DOI: 10.15825/1995-1191-2023-2-148-157

DIAGNOSTIC AND THERAPEUTIC POTENTIAL OF TRANSFORMING GROWTH FACTOR BETA 1 IN SOLID ORGAN TRANSPLANTATION: RECENT RESEARCH FINDINGS

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Clinical outcomes of solid organ transplantation depend on many factors. One of the main factors is the risk of post-transplant complications, which affect allograft and recipient survival. Multifactorial organ damage in post-transplant complications and the search for diagnostic and prognostic indicators of the condition have contributed to the study and selection of a wide range of proteomic and molecular genetic biomarkers, which have shown to be effective in solid organ transplantation. The use of biomarkers opens up additional possibilities for assessing the risk of complications and their early diagnosis. This potentially reduces the frequency of invasive diagnostic procedures. Transforming growth factor beta 1 (TGF-β1) regulates many biological processes, has anti-inflammatory and immunosuppressive effects, participates in immune response, and plays a key role in extracellular matrix (ECM) protein synthesis. ECM dysregulation leads to fibroblast hyperproliferation and increased collagen synthesis and, consequently, tissue fibrosis. The variability of the diagnostic and prognostic potential of TGF-β1 has been demonstrated in studies on recipients of various solid organs. The objective of this review is to analyze recent evidence on the role of TGF-β1 in the development of post-transplant complications and to assess its prospects as a marker of graft pathology or as a target for therapy.

Keywords: solid organ transplantation, complications diagnosis, transforming growth factor beta, TGF-β1, biomarkers, fibrosis, rejection, nephrotoxicity.

INTRODUCTION

Clinical outcomes of solid organ transplantation depend on many factors. One of the main ones is the risk of post-transplant complications, which affect allograft and recipient survival. Multifactorial organ damage in post-transplant complications and the search for diagnostic and prognostic indicators of the pathology have contributed to the study and selection of a wide range of proteomic and molecular genetic biomarkers, which have shown to be effective in heart, liver, kidney and lung transplantation.

The use of biomarkers opens up additional possibilities for assessing the risk of complications and their early diagnosis. This potentially reduces the frequency of invasive diagnostic procedures [1].

TGF-β1 is an important biomarker of post-transplant complications. It regulates many biological processes, has anti-inflammatory and immunosuppressive effects, participates in immune response, and plays a key role in ECM protein synthesis synthesis – ECM dysregulation leads to fibroblast hyperproliferation and increased collagen synthesis and, consequently, tissue fibrosis [2]. TGF-β1 is involved in the pathogenesis of many diseases and, what is particularly attractive, is that it has a high therapeutic potential [3].

The aim of this review was to analyze recent data on the role of TGF-β1 in post-transplant complications in solid organ recipients and to assess its prospects as a marker of graft pathology or as a target for therapy.

STRUCTURE AND BIOLOGICAL ROLE

TGF-β1 is one of the components of the TGF-β superfamily, whose members received their names according to the history of their molecular identification. They include activins (ACT), inhibins (INH), bone morphogenetic proteins (BMP), growth differentiation factors (GDF), and Müller inhibitory substance (MIS) [4]. TGF-β is a homodimer consisting of two polypeptide chains, each containing 112 amino acid residues, connected by a disulphide bond and forming a complex of a total molecular weight of 25 kDa. Currently, three TGF-β isoforms are known: TGF-β1 (the most common), TGF-β2, and TGF-β3 [5].

TGF-β was originally classified as an immunomodulatory cytokine that induces and maintains immune tolerance. TGF-β1 has anti-inflammatory and immunosuppressive effects due to cytokine production by T-lymphocytes; TGF-β2 is involved in the development of immune tolerance and is effective in suppressing macrophage inflammatory responses. However, despi-

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te the structural similarities of the isoforms, more and more evidence point to differences in their biological properties: TGF-β1 and TGF-β2 have been shown to have predominantly profibrotic effects, while TGF-β3, in contrast, has been characterized as a fibromodulatory partner for the other two isoforms [6].

The biofunctions of TGF-β are realized through the TGFβRI, -II and -III receptors of the same name. When TGF-β binds to the receptors, activation of signaling pathways, including Smad-dependent ones, is triggered.

Activated TGF-β exerts its effects on cell proliferation, differentiation, and migration in part through its capacity to modulate the deposition of ECM components such as collagen, elastin, and fibronectin. Specifically, TGF-β isoforms have the ability to induce the expression of these proteins in mesenchymal cells and to stimulate the production of protease inhibitors that prevent enzymatic breakdown of the ECM. Dysregulation of these functions is associated with a change in the cellular structure, the cells acquire mesenchymal instead of epithelial properties [7].

Thus, the differences in the effects of TGF-β may be due to a variety of activation cascades in different cell types, peculiarities of the cellular environment, and the influence of other regulatory molecules.

TGF-β1 AND SMAD IN THE DEVELOPMENT OF PATHOLOGICAL PROCESSES

Smad is the main group of mediators of the biological action of TGF-β, which are activated in the development of a wide range of pathological processes in both humans and animals [8].

Three classes of Smads transcription factors have been identified: receptor-regulated Smads (R-Smads), common Smads (Co-Smads), and inhibitory Smads (I-Smads). R-Smads, including Smad1, Smad2, Smad3, Smad5, and Smad8, are directly activated through TGFβRI phosphorylation. Once Smad2 and Smad3 are activated, they bind to Smad4 to form a hetero-oligomeric complex Smad2–Smad3–Smad4, which translocates into the nucleus where it interacts with DNA directly or indirectly through other DNA-binding proteins, regulating transcription of target genes [9].

It is known that Smad3 has a profibrotic effect and is involved in the pathogenesis of kidney disease; Smad2 and Smad7 perform protective functions; Smad4 has a dual role, on one hand, it promotes Smad3-dependent renal fibrosis, on the other hand, it suppresses nuclear factor kappa B (NF-κB)--mediated inflammation via a Smad7-dependent mechanism [10].

Since the TGF-β/Smad3 complex is involved in transcription of a number of genes, this allows us to consider TGF-β as a promising marker of structural changes in organs, and Smad transcription factors as a target for correction of these processes [11].

ASSOCIATION OF TGF-β1 WITH BIOMARKERS OF POST-TRANSPLANT COMPLICATIONS: MIRNAS

MicroRNAs (miRNAs) are a group of small non-coding RNAs about 22 nucleotides long that circulate in biological fluids and regulate post-transcriptional gene expression [12]. Recent studies have shown promising applications of this class of signaling molecules for diagnosis of post-transplant complications, as well as potential targets for therapy [13].

A number of microRNAs involved in immune response reactions and development of structural changes of transplanted organs (primary dysfunction, fibrosis, acute cellular and humoral rejection) have been identified. An analysis of the mechanisms of action of some miRNAs revealed a link with TGF-β signaling pathways.

There is evidence of the involvement of miRNAs in renal inflammation and fibrogenesis and regulated by TGF-β1 via the Smad3 mechanism: miR-21, miR-93, miR-192, miR-216a, miR-377, miR-29, miR-200 [14].

A study by Zhang et al. showed that miR-27 upregulation increases cardiomyocyte activity and inhibits apoptosis, an effect mediated through TGFβRI receptors [15].

Suzuki et al. showed that miR-27 positively regulates mesenchymal gene induction with TGF-β participation [16]. Wang et al. described circulating miR-27 also as a regulator of myogenesis through TGF-β signaling pathway: miR-27 upregulation was associated with reduced myostatin level and muscle cell proliferation [17].

The effect of miR-27 on the development of bronchiolitis obliterans (BO), chronic rejection and fibrous obliteration of small airways after lung transplantation was investigated. In experiments on a model of orthotopic tracheal transplantation in mice, a protective effect of miR-27a-3p was shown by regulating TGF-β and Smad2/Smad4, as well as by maintaining dendritic cells in an immature state [18]. The authors point out the dual role of TGF-β, consisting, on one hand, in induction of tolerance and, on the other hand, in stimulation of myofibroblast transdifferentiation.

Participation of miR-27 in the mechanisms of myocardial fibrosis, BO, as well as the formation of immune response through influence on TGF-β reflects the prospects of the latter as a marker of structural changes in transplanted organs. This is supported by our earlier studies, which showed that plasma miR-27 and -339 upregulation in recipients was associated with the presence of histological signs of transplanted heart myocardial fibrosis [19]. At the same time, a significant decrease in miR-27 levels was observed in heart recipients with acute cellular rejection compared to recipients without rejection signs [20].

Recent studies by Cuiqiong et al. showed that the miR-101 family members play an important role in the pathogenesis of liver fibrosis. Through the TGF-β sig-

naling pathway, miR-101 regulates hepatic stellate cell activation and induces accumulation of extracellular matrix proteins in them [21].

The works of Li [22] and Pan [23] showed that miR-101 blocks TGF-β1/Smad2 signaling pathway by inhibiting RUNX1, which prevents development of postinfarction myocardial remodeling.

The overexpression of miR-142-3p in alveolar epithelial cells and lung fibroblasts is able to reduce the expression of transforming growth factor beta receptor 1 (TGFβ-R1) and profibrotic genes. Furthermore, exosomes isolated from macrophages present antifibrotic properties due in part to the repression of TGFβ-R1 by miR-142-3p transfer in target cells. Thus, macrophage-derived exosomes may fight against pulmonary fibrosis progression via the delivery of antifibrotic miR-142-3p to alveolar epithelial cells and lung fibroblasts [24].

TGF-β1 IN SOLID ORGAN RECIPIENTS

A wide range of immune and nonimmune cells, such as T-lymphocytes, monocytes, vascular endothelium and stromal cells, produce TGF-β under various conditions. According to numerous data, blood levels of TGF-β1 in healthy individuals vary widely (from 0.5 to 80 ng/mL) and are independent of gender [25, 26], but may vary with age. This fact was elucidated in more detail in the work of Okamoto et al.: the serum TGF-β1 level of healthy children under 14 years of age was significantly higher than that of healthy adults ($p < 0.01$), which obviously provides a reasonable basis for studying TGF-β1 in patients according to their belonging to the appropriate age group [27]. These results are also supported by our studies showing almost three-fold differences in the plasma TGF-β1 levels of healthy children and adults [28].

As a multifunctional cytokine, TGF-β1 is synthesized by a wide range of cells in various tissues and organs, stimulating the accumulation of ECM proteins.

TGF-β1 IN LIVER RECIPIENTS

The main source of TGF-β1 in liver tissues is stellate cells, whose profibrotic properties are activated under the influence of various factors [29].

In this aspect, TGF-β1 levels can be considered as a diagnostic or prognostic marker of liver pathology. Studies of a pediatric group of patients with end-stage liver failure, conducted at Shumakov National Medical Research Center of Transplantology and Artificial Organs, showed that TGF-β1 levels are associated with the presence of pathology and, moreover, the degree of organ damage [30].

Clinical studies of liver recipients have shown a tendency for increased TGF-β1 levels in the blood with preserved graft function (44.7 ± 7 ng/mL) compared to recipients with a history of rejection crises (32.7 ± 3 ng/mL) [31].

TGF-β1 IN KIDNEY RECIPIENTS

The source of TGF-β in the kidney is parenchymal cells, lymphocytes, or circulating TGF-β molecules in the blood. The extracellular concentration of TGF-β is primarily regulated by the conversion of inactive TGF-β to the active form, which is often overlooked by researchers because of the complex biological nature of TGF-β [32].

Plasma TGF-β1 level is a potential indicator of the progression of chronic kidney disease [33]. Experiments on animals have shown that TGF-β1 overexpression in the kidneys induced interstitial proliferation, tubular epithelial aerophagia and renal fibrosis with ECM accumulation in tubulointerstitium, capillaries and glomerulus, accompanied by decreased glomerular filtration rate [34]. Progressive renal fibrosis contributed to nephron dysfunction and albuminuria [35]. Genetically determined TGF-β1 deficiency in mice also led to inflammation of several organs, including the kidneys [36].

The effect of TGF-β on transplanted kidney has not been sufficiently studied. A study of TGF-β1 levels at 6 months after kidney transplantation showed higher TGF-β1 concentrations in the group of recipients with chronic rejection compared to the group without it; there was also a positive correlation between TGF-β1 levels and the total cellular infiltrate in kidney biopsies. Importantly, both groups had a history of biopsy-proven acute rejection, which characterizes TGF-β as a marker of chronic rejection [37].

However, Du et al. found that TGF-β1 levels in blood correlated directly with the duration of graft survival [38]. At the same time, TGF-β1 levels correlated positively with estimated glomerular filtration rate, and negatively with serum creatinine levels.

A recent observational cohort study of 1271 kidney transplant pairs showed that donor genotype frequencies of rs1800472 in TGF-β1 differed significantly between patients with and without graft loss ($p = 0.014$), and recipients carrying the T-allele of the TGF-β1 variant showed had a higher risk of graft loss in the long-term. Given that the T allele has a lower level of TGF-β1 expression, these results suggest a positive effect of TGF-β1 signal transduction on the long-term survival of transplanted kidney [39].

Due to both the profibrogenic and protective effects of TGF-β1, there are conflicting data in the literature on the effect of this growth factor on renal transplant survival.

TGF-β1 IN HEART RECIPIENTS

Infiltration by macrophages, suppression of lymphocyte function, fibroblast proliferation, and collagen synthesis are important processes regulated by TGF-β1 and leading to chronic heart failure (CHF). In the heart, TGF-β1 is synthesized by cardiomyocytes and fibroblasts and is released during myocardial infarction, pressure

overload, angiotensin II and noradrenaline administration, and inhibited by nitric oxide [40].

Numerous studies of patients with CHF show that type I and type III collagen gene expression is associated with TGF-β1 [41]. A study of patients with dilated cardiomyopathy at Shumakov National Medical Research Center of Transplantology and Artificial Organs showed the relevant results: plasma TGF-β1 levels of CHF patients was higher than that of healthy subjects (29.9 ± 19.7 ng/mL vs. 8.7 ± 7.5 ng/mL, p = 0.001) [42]. At the same time, after heart transplantation, the TGF-β1 level in the recipients' blood plasma significantly decreased, and in the long term reached the level characteristic of healthy individuals.

Obviously, the role of TGF-β1 in graft pathology in heart recipients is of particular practical interest. The detection of myocardial fibrosis associated with cyclosporine therapy has provided the basis for the assumption that this drug may contribute to diastolic dysfunction of the cardiac allograft [43]. Meanwhile, the effects of TGF-β1 on cardiac fibroblast proliferation vary. Some studies have reported that TGF-β1 stimulates cardiac fibroblast proliferation, while others have demonstrated its antiproliferative effects [44]. Such different results can be down to possible differences in differentiation of fibroblast populations, as well as to the influence of other growth factors.

A five-year follow-up by E. Aziz in 152 heart recipients allowed to assess the magnitude of TGF-β1 expression in cardiac transplants [45]. According to the results obtained, frequent episodes of cellular rejection during the first two years after transplantation were accompanied by higher TGF-β1 levels in tissues, and initiated a series of inflammatory and immune responses with subsequent diastolic dysfunction and myocardial fibrosis. In another study, the authors were able to show the association of TGF-β1 expression in biopsy specimens of transplanted hearts with the development of vasculopathy and low survival rates [46].

In heart recipients with the AA genotype of the rs1800470 polymorphism of the TGF-β1 gene, myocardial fibrosis verified by endomyocardial biopsy was detected more frequently than in G allele carriers, which may also indicate the association of the TGF-β1 gene polymorphism with transplant myocardial fibrosis [47].

TGF-β1 IN LUNG RECIPIENTS

According to the International Society for Heart and Lung Transplantation, the 5-year survival rate of lung recipients is about 53%. The main factor affecting long-term survival is chronic graft rejection, histologically characterized by BO, caused by inflammatory or fibrotic processes in the bronchioles [48]. Physiologically, BO is accompanied by airflow limitation due to significant structural changes in the graft (partial or complete occlusion of the airway lumen). Occlusion is often associated with destruction of the airway smooth muscle and elastin fibers. Among a variety of cytokines and growth factors, TGF-β plays the most significant role in this process [49].

In a number of studies, the authors studied the mechanisms of BO development, and established a positive correlation between the frequency of this complication and the level of TGF-β1 expression [50]. Moreover, as early as 1997, in an experiment on mice, TGF-β expression was found to have a direct influence on the development of severe interstitial fibrosis [51]. A study by Charpin et al. in 1998 showed that TGF-β expression increases in lung recipients even before manifestation of obvious clinical signs of BO [52]. Thus, in several patients, the maximum TGF-β1 levels in the tissue were recorded several months before BO was diagnosed, and these patients died within 2 years after diagnosis. These results suggest that increased TGF-β levels are an early prognostic marker of chronic rejection of transplanted lungs.

A number of studies also demonstrate the mechanism of TGFβ/Smad signaling cascade activation in fibrogenesis processes in a lung transplant, and the concept of TGF-β inhibition as a target for therapy underlies most ideas aimed at improving lung transplant outcomes [53, 54].

TGF-β1 AND IMMUNOSUPPRESSION

The use of calcineurin inhibitors has led to significant advances in transplantation with excellent short-term outcomes. For example, cyclosporine A (CsA) has revolutionized transplantology since the 1970s due to its immunosuppressive effect [55]. The powerful immunosuppressive properties of tacrolimus were discovered later in 1984 [56].

However, despite almost half a century of successful use of these drugs, a wide range of side effects that reduce recipient long-term survival, the main one being nephrotoxicity, remains the Achilles heel of most immunosuppressive regimens.

Fibrosis occurs through induction of epithelial-mesenchymal transition (EMT) processes by TGF-β1 produced by damaged parenchymal cells and macrophages. The triggering of PI3K/Akt/GSK-3β signaling cascade leads to increased expression of Ser-9-phosphorylated inactive form of GSK-3β and accumulation of β-catenin in cytoplasm followed by nuclear translocation [57]. The fact that TGF-β1 is involved in CsA-induced EMT allows us to evaluate possible ways of inhibiting this process.

A recent study by Nagavally et al. described the nephroprotective role of a natural flavonoid, chrysin, which inhibited TGF-β1 signal transduction and prevented cytoplasmic β-catenin accumulation. The authors cite promising results and point to the efficacy and safety of CsA in combination with chrysin, which will significantly

reduce the undesirable effects of immunosuppressive therapy [58].

Since tacrolimus directly or indirectly induces TGF-β1 expression, ways to combat chronic nephropathy are also being developed in relation to immunosuppressive therapy regimens [59].

Zhang et al. studied the effect of traditional Chinese phytotherapeutic agent, used in various inflammatory processes in the kidneys on the action of tacrolimus in rats. During the experiments, combined use of the drugs suppressed the expression of TGF-β1/Smad2/3/βig-h3 and proinflammatory cytokines, and weakened oxidative stress and apoptosis [60].

In addition, it should be noted that opportunistic infection (OI) remains a serious complication throughout the post-transplant period, jeopardizing the benefits of any long-term immunosuppressive therapy. From this point of view, evidence from Boix et al. suggesting TGF-β1 as a predictor of OI in the first year after liver and kidney transplantation are of great interest [61]. In their study, the authors showed that concentration of TGF-β >363.25 pg/mL in liver and TGF-β >808.51 pg/mL in kidney recipients were able to stratify patients at high risk of OI with a sensitivity and specificity above 70% in both types of solid organ transplantations.

Thus, TGF-β regulation acts as an important etiological factor in chronic nephrotoxicity and other immunosuppressant-induced complications, and the impact on this pathway can reduce the undesirable effects of therapy and potentially improve long-term transplant outcomes.

TGF-β1 AS A TARGET FOR THERAPY

One of the main tasks of transplantology is to achieve long-term graft and recipient survival, reducing possible post-transplant risks. Along with the development of new effective methods for diagnosing complications, there is an active search for therapeutic targets as a possible way to solve the problem.

Evidence of the role of TGF-β in fibrosis development became a breakthrough and led to increase in the number of studies aimed at searching for new drugs such as antisense oligonucleotides, neutralizing antibodies, cyclic pentapeptides, TGF-β ligand traps and small-molecule kinase inhibitor drugs, etc. [62].

To stop progressive fibrosis in experimental glomerulonephritis, Border et al. for the first time used *in vivo* injections of anti-TGF-β1 neutralizing antibodies. Injection of anti-TGF-β1 antibodies in acute mesenchymal proliferative glomerulonephritis suppressed ECM protein production and slowed fibrosis progression, which was histologically confirmed [63].

Another study aimed at developing an experimental therapy was the introduction of antibodies against TGF-β receptor type II (TGFβRII) inhibiting mesenchymal matrix growth, which was confirmed by decreased proteinuria compared to the control group of rats with glomerulonephritis [64].

Currently, drugs targeting members of the TGF-β superfamily or their receptors are under development, and dozens of antifibrotic agents with different targets are being tested; they are mostly chemically synthesized oligonucleotides. For example, injections of miR-326 into mice with induced pulmonary fibrosis were accompanied by antifibrotic effects through Smad7 regulation and a significant decrease in TGF-β1, Smad3, matrix metalloproteinase-9 (MMP-9) [65].

Preclinical trials have shown the effectiveness of therapy with antisense oligonucleotides that inhibit TGF-β gene expression and reduce tissue fibrosis in glomerulonephritis [66]. The possibility of highly selective antibodies SRK-181, whose key mechanism is to prevent the cleavage of TGF-β1 precursor and the release of mature TGF-β1, is also being studied [67].

Another highly effective selective inhibitor, AVID200, targets an altered TGFβRII receptor. It enhances the binding activity of TGFβRII to TGF-β1 and TGF-β3 and thus greatly reduces the binding activity to TGF-β2 [68].

Luangmonkong et al. found no visible side effects of lifelong blocking of TGF-β activity in mice, although in humans, its long-term systemic suppression was associated with a high risk of therapy toxicity and decreased regenerative tissue function, which is due to the functional pleiotropy of TGF-β [69].

The results published to date reveal the complexity of the process of developing therapeutic approaches using TGF-β1 in humans, which require a comprehensive study to minimize undesirable effects.

CONCLUSION

The validity of the concept of TGF-β1 as a diagnostic and prognostic marker of the risk of adverse events in solid organ transplantation has been confirmed by numerous studies in recent years. Both in experimental models and in the clinic, the key role of TGF-β1 in a wide range of biological processes, namely mechanisms of allograft tissue fibrosis, formation of immune tolerance, and in the cascade of inflammatory reactions against nephrotoxicity of immunosuppressive drugs used, has been shown. The role of TGF-β1 in very severe post-transplant complications – transplant rejection and fibrosis – is still the focus of research.

The variability of the diagnostic and prognostic potential of TGF-β1 has been demonstrated in studies of recipients of various solid organs. For example, post-lung transplant bronchiolitis obliterans and chronic rejection is associated with increased blood TGF-β1 levels, while in contrast, adverse events (progression of liver failure and fibrosis) in liver transplantation are associated with decreased TGF-β1 levels, and higher levels are typical for recipients with intact liver graft function. Studies in heart and kidney transplantation show conflicting evi-

dence on the profibrotic and antiproliferative effects of TGF-β1.

Equally important is the fact that TGF-β1 levels change with age, which necessitates a separate study of TGF-β1 in pediatric recipients.

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The authors declare no conflict of interest.

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The article was submitted to the journal on 05.05.2023