Role of Transforming growth factor-beta 1 Gene Polymorphism in Congenital Heart Disease

***1 Alaa Refaat Mohamed Ibrahim, 1 Bashir Abd Allah Hassan, 2 Amal Fawzy, 1 Ahmed Abd El Samad El Hewala**
1 Pediatrics Department, 2 Medical Biochemistry Department, Faculty of Medicine, Zagazig University, Egypt
***Corresponding author: AlaaRefaat Mohamed Ibrahim**
Email: alaaarefaat578@gmail.com, Mobile: 01006259587.

Abstract

Congenital heart diseases (CHDs) are believed to be caused by abnormal gene functioning during embryonic heart development. Transforming growth factor-beta1 (TGFB1) is known to express in the early embryonic heart and regulates heart development.

KeyWords:.

DOINUMBER:10.48047/NQ.2022.20.19.NQ99368 **NEUROQUANTOLOGY2022;20(19):4046-4053**

Introduction:

In the early 1980s, it had become apparent that cell growth is controlled by many polypeptides and hormones. A new hypothesis of 'autocrine secretion' was postulated, which suggested that polypeptide growth factors are able to cause malignant transformation of cells **(1).**

A new polypeptide called SGF (Sarcoma Growth Factor) was discovered in cultures of transformed rat kidney fibroblasts (**2)**; soon it became apparent that this factor is a mixture of at least two substances with different functions. They were called Transforming Growth Factor-α (TGF-α) and Transforming Growth Factor-β (TGF-β) (**3).**

TGF-β was further described by Roberts and Sporn as a secreted polypeptide capable of inducing fibroblast growth and collagen production (**4).** Soon after its discovery, TGF-β was found to inhibit cell proliferation as well; thus, a dual role of this cytokine was recognized (**5).**

TGF-β family and isoforms

The TGF-β superfamily is composed of a large group of proteins, including the activin/inhibin family, bone morphogenetic proteins (BMPs), growth differentiation factors (GDFs), the TGF-β subfamily, and the glial cell line-derived neurotrophic factor (GDNF) family. This review will focus solely on the TGF-β family. The TGF-β proteins have been discovered in a variety of species, including invertebrates as well as vertebrates. TGF-β superfamily is fundamental in regulation of various biological processes, such as growth, development, tissue homeostasis and regulation of the immune system (**6).**

Beta-type subfamily growth factors are homodimeric or heterodimeric polypeptides with multiple regulatory properties depending on cell type, growth conditions and presence of other polypeptide growth factors. Since their expression is also controlled by distinct promoters, their secretion is temporal and tissue specific (**4).**

There are three known isoforms of TGF-β (TGF-β1, TGF-β2 and TGF-β3) expressed in mammalian tissues; they contain highly conserved regions but diverge in several amino acid regions. All of them function through the same receptor signaling pathways (**7).**

TGF-β1, the most abundant and ubiquitously expressed isoform, was cloned from human term placenta mRNA (**8).** In mouse development, Tgf-β1 mRNA and/or protein have been localized in cartilage, endochondral and membrane bone and skin, suggesting a role in the growth and differentiation of these tissues (**9).**

TGF-β2 was first described in human glioblastoma cells. It was found that TGF-β2 is

capable of suppressing interleukin-2-dependent growth of T lymphocytes. Thereby, it was named glioblastoma-derived T cell suppressor factor (G-TsF). Physiologically, TGF-β2 is expressed by neurons and astroglial cells in embryonic nervous system (**10).**

It is also important in tumor growth enhancing cell proliferation in an autocrine way and/or reducing immune-surveillance of tumor development (**11).** Their mature forms, which consist of the C-terminal 112 amino acids, TGF-β1 and TGF-β2 share 71% sequence similarity (**12).**

The third isoform, TGF-β3, was isolated from a cDNA library of human rhabdomyosarcoma cell line; it shares 80% of amino acid sequence with TGF-β1 and TGF-β2. Studies on mice demonstrated essential function of Tgf-β3 in normal palate and lung morphogenesis and implicate this cytokine in epithelial-mesenchymal interaction (**10).** Its mRNA is present in lung adenocarcinoma and kidney carcinoma cell lines; interestingly, umbilical cord expresses very high level of TGF-β3 (**13).**

TGF-β synthesis and activation

Mature dimeric form of TGF-β, composed of two monomers stabilized by hydrophobic interactions and disulphide bridge, initiates intracellular signaling. The three TGF-βs are synthesized as pro-proteins (pro-TGF-βs) with large amino-terminal pro-domains (called latency associated proteins – LAPs), which are required for proper folding and dimerization of carboxy-terminal growth-factor domain (mature peptide) (**4).**

This complex is called 'small latent complex' (SLC). After folding and dimerization, TGF-β dimer is cleaved from its pro-peptides in trans-Golgi apparatus by furin type enzymes; however, it remains associated with its pro-peptide through noncovalent interactions, creating 'large latent complex' (LLC). Most cultured cell types release latent TGF-β into extracellular matrix as LLC which in addition includes a 120–240 kDa glycoprotein called latent TGF-β binding protein (LTBP) (**14).**

LTBP is composed primarily of two kinds of cysteine-rich domains: EGF-like repeats (most of which are calcium-binding) and eight-cysteine domains. LTBP participates in the regulation of latent TGF-β bioavailability by addressing it to the extracellular matrix (ECM). Non-active TGF-β stays in ECM; its further activation is a critical step in the regulation of its activity (Figure 1) (**15).**

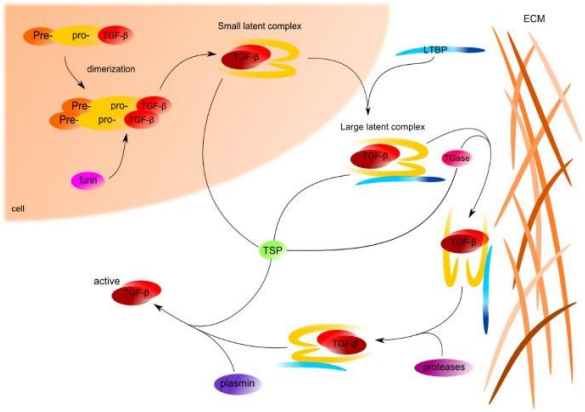

Figure (1): TGF-β synthesis and activation **(6).**

TGF-βs are synthesized as inactive precursors that contain pre-region (Signal peptide) and pro-region (N terminal peptide - LAP). Processing of inactive form starts with proteolytic cleavage that removes signal peptide from pre-pro-TGF-βs form. After dimerization, TGF-βs are cleaved by proteases (eg. Furin) into C-terminal mature peptides and N-terminal LAP (Latency Associated Peptide). TGF-βs with LAP form small latent complexes (SLP) that are transported to extracellular matrix where can further covalently bind to latent TGF-β binding protein (LTBP) to form a large latent complexes (LLC). LTBP is able to connect inactive TGF-β forms to ECM proteins. This interaction is further supported by covalent transglutaminase-induced (TGase) crosslinks. Activation of TGF-β starts with release of LCC from ECM by proteases. Then, the mature protein is cleaved from LTBP, which is provided *in vitro* by acidic condition, pH or plasmin or *in vivo* by thrombospondin (TSP). Once the active TGF-β family member is released from the ECM, it is capable of signaling **(6).**

A number of papers have reported TGF-β activation by retinoic acid and fibroblast growth factor-2 (FGF-2) in endothelial cells(**16)**, or by endotoxin and bleomycin in macrophages (**17).**

Further, a variety of molecules is involved in TGF-β activation. Proteases including plasmin, matrix metaloproteases MMP-2 and MMP-9, are TGF-β activators *in vitro* **(18).** Other molecules involved in the mechanism of activation are thrombospondin-1, integrins, such as αVβ6 or αVβ8, or reactive oxygen species (ROS) (**19).**

Moreover, latent TGF-β present in conditional medium is activated by acid treatment (pH 4.5) *in vitro*. *In vivo*, a similar pH is generated by osteoclasts during bone resorption. Since the bone matrix deposited by osteoblasts is rich in latent TGF-β, the acidic environment created by osteoclasts *in vitro* might result in latent TGF-β activation (**6).**

TGF-β receptors

In most cells, three types of cell surface proteins mediate TGF-β signaling: TGF-β receptor I (TβRI), II (TβRII) and III (TβRIII) (**20).** Out of these three receptors, TβRIII, also called betaglycan, is the largest (250–350 kDa) and most abundant binding molecule. This cell-surface chondroitin sulfate / heparan sulfate proteoglycan is expressed on both fetal and adult tissues and most cell types (**21).**

Endoglin (CD105) was shown to act as type III receptor for TGF-β as well (**22).**Endoglin is a membrane, an RGD-containing glycoprotein, which is expressed in a limited set of cell types, primarily vascular endothelial cells, several hematopoietic cell types, bone marrow stromal cells and chondrocytes. Its expression strongly increases in active vascular endothelial cells upon tumor angiogenesis (**23).**

Moreover, in normal brain, it was found to be expressed in the adventitia of arteries and arterioles, and it is expressed on several types of tumor cells, such as invasive breast cancers and cell lines or renal cell carcinoma **(4).**

Although betaglycan and endoglin are co-receptors not directly involved in intracellular TGF-β signaling due to lack of kinase domain, they can control access of TGF-β to TGF-β receptors and consequently modulate intracellular TGF-β activity (**24).**

Betaglycan binds all three isoforms of TGF-β, with higher affinity for TGF- β2; however, endoglin binds TGF-β1 and -β3 with constant affinity and has only weak affinity for TGF-β2. TβRI and TβRII mediate signal transduction. Both receptors are transmembrane serine/theronine kinases, which associate in a homo- or heteromeric complex and act as tetramers. They are organized sequentially into an N-terminal extracellular ligand-binding domain, a transmembrane region, and a C-terminal serine/threonine kinase domain. The type II receptors range from 85 to 110 kDa, while the type I receptors are smaller and their size ranges from 65 to 70 kDa**(4).**

Moreover, TβRI contains a characteristic, highly conserved 30 amino acids long GS domain in the cytoplasmic part, which needs to be phosphorylated to fully activate TβRI. TβRII contains 10 bppolyadenine repeat in the coding region of the extracellular domain. This region is frequently a target of changes leading to frameshift missense mutations or early protein terminations that result in truncated or inactive products **(4).**

TGF-β receptors activation

Bioactive forms of TGF-βs are dimers held together by hydrophobic interactions and, in most cases, by an intersubunit disulfide bond as well. The dimeric structure of these ligands suggests that they function by bringing together pairs of type I and II receptors, forming heterotetrameric receptor complexes. Binding of TGF-β to extracellular domains of both receptors also induces proper conformation of the intracellular kinase domains. These receptors are subject to reversible post-translational modifications (phosphorylation, ubiquitylation and sumoylation) that regulate stability and availability of receptors as well as SMAD and non-SMAD pathway activation **(6).**

Receptor phosphorylation activates TGF-β signaling pathway – the ligand binds to TβRII first, followed by subsequent phosphorylation of a Gly-Ser regulatory region (GS-domain) within TβRI. This leads to incorporation of TβRI and formation of a large ligand-receptor complex that consists of dimeric TGF-β ligand and two pairs of TβRI and TβRII (**24).**

The TGF-β receptor complex is extremely stable upon solubilization. TGF-β1 and TGF-β3 bind to TβRII without participation of type I receptor, whereas TGF-β2 interacts only

with combination of both receptors. Although ligand binding may induce autophosphorylation of TβRII cytoplasmic domain, signaling in the absence of TβRI has not been reported. TβRIII betaglycan promotes binding of TGF-β2 to TβRII, since the affinity of TGF-β2 to TβRII is low in the absence of betaglycan**(6).**

Endoglin binds TGF-β1, TGF-β3 but not TGF-β2 in the presence of the TβRI and TβRII. In some cell types, endoglin was found to inhibit TGF-β signaling – for example in chondrocytes, it enhances TGF-β1-induced SMAD1/5 phosphorylation but inhibits TGF-β1-induced SMAD2 phosphorylation (**25).**

Ubiquitylation and ubiquitin-mediated degradation define stability and turnover of receptors. Ubiquitylation occurs through sequential actions of E1, E2 and E3 ubiquitin ligases that provide specificity in the ubiquitylationproces. The E3 ubiquitin ligases such as Smurf1 and Smurf2 (SMAD ubiquitylation-related factor 1 and 2) regulate the stability of TβRI and heteromeric TGF-β receptor complex.Sumoylation, similarly to ubiquitylation, requires E1, E2 and E3 ligases which results in SUMO polypeptide attachment. Although sumoylation has not been observed for any other transmembrane receptor kinases, it was shown to modify TβRI function by facilitating the recruitment and phosphorylation of SMAD3. TGF-β receptors are also constitutively internalized via clathrin-dependent or lipid-raft-dependent endocytic pathways (**26).**

TGF-beta play crucial role in cardiac development:

Transforming growth factor-β (TGFβ), a group of ubiquitously-expressed pluripotent cytokines, is implicated in a wide variety of physiological and pathological processes (**27).**

A previous study revealed that TGFβ promoted valve remodeling and differentiation via inducing matrix organization and suppressing cushion mesenchyme differentiation into cartilage cell lineage during heart development (**28).**

TGFβ1, one isoform of TGFβ, has been demonstrated to play a functional role in the development, physiology and pathology of vascular system, through participating in cell cycle, proliferation, differentiation, maturation and apoptosis (**29).**

Besides, its over-expression can lead to organ fibrosis and dysfunction. TGFβ1 gene is located on chromosome 19q13.1-q13.3, containing seven exons and six large introns (**30).**

Polymorphisms in TGFβ1 gene may change the expression and function of TGFβ1 protein, leading to many cardiovascular diseases. However, the correlation of TGFβ1 gene polymorphisms with CHD susceptibility is still unclear. Many polymorphisms in the gene TGFβ1 have been identified, including rs1982073 and rs1800471, which could change the protein expression or structure (**31).**

TGFβ1, a profibrogenic cytokine, has close relation with diabetic nephropathy (DN), congenital hepatic fibrosis and many other congenital diseases. Although the amino acid sequence of TGFβ1 is highly conserved in mammals, common genetic variations in TGFβ1 gene can alter the expression or its protein structure (**32).**

Several polymorphism sites have already been reported, including rs1800468 (G/A) and rs1800469 (C/T) in the promoter region, rs1982073 (T/C, Leu10Pro) and rs1800471 (G/C, Arg25Pro) in the signal peptide region, and rs1800472 (C/T, Thr263Ile) in the region encoding the precursor part of the protein (**33, 34).**

A study on TGFβ1 SNP rs1982073 in hypertension patients with left ventricular hypertrophy revealed that the C allele of the polymorphism promoted the gene expression and the risk of hypertensive left ventricular hypertrophy **(35).**

Some scholars have reported that TGFβ1 could inhibit the formation of atherosclerosis, being a risk factor for such disease (**36).**

TGF-beta (Transforming Growth Factor-beta) signaling plays a crucial role in cardiac development by regulating various processes involved in cardiomyocyte proliferation, differentiation, and morphogenesis.

Here's how TGF-beta affects cardiac development: Cardiomyocyte Proliferation: During early cardiac development, TGF-beta

signaling promotes cardiomyocyte proliferation, contributing to the expansion of the developing heart. TGF-beta ligands bind to their receptors (TGFBR1 and TGFBR2), activating downstream signaling pathways that promote cell cycle progression and mitotic activity in cardiomyocytes.Cardiomyocyte Differentiation: TGF-beta signaling is involved in the differentiation of progenitor cells into functional cardiomyocytes. TGF-beta ligands, in coordination with other signaling pathways, help drive the differentiation of cardiac progenitor cells, guiding their commitment towards the cardiac lineage.Endocardial Cushion Formation: TGF-beta signaling is critical for the formation of endocardial cushions, which are essential structures in cardiac septation and valve development. TGF-beta signaling promotes the transformation of endocardial cells into mesenchymal cells, a process known as endothelial-mesenchymal transition (EndMT). This transition is necessary for the formation of endocardial cushions.Cardiac Morphogenesis: TGF-beta signaling influences the morphogenesis of the developing heart by regulating cardiac cell migration, tissue remodeling, and patterning. TGF-beta signaling pathways interact with other signaling networks, such as BMP (Bone Morphogenetic Protein) and Wnt, to coordinate complex morphogenetic events like chamber formation, septation, and cardiac looping.Valve Formation: TGF-beta signaling is involved in the formation and remodeling of cardiac valves. TGF-beta ligands regulate the differentiation and migration of valve progenitor cells, as well as the synthesis and organization of extracellular matrix components within the valves.6. Regulation of Cardiac Extracellular Matrix: TGF-beta signaling influences the production and remodeling of the cardiac extracellular matrix (ECM). TGF-beta promotes the synthesis of ECM proteins, such as collagens and fibronectin, and regulates the balance between ECM deposition and degradation, essential for cardiac tissue organization and function **(37,38)**.

Some specific examples of CHD that has been associated with abnormal TGF-beta signaling:

Atrioventricular Septal Defect (AVSD): AVSD is a common congenital heart defect characterized by a malformation of the atrial and ventricular septa, as well as abnormalities in the atrioventricular valves. Studies have shown that mutations in genes involved in the TGF-beta signaling pathway, such as GATA4 and NKX2.5, can disrupt normal cardiac development and lead to AVSD.Tetralogy of Fallot (TOF): TOF is a complex congenital heart defect characterized by a combination of four abnormalities: ventricular septal defect (VSD), overriding aorta, pulmonary stenosis, and right ventricular hypertrophy. Dysregulation of TGF-beta signaling has been implicated in the pathogenesis of TOF, particularly through its influence on cardiac neural crest cell migration and differentiation.Hypoplastic Left Heart Syndrome (HLHS): HLHS is a severe congenital heart defect in which the left side of the heart is underdeveloped. Studies have identified abnormalities in TGF-beta signaling components, such as the NOTCH1-TGFBR2 signaling axis, that play a role in the development of HLHS.Bicuspid Aortic Valve (BAV): BAV is a common congenital heart defect characterized by the presence of two instead of three aortic valve leaflets. Disruptions in TGF-beta signaling, particularly mutations in TGFBR1 and TGFBR2 genes, have been implicated in the pathogenesis of BAV and the associated aortopathy.Pulmonary Valve Stenosis:Pulmonary valve stenosis is a congenital heart defect characterized by narrowing of the pulmonary valve, which restricts blood flow from the right ventricle to the pulmonary artery.Studies have suggested that dysregulation of TGF-beta signaling, particularly alterations in the expression of TGF-beta ligands and receptors, can contribute to the development of pulmonary valve stenosis.Hypoplastic Right Heart Syndrome (HRHS): HRHS is a rare congenital heart defect in which the right side of the heart, including the right ventricle and tricuspid valve, is underdeveloped. Abnormalities in TGF-beta signaling, such as

mutations in genes like GATA4 and NKX2.5, have been implicated in the pathogenesis of HRHS.Transposition of the Great Arteries (TGA): TGA is a congenital heart defect in which the positions of the pulmonary artery and the aorta are switched, resulting in parallel circulations instead of the normal series circulation. Studies have shown that dysregulated TGF-beta signaling can disrupt the development of the cardiac outflow tract, contributing to the development of TGA.Aortic Aneurysm and Dissection: Aortic aneurysm and dissection are conditions characterized by the weakening and expansion of the aorta, which can lead to life-threatening complications. Abnormalities in TGF-beta signaling, including mutations in TGFBR1 and TGFBR2 genes, have been associated with aortic aneurysm and dissection, suggesting its involvement in the pathogenesis of these conditions.These examples highlight the diverse range of congenital heart diseases that have been linked to abnormal TGF-beta signaling. It's important to note that the specific mechanisms by which TGF-beta signaling disruptions contribute to each condition may vary and require further investigation**(39, 40).**

Potential therapeutic targets within the TGF-beta signaling pathway for intervention and treatment of CHD:

The identification of potential therapeutic targets within the TGF-beta signaling pathway offers promising avenues for intervention and treatment of congenital heart diseases (CHD). Here are some potential targets that have been explored:TGF-beta Receptor Inhibitors: Targeting the TGF-beta receptors, such as TGFBR1 and TGFBR2, has been investigated as a potential therapeutic strategy. Small molecule inhibitors or antibodies that block the activity of these receptors could help modulate aberrant TGF-beta signaling and potentially mitigate the progression of CHD.Smad Proteins and Co-Factors: Smad proteins play a crucial role in transmitting TGF-beta signals from the cell surface to the nucleus. Modulating the activity of Smad proteins or their co-factors could offer a targeted approach for manipulating TGF-beta signaling in specific contexts of CHD.Downstream Signaling Effectors: Targeting downstream effectors of TGF-beta signaling, such as transcription factors and other signaling molecules, could be explored to restore normal signaling patterns in CHD. Identifying specific molecules that interact with TGF-beta signaling and contribute to CHD pathogenesis may offer opportunities for therapeutic intervention.Modulators of TGF-beta Signaling Crosstalk: TGF-beta signaling interacts with other signaling pathways, such as BMP and Wnt, during cardiac development. Targeting molecules involved in the crosstalk between TGF-beta and other signaling pathways could potentially restore balance and correct abnormalities associated with CHD.Gene Therapies: Gene therapies hold promise for correcting genetic mutations and dysregulation of TGF-beta signaling genes implicated in CHD. Techniques such as CRISPR/Cas9 gene editing or gene replacement strategies could be utilized to restore normal TGF-beta signaling function.Pharmacological Approaches: Small molecules, drugs, or compounds that can modulate TGF-beta signaling components or downstream targets may offer therapeutic potential. High-throughput screening and drug discovery efforts can help identify molecules with the ability to modulate TGF-beta signaling for the treatment of CHD.t's important to note that therapeutic strategies targeting the TGF-beta signaling pathway for CHD are still in the early stages of research and development. Further studies are needed to evaluate the safety, efficacy, and specificity of these potential targets. Clinical trials and preclinical models will play a crucial role in determining the feasibility and effectiveness of such interventions**(41-43).**

References:

1. **Gallo, G. O. (2016).** Characterization of cellular and molecular responses of human glioblastoma to Transforming Growth Factor-β signalling pathway inhibition.
2. **Alzawi, A., Iftikhar, A., Shalgm, B., et al. (2022).** Receptor, Signal, Nucleus, Action: Signals That Pass through Akt on the Road to Head and Neck Cancer Cell Migration. Cancers, 14(11), 2606.
3. **Cochet, C. (2019).** Secretion of Epidermal Growth Factor and Transforming Growth Factor α by Normal and Malignant Cells.

In Malignant Cell Secretion (pp. 207-214). CRC Press.

4. **Hanafy, N. A., & El Kemary, M. (2019).** TGFβ1 as a Good and Bad Biological Molecule: Structure and Function. Biomed. J. Sci. Tech. Res. BJSTR.
5. **Fabregat, I., Moreno-Càceres, J., Sánchez, A., et al. (2016).** TGF-β signalling and liver disease. The FEBS journal, 283(12), 2219-2232.
6. **Kubiczkova, L., Sedlarikova, L., Hajek, R., et al. (2012).** TGF-β–an excellent servant but a bad master. Journal of translational medicine, 10(1), 1-24.
7. **Mukundan, A., Byeon, C. H., Hinck, C. S., et al. (2022).** Convergent evolution of a parasite-encoded complement control protein-scaffold to mimic binding of mammalian TGF-β to its receptors, TβRI and TβRII. Journal of Biological Chemistry, 298(6), 101994.
8. **Robertson, D. (2019).** Hydrocortisone and TGF-β1 regulate the glucocorticoid receptor and the serotonin transporter in SWAN-71 and JEG-3 cells (Doctoral dissertation, Griffith University).
9. **Goessler, U. R., Bugert, P., Bieback, K., et al. (2006).** In vitro analysis of differential expression of collagens, integrins, and growth factors in cultured human chondrocytes. Otolaryngology—Head and Neck Surgery, 134(3), 510-515.
10. **Merkel, A., Guirado, E., Narayanan, K., et al. (2021).** Non-collagenous ECM Matrix Components Growth Factors and Cytokines Involved in Matrix Mineralization. Extracellular Matrix Biomineralization of Dental Tissue Structures, 75-108.
11. **Syed, V. (2016).** TGF-β Signaling in Cancer. Journal of cellular biochemistry, 117(6), 1279-1287.
12. **Poniatowski, Ł. A., Wojdasiewicz, P., Gasik, R., et al. (2015).** Transforming growth factor Beta family: insight into the role of growth factors in regulation of fracture healing biology and potential clinical applications. Mediators of inflammation, 2015.
13. **Hussain, A. F., Sulaiman, G. M., Dheeb, B. I., et al. (2020).** Histopathological changes and expression of transforming growth factor beta (TGF-β3) in mice exposed to gliotoxin. Journal of King Saud University-Science, 32(1), 716-725.
14. **Efthymiou, G. (2019).** Deciphering the role of oncofetal fibronectin isoforms in matrix assembly and cellular function (Doctoral dissertation, COMUE Université Côte d'Azur (2015-2019)).
15. **McGarty, T. P. (2019).** IMMUNOTHERAPY: POSSIBLE DIRECTIONS.
16. **Mao, X., Xu, J., Wang, W., et al. (2021).** Crosstalk between cancer-associated fibroblasts and immune cells in the tumor microenvironment: new findings and future perspectives. Molecular cancer, 20(1), 1-30.
17. **Lee, J. W., Chun, W., Lee, H. J., et al. (2021).** The role of macrophages in the development of acute and chronic inflammatory lung diseases. Cells, 10(4), 897.
18. **Riihilä, P., Nissinen, L., &Kähäri, V. M. (2021).** Matrix metalloproteinases in keratinocyte carcinomas. Experimental Dermatology, 30(1), 50-61.
19. **Peng, D., Fu, M., Wang, M., et al. (2022).** Targeting TGF-β signal transduction for fibrosis and cancer therapy. Molecular Cancer, 21(1), 1-20.
20. **Tazat, K., Hector-Greene, M., Blobe, G. C., et al. (2015).** TβRIII independently binds type I and type II TGF-β receptors to inhibit TGF-β signaling. Molecular biology of the cell, 26(19), 3535-3545.
21. **Goulet, C. R., &Pouliot, F. (2021).** TGFβ signaling in the tumor microenvironment. Tumor Microenvironment, 89-105.
22. **Pawlak, J. B., &Blobe, G. C. (2022).** TGF-β superfamily co-receptors in cancer. Developmental Dynamics, 251(1), 137-163.
23. **Margioula-Siarkou, G., Margioula-Siarkou, C., Petousis, S., et al. (2022).** The role of endoglin and its soluble form in pathogenesis of preeclampsia. Molecular and Cellular Biochemistry, 477(2), 479-491.
24. **Nickel, J., Ten Dijke, P., & Mueller, T. D. (2018).** TGF-β family co-receptor function and signaling. ActaBiochimica et BiophysicaSinica, 50(1), 12-36.
25. **Villarreal, M. M., Kim, S. K., Barron, L., et al. (2016).** Binding properties of the transforming growth factor-β

coreceptorbetaglycan: proposed mechanism for potentiation of receptor complex assembly and signaling. Biochemistry, 55(49), 6880-6896.

26. **Kang, J. S., Liu, C., &Derynck, R. (2009).** New regulatory mechanisms of TGF-β receptor function. Trends in cell biology, 19(8), 385-394.
27. **Porte, J., Jenkins, G., &Tatler, A. L. (2021).** Myofibroblast TGF-β Activation Measurement In Vitro. In Myofibroblasts (pp. 99-108). Humana, New York, NY.
28. **Dituri, F., Cossu, C., Mancarella, S., et al. (2019).** The interactivity between TGFβ and BMP signaling in organogenesis, fibrosis, and cancer. Cells, 8(10), 1130.
29. **Park J, Lee J, Kang W, Chang S, Shin EC and Choi C. (2013):** TGF-beta1 and hypoxia-dependent expression of MKP-1 leads tumor resistance to death receptor-mediated cell death. Cell Death Dis; 4: e521.
30. **Zheng, R., Fu, Z., & Zhao, Z. (2021).** Association of Transforming Growth Factor β1 Gene Polymorphisms and Inflammatory Factor Levels with Susceptibility to Sepsis. Genetic Testing and Molecular Biomarkers, 25(3), 187-198.
31. **Wang E, Wang Z, Liu S,et al. (2014):** Polymorphisms of VEGF, TGFbeta1, TGFbetaR2 and conotruncal heart defects in a Chinese population. MolBiol Rep; 41: 1763-1770.
32. **Lee CG, Kang HR, Homer RJ, et al. (2006):** Transgenic modeling of transforming growth factor-beta(1): role of apoptosis in fibrosis and alveolar remodeling. Proc Am ThoracSoc; 3: 418-423.
33. **Shah R, Rahaman B, Hurley CK et al. (2006):** Allelic diversity in the TGFB1 regulatory region: characterization of novel functional single nucleotide polymorphisms. Hum Genet 2006; 119: 61-74.
34. **Vitiello, G. A. F., Guembarovski, R. L., Hirata, B. K. B. et al. (2018).** Transforming growth factor beta 1 (TGFβ1) polymorphisms and haplotype structures have dual roles in breast cancer pathogenesis. Journal of cancer research and clinical oncology, 144(4), 645-655.
35. **Shi, Y., Zhang, J., Xu, W., et al. (2019).** The correlation of TGFβ1 gene polymorphisms with congenital heart disease susceptibility. *Gene*, *686*, 160-163.
36. **Terada, K., Yamada, H., Kikai, M., et al. (2018).** Transplantation of periaortic adipose tissue inhibits atherosclerosis in apoE−/− mice by evoking TGF-β1-mediated anti-inflammatory response in transplanted graft. Biochemical and Biophysical Research Communications, 501(1), 145-151.
37. **Doetschman T, Barnett V, Runyan B *et al*. (2012):** Transforming growth factor beta signaling in adult cardiovascular diseases and repair. Cell and tissue research, 347: 203-223.
38. **Sorensen W, van Berlo H (2020):** The Role of TGF—β Signaling in Cardiomyocyte Proliferation. Current heart failure reports, 17: 225-233.
39. **Gottlieb, E. A., Matisoff, A., &Andropoulos, D. B. (2023).** Genetic Syndromes and Associations in Congenital Heart Disease. *Anesthesia for Congenital Heart Disease*, 131-165.
40. **Liu, T., Xie, M., Lv, Q., Li, Y., Fang, L., Zhang, L., ... & Wang, J. (2019).** Bicuspid aortic valve: an update in morphology, genetics, biomarker, complications, imaging diagnosis and treatment. *Frontiers in physiology*, *9*, 1921.
41. **Leask, A. (2010).** Potential therapeutic targets for cardiac fibrosis: TGFβ, angiotensin, endothelin, CCN2, and PDGF, partners in fibroblast activation. *Circulation research*, *106*(11), 1675-1680.
42. **Tie, Y., Tang, F., Peng, D., Zhang, Y., & Shi, H. (2022).** TGF-beta signal transduction: Biology, function and therapy for diseases. Molecular Biomedicine, 3(1), 45.
43. **Akhurst, R. J. (2017).** Targeting TGF-β signaling for therapeutic gain. *Cold Spring Harbor perspectives in biology*, *9*(10), a022301.

*e*ISSN1303-5150