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Transforming growth factor (TGF) – is it a key protein in mammalian reproductive biology?

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Abstract

The superfamily of transforming growth factors β (TGF-β) consists of cytokines that are crucial in regulating the organism's biological functions and includes three isoforms of TGF-β protein, Anti-Müllerian Hormone (AMH), inhibin A and B, activins, 20 bone morphogenetic proteins (BMP1-20) and 9 growth factors (GDF1-9). Their signal transduction pathway involves three types of membrane receptors that exhibit a serine/threonine kinase activity, as well as the Smad proteins. After ligand binding, the Smad proteins are phosphorylated and translocated to the nucleus, where they interact with transcription factors and affect gene expression. TGF-β family members are involved in cell growth and differentiation, as well as chemotaxis and apoptosis, and play an important role during an inflammation. Defects in TGF-β proteins or in their signalling pathway underlie many severe diseases, such as systemic lupus, systemic scleroderma, bronchial asthma, atherosclerosis, hyperthyroidism or cancer. These factors are also crucial in mammal reproductive functions, as they are involved in folliculogenesis, steroidogenesis, ovulation, maternal-embryo interaction, embryo development and uterine decidualization. Their defects result in issues with fertility. This review focuses on the relevance of TGF-β family members in a mammal reproduction with an emphasis on three TGF-β isoforms, inhibins A and B, GDF-9 and their signal transduction pathway.

Running title: TGFB in mammalian reproduction

Keywords: mammals, reproduction, growth factors, TGFB

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TGF - a large family

The family of transforming TGF-β growth factors is a group of cytokines responsible for many important functions in the body. Produced by cells of different origins, they are important mediators of body processes. They influence growth, differentiation, cell migration, formation and degradation of cell matrix components, as well as processes of chemotaxis and apoptosis [1]. Cytokines are mainly produced by mononuclear cells, including activated monocytes or lymphocytes. The increase in activity of these factors was observed in inflammatory sites [2–4]. TGF-β family is comprised of 3 TGF-β1,2,3 isoforms synthesized in mammals, Anti-Müllerian Hormone (AMH), inhibin A and B (A, B and AB), 20 bone morphogenetic proteins (BMP1- 20) and 9 different growth factors (GDF 1-9) [5–9].

TGF-βs are low molecular weight proteins that control both the function of the healthy organism and the course of many diseases. Abnormalities in the TGF-β signal transduction pathway are the underlying immunological disorders associated with neoplasms, hyperthyroidism pathologies, and autoimmune diseases. These cytokines are also factors promoting atherosclerosis and are associated with abnormalities of the blood system or chronic transplant rejection [4, 10–13].

Expression of TGF-β family members also occurs in the ovarian follicle, granulosa cells and the oocyte itself. They are responsible for the differentiation of follicles, proliferation or atresia of granulosa; steroidogenesis, oocyte maturation, and follicular luteinisation. TGF-β expression disorders have been reported in a number of autoimmune diseases (TGF-β1) or cancers, including ovarian cancer (inhibin B). This creates the possibility of using these factors as molecular markers for the detection of developmental disorders in both somatic and sex cells [3].

TGF-β family proteins are also involved in ovulation, maternal-embryo interactions, embryonic development, such as gastrulation and morphogenesis, and uterine decidualization as well as regulation of male reproductive functions, e.g. testis development [14].

Transforming growth factor beta

In mammals, there are three TGF-β isoforms encoded by different genes: TGF-β1, TGF-β2, TGF-β3. These isoforms exhibit a 70% identical amino acid sequence and their molecular weight is about 25 kDa. They are proteins consisting of two subunits, each containing 112 amino acids, bound by disulfide bonds [3, 7, 15, 16].

Of the three isoforms, TGF-β1 is the best known. This cytokine was found to be expressed in dendritic cells, leukocytes, NK cells, and epithelial cells. The lack of TGF-β1 predisposes to the development of such disorders like systemic lupus or systemic scleroderma [17–19]. Impairment in the expression of this factor was found in the pathogenesis of bronchial asthma, atherosclerosis, vascular inflammation and cirrhosis associated with HCV infection [20, 21]. In addition, elevated TGF-β1 levels were noted in conditions of increased tissue regeneration, and also in the development of vascular lesions characteristic for Alzheimer's disease [22].

TGF-β1 is mostly secreted in its inactive form called the latent complex. There can be two types of latent complexes distinguished: small and large, where the first one consists of TGF-β1 bound by latent TGF-β1-associated peptide (LAP) and the latter contains small latent complex bound by LTBP protein. The latent form of TGF-β1 is secreted to the extracellular matrix where it is stored until it is needed [23].

The presence of TGF-β2 was found in epithelial cells and nerve cells. Deficiency of this cytokine leads to developmental disturbances of the visual, auditory, spinal and urogenital tract [21]. In the nerve and glial cells, the TGF-β2 molecule is accompanied by TGF-β3. The absence of this third isoform may also lead to defects in the development of the palate and lungs [1, 4].

In follicles, TGF-β is responsible for the differentiation and proliferation of granulosa and theca cells, as well as inhibition of androgen production [7].

There are TGF-β receptors present in both theca and granulosa cells. This factor's effect on developing follicle is dependent on species and the stage of folliculogenesis. It can act as an inhibitor or stimulator of granulosa and theca cells proliferation. Also, TGF-β's role had been demonstrated in progesterone and inhibin secretion by granulosa cells [15].

TGF-β1 has a regulatory effect on primordial follicle growth in mice and is involved in maintaining the primordial follicle pool. It was observed that there was a decrease in the amount of primordial and growing follicles when ovaries were cultured with TGF-β1. Therefore, there is a possibility that this protein's relatively low level enables the activation of primordial follicles [24, 25].

Conditional ablation of TGF-β1's receptor in mice results in the altered functionality of the female reproductive tract, such as defective oviduct and uterus development, which prevents embryo implantation, thus causes problems with fertility [26].

TGF-β1 is also involved in uterus decidualization and is expressed in endothelial cells, in the decidua, as well as endometrial epithelial cells. Its liberation from the latent complex is vital in embryonic implantation, as it activates the Smad3 signalling pathway. Mouse study shows that estradiol plays an important role in TGF-β1 activation in the uterus [23]. However, its role in decidualization in humans remains unclear; several studies have shown that it acts as an inhibitor of decidualization, decreasing PRL, IGFBP-1 and TF expression [14]. However,

other findings suggest that TGF-β1 secreted by endometrial epithelial cells reinforces decidualization and is highly expressed in its active form during this process [23].

Inhibin A and B

Inhibins are glycoproteins, active in the form of dimers. They occur as several isoforms composed of α and βA, βB subunits that dimerize through disulfide bonds. Inhibin A contains the αβA subunits whereas inhibin B is composed of αβB. Inhibins participate in the regulation of many processes in the body, including the regulation of reproductive functions [27, 28]. Inhibin activation remains under the control of FSH and LH. It is mainly expressed in Sertoli and granulosa cells, but also in the placenta and other tissues, acting as activin antagonist, likely by binding to activin receptors. Although inhibin has lower affinity to activin receptor than activin, the role of TGBR3 as a coreceptor in this antagonism has been reported [29].

Inhibin A is secreted by the pituitary, adrenal glands, spleen, bone marrow, placenta and fetal membranes. In the ovary, the source of this glycoprotein is the corpus luteum and the dominant follicles. Inhibin A is involved in androgen synthesis regulation by increasing its secretion in theca cells [15]. It reduces FSHR (FSH receptor) mRNA level in granulosa cells, thus serves as a regulator of FSH responsiveness [29]. The levels of inhibin A concentrations in plasma serve an important role in the diagnosis of pathology of pregnant women such as ectopic pregnancy, hydatidiform mole, miscarriage, or Down's syndrome [30]. Inhibin A plasma level increases during pregnancy, reaching the peak in the third trimester. Lower levels of inhibin A has been reported in women who had a miscarriage. During the menstrual cycle, the highest level of inhibin A is observed at the mid-luteal phase in the female, while in the male it is absent [29].

Inhibin B is produced in granulosa cells of the early antral follicles. This glycoprotein may be an important marker of follicle growth as well as a useful tool for evaluating the response to induction of ovulation as a result of LH surge. In addition, changes in inhibin B levels may indicate premature ovarian failure and hypothalamic-related disorders [28, 31]. Inhibin B reaches its maximal level during the early follicular and early luteal phases in the female. In the male, it is expressed in Sertoli and Leydig cells, with its levels possibly influenced by IL-1 [29]. Total inhibin, which consists of the sum of precursors, subunits and inhibin particles is used as a potential marker for PCOS or ovarian cancer in humans.

Inhibins play a significant role in regulating the hormonal cycle of the female rats, affecting the secretion of FSH from the pituitary gland. Normalization of hormone secretion is based on negative feedback. Increased concentrations of ovarian glycoproteins secreted into the peripheral blood, inhibit the secretion of FSH [32] and decrease the half-life of FSHβ mRNA. Such feedback is also apparent in males [29].

Disruption of inhibin α gene, which encodes subunit normally present in both inhibin A and B, causes granulosa cell and testicular tumours, as well as an increased rate of follicular development, and disturbs the communication between the oocyte and somatic cells. However, the formation of primordial follicles remains unaffected. Also, a decrease in Leydig cells amount and enlarged testes can be observed in males. On the other hand, overexpression of α subunit causes ovarian cysts and a decrease in ovulation in females, as well as smaller testes and lower sperm number in males. Adult mice deprived of inhibin are infertile [29, 33].

Taken together, these observations show that inhibins are crucial factors in mammal reproduction.

Growth differentiation factor 9 (GDF9)

The GDF-9 gene, encoding the Growth Differentiation Factor 9, is 2.94 bp long and has two exons. GDF-9 differs from other TGF-β family members because it contains serine instead of a fourth cysteine residue, which is normally responsible for disulfide bond formation [34]. This factor is involved in the regulation of oogenesis and folliculogenesis. GDF-9 may play a role in the pathogenesis of polycystic ovary syndrome and premature ovarian dysfunction. Decreased mRNA expression of the gene encoding this factor was found in patients suffering from PCOS [35, 36].

This factor is synthesized in the oocytes, starting from the nucleation stage of primary follicles, as well as in cumulus granulosa cells. GDF-9 plays an important role in oocyte and granulosa cell communication, which enables correct folliculogenesis [37]. Female homozygous mice experimentally deprived of this gene were characterized by primary infertility due to inhibition of oocyte development and degeneration of reproductive cells. Moreover, smaller ovaries and a higher number of primordial and primary follicles have been observed, while there were no defects in male fertility [34]. It has been shown that the GDF-9 factor and complex kit/kit ligand interactions affect the regulation of oocyte growth, facilitating their cytoplasmic maturation, as well as the proliferation and differentiation of granulosa cells [15]. Also, GDF-9 influences somatic cells surrounding the oocyte and is required for normal granulosa and theca cells generation [15]. Rat ovaries treated with GDF-9 exhibit a higher amount of preantral follicles and fewer primordial ones, which shows GDF-9's role in promoting follicular development [38]. Moreover, this factor is involved in steroid synthesis, inhibition of luteinization and cumulus expansion. It also enhances FSHR expression, while decreasing LHR level [37].

GDF-9 acts synergistically with BMP15, another TGF-β family protein, in providing the correct developmental competence of the oocyte. Nonsynonymous mutations in this factor may contribute to its misfolding and dizygotic twinning, through the increase in a number of dominant follicles, as well as to primary ovarian insufficiency. Thus, GDF-9 is essential in female fertility [34].

TGFB – signalling pathway

The signal transduction pathways of transforming growth factors involve 3 types of membrane receptors, TGFβR -I, -II, -III, present in all cell types. The molecular weight of TβR-I is 53kDa, while TβR-II is approximately 75 kDa. It should be emphasized that the three receptors in a mammal are characterized by high similarity in construction. In humans, rats and mice they demonstrate > 98% homology in their amino acid sequence [39, 40]. In their structure, the receptors contain a domain that exhibits serine/threonine kinase activity, the transmembrane region, and the ligand-binding domain [41]. As a result of ligand attachment, TβR-II is activated, which in turn activates TβR-I by transphosphorylation of its extracellular glycine-serine domain (the so-called GS domain). TβR-III is a highly glycosylated transmembrane proteoglycan with a molecular weight of 280-330 kDa. The primary role of this receptor is to facilitate ligand access to TβR-I and TβR-II [42]. Receptors can also form heteromeric complexes, which gives rise to versatility in response to ligands and enables the induction of different signalling pathways as a result [43].

Activation of the TβR-I membrane receptor implies the activation of Smad proteins in the cytoplasm, via phosphorylation and oligomeric complexes formation, that act on many transcription factors in the cell nucleus, inducing gene expression. Among the proteins signalling to the nucleus, we distinguish the so-called R-Smad (including Smad 1,2,3,5,8) and co-Smad 4 (Smad 4). Smad 2 and 3 are activated through TβR-I and ActRIB and Smad 1, 5 and 8 are activated by Alk1, Alk2, Alk3 and Alk6 [43]. R-Smad proteins are mainly found in the cytoplasm and, after activation by TβR-I, associate with Smad 4 and are transported to the nucleus of the cell which results in altered gene expression [9]. R-Smads contain domains responsible for interaction with transcription factors and DNA binding, as well as sites that interact with transcriptional coactivators, such as p300 or CBP. They can also undergo ubiquitin-mediated degradation [43]. Smad antagonists include inhibitory expression in the oncoprotein-c-ski nucleus, c-Sno, which binds R-Smad and Co-Smad proteins [21]. After R-Smads fulfil their role, they are dephosphorylated in the nucleus and translocated back to the cytoplasm [43].

The signal transduction can proceed in a Smad-independent manner [14]. TGF-β may be involved in the transgenic MAPK kinase (mitogen-activated protein kinase) transduction pathway as well as Erk and JNK pathway activation [14]. Moreover, ligands of the TGF-β receptors may interact outside of TβR with endoglin, or beta-linked glycans eg. in endothelial cells [42, 44].

Except for the aforementioned receptors, there have been ancillary binding proteins identified that can influence TGF-β signalling pathways, such as α2-macroglobulin and follistatin. α2-macroglobulin binds to TGF-β, activin and inhibin, which prevents their degradation, whereas follistatin mainly binds activin and as a result disrupts its binding with the receptor [15].

The TGF-β signalling pathway is proven to be necessary in many processes involved in oogenesis, folliculogenesis, fertilization and embryonic development. For example, its role in successful uterine decidualization was observed in Smad-null mice, where oviductal and myometrial development was impaired [14].

Research perspectives

The superfamily of transforming growth factors β is involved in many processes that are crucial for both female and male reproduction ability. Unveiling the roles of TGF-β's ligands and receptors in reproduction may be helpful in infertility diagnosis and therapy. There are many mouse studies, that uncover the role of TGF-β family's components in reproduction. However, there are further studies needed to understand its role in humans.

TGF-β family proteins can be utilized as diagnostic markers of fertility and pregnancy disorders. Measuring inhibin A serum level is helpful in predicting spontaneous abortion, pre-eclampsia or Down's syndrome, as well as monitoring the ovarian reserve, thus there is a need to elaborate tests, where inhibin levels would be analyzed along with other markers in a non-invasive manner.

Moreover, the higher inhibin serum levels have been reported in women with PCOS compared to the control group. However, the exact role of inhibins in PCOS remains elusive and there are further studies required.

The decrease in inhibin activity is suspected to promote tumour formation in ovarian cancer. However, the molecular role of inhibin in is not entirely understood. Also, its role in pregnancy is unclear, but it is known to be secreted by corpus luteum in humans. It is suggested that both inhibins and activins may be involved in embryo transport and implantation, as their serum levels are lower in ectopic pregnancies, but there are further studies needed to elucidate that process [29].

The fact that TGF-β1 in its active form is crucial for embryo implantation can be helpful in determining the reasons of fertility problems as well as may be a potential therapeutic target, as it may

cause infertility when not dissociating from the latent complex. There is also a need to investigate how TGF-β Smad-independent signalling affects decidualization [14].

It is well established that a protein has to be folded correctly in order to fulfil its biological function. In the case of nonsynonymous mutations of GDF-9, this factor may be misfolded, which can cause many fertility disorders, such as PCOS or primary ovarian insufficiency. A potential solution to this problem can be the utilization of exogenous chaperones, which would ensure the correct GDF-9 conformation [34].

In summary, the TGF-β family contains proteins that are essential for both male and female fertility. They are involved in oogenesis, folliculogenesis, decidualization, fertilization and embryo implantation, as well as the correct development of the reproductive tract. These factors can be utilized as markers in infertility diagnosis and also serve as potential therapeutic targets.

Ethical approval

The conducted research is not related to either human or animal use.

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Conflict of interest statement

The authors declare they have no conflict of interest.

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