represent important future research directions.

References

[1] JIN Hua, DING Jiayi, ZHU Qingwen. Effect of granulosa cells on the development of immature oocytes during in vitro culture [J]. Journal of Qiqihar Medical College, 2006, 27(3): 309-310.
[2] SHULL M M, DOETSCHMAN T. Transforming growth factor- 1 in reproduction [J]. Molecular Reproduction and Development, 1994, 39(2): 239-246.
[3] GUO Yonghong, LUO Jinyan. Research progress on TGF- superfamily and Smad signal transduction [J]. Medical Recapitulate, 2005, 11(8): 685-688.
[4] DIJKE P T, GOUMANS M J, ITOH F, et al. Regulation of cell proliferation by Smad proteins [J]. Journal of Cellular Physiology, 2002, 191(1): 1-16.
[5] GRÖNROOS E, KINGSTON IJ, RAMACHANDRAN A, et al. Transforming Growth Factor inhibits Bone Morphogenetic Protein-induced transcription through novel phosphorylated Smad1/5-Smad3 complexes [J]. Molecular and Cellular Biology, 2012, 32(14): 2904-2916.
[6] XU Mengsi. Study on the effect of TGF -SMAD signaling pathway on porcine granulosa cells and reproductive traits [D]. Master' s Thesis. Shihezi: Shihezi University, 2015: 1-20.
[7] ZHAO Ying, LUO Yuyu, ZHANG Wencheng. Role of TGF- 1/Smads signaling pathway and eNOS in endothelial cell homeostasis and atherosclerosis [J]. Journal of Logistics University of PAP: Medical Sciences, 2013, 22(6): 560-563.
[8] ZHANG Yong, QIN Na, YU Bin. Research progress on TGF- /Smads signal transduction pathway [J]. Journal of Guangxi Medical University, 2009, 26(1): 155-157.
[9] GAO Na, WU Tao. Research progress on the role of TGF- in ovarian cancer [J]. Journal of Dalian Medical University, 2012, 34(4): 409-413.
[10] LIU S J, DE BOECK M, VAN DAM H, et al. Regulation of the TGF- pathway by deubiquitinases in cancer [J]. The International Journal of Biochemistry & Cell Biology, 2016, 76: 135-145.
[11] BORSUTZKY S, CAZAC B B, ROES J, et al. TGF- receptor signaling is critical for mucosal IgA responses [J]. Journal of Immunology, 2004, 173(5): 3305-3309.
[12] DE CAESTECKER M. The transforming growth factor- superfamily of receptors [J]. Cytokine & Growth Factor Reviews, 2004, 15(1): 1-11.
[13] WANG X F, LIN H Y, NG-EATON E, et al. Expression cloning and characterization of the TGF- type III receptor [J]. Cell, 1991, 67(4): 797-805.
[14] CHENG Min, WEN Liangzhen. Transforming growth factor and its recep-

tors in follicular development [J]. Foreign Medicine: Obstetrics and Gynecology, 2001, 28(5): 261-264.
[15] PANGAS S A, MATZUK M M. Genetic models for transforming growth factor superfamily signaling in ovarian follicle development [J]. Molecular and Cellular Endocrinology, 2004, 225(1/2): 83-91.
[16] MING Jia. Smads protein family and TGF- intracellular signal transduction [J]. Foreign Medicine: Molecular Biology, 2003, 25(2): 89-93.
[17] CHEN Feng, ZHENG Min, CHEN Zhi. Role of Smad2 and Smad3 in TGF- 1 signal transduction [J]. International Journal of Epidemiology and Infectious Diseases, 2006, 33(3): 187-189.
[18] CHEN Jun, QIAN Yunliang. TGF- /Smads pathway and myofibroblast differentiation in hypertrophic scars [J]. Chinese Journal of Aesthetic Medicine, 2007, 16(7): 1000-1003.
[19] TOMIC D, MILLER K P, KENNY H A, et al. Ovarian follicle development requires Smad3 [J]. Molecular Endocrinology, 2004, 18(9): 2224-2240.
[20] SASSEVILLE M, RITTER L J, NGUYEN T M, et al. Growth differentiation factor 9 signaling requires ERK1/2 activity in mouse granulosa and cumulus cells [J]. Journal of Cell Science, 2010, 123(18): 3166-3176.
[21] PANGAS S A, LI X H, ROBERTSON E J, et al. Premature luteinization and cumulus cell defects in ovarian-specific Smad4 knockout mice [J]. Molecular Endocrinology, 2006, 20(6): 1406-1422.
[22] AN Xinling, HAN Jinxiang, WANG Shili. Research progress on bone morphogenetic proteins [J]. Food and Drug, 2009, 11(6): 69-73.
[23] WANG Wei. Effect of BMP/Smad signaling pathway on porcine follicular granulosa cells [D]. Doctoral Dissertation. Nanjing: Nanjing Agricultural University, 2010: 1-13.
[24] WANG Wei, WANG Shaobing, XU Yinxue. BMP/Smad signaling pathway and mammalian folliculogenesis [J]. Hereditas, 2009, 31(3): 245-254.
[25] ERICKSON G F, SHIMASAKI S. The spatiotemporal expression pattern of the bone morphogenetic protein family in rat ovary cell types during the estrous cycle [J]. Reproductive Biology and Endocrinology, 2003, 1(1): 9.
[26] FENG N P, WANG Z F, ZHANG Z, et al. miR-487b promotes human umbilical vein endothelial proliferation, migration, invasion formation through regulating THBS1 [J]. Neuroscience Letters, 2015, 591: 1-7.
[27] ADAMS J C, LAWLER J. The thrombospondins [J]. The International Journal of Biochemistry & Cell Biology, 2004, 36(6): 961-968.
[28] NAGANUMA H, SATOH E, KAWATAKI T, et al. Cell density regulates thrombospondin-1 production in malignant glioma cells [J]. Journal of Neuro-Oncology, 2003, 63(2): 147-153.
[29] HUGO C, DANIEL C. Thrombospondin in renal disease [J]. Nephron Experimental Nephrology, 2009, 111(3): e61-e66.
[30] LAWLER J. Thrombospondin-1 as an endogenous inhibitor of angiogenesis and tumor growth [J]. Journal of Cellular and Molecular Medicine, 2002, 6(1): 1-12.
[31] ZHOU Z Q, CAO W H, XIE J J, et al. Expression and prognostic significance of THBS1, Cyr61 and CTGF in esophageal squamous cell carcinoma [J].

BMC Cancer, 2009, 9(1): 291.
[32] MURPHY-ULLRICH J E, POCZATEK M. Activation of latent TGF- by thrombospondin-1: mechanisms and physiology [J]. Cytokine & Growth Factor Reviews, 2000, 11(1/2): 59-69.
[33] LAWLER J, SUNDAY M, THIBERT V, et al. Thrombospondin-1 is required for normal murine pulmonary homeostasis and its absence causes pneumonia [J]. Journal of Clinical Investigation, 1998, 101(5): 982-992.
[34] BAI C, SEN P, HOFMANN K, et al. SKP1 connects cell cycle regulators to the ubiquitin proteolysis machinery through a novel motif, the F-box [J]. Cell, 1996, 86(2): 263-274.
[35] FELDMAN R M R, CORRELL C C, KAPLAN K B, et al. A complex of Cdc4p, Skp1p, and Cdc53p/cullin catalyzes ubiquitination of the phosphorylated CDK inhibitor Sic1p [J]. Cell, 1997, 91(2): 221-230.
[36] CONNELLY C, HIETER P. Budding yeast SKP1 encodes an evolutionarily conserved kinetochore protein required for cell cycle progression [J]. Cell, 1996, 86(2): 275-285.
[37] WANG Yufeng. Functional study of Skp1 protein in mouse early embryonic development [D]. Master' s Thesis. Nanjing: Nanjing Medical University, 2008.

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