Minireview Article

ROLE OF TRANSFORMING GROWTH FACTOR-β IN EPITHELIAL-MESENCHYMAL TRANSITION OF HUMAN CANCERS - A BRIEF REVIEW

UNDER REVIEW

ABSTRACT

Epithelial Mesenchymal Transition (EMT) is a crucial process in embryogenesis, however it also plays an important role in pathologies including inflammation, wound healing and cancer. EMT is a determining step in cancer metastasis as tumour cells utilise the process of EMT for invasion, migration and colonization at distant sites. The transforming growth factor-β (TGF-β) is a pleiotropic inflammatory cytokine belonging to the TGF-β super family of growth and differentiation factors secreted by immune, non-hematopoietic and tumour cells. Three TGF-β isoforms, TGF-β1, TGF-β2, and TGF-β3 are recognized in mammals. TGF-β induced signalling pathways remains the most prominent among other pathways inducing EMT. The TGF-β induced signalling pathways are inducted in all three types of EMT including the physiological embryogenesis process and pathological process such as wound healing and tumorogenesis. TGF-β acts via the SMAD and Non-SMAD pathways and plays major roles in tumour infiltration and metastasis. This article aims to briefly review the role of TGF- β induced signalling pathways and its role in tumour progression in human cancers such as lung cancer, breast cancer and oral squamous cell carcinoma.

Key words: Epithelial mesenchymal transition, TGF- β, Cancer

INTRODUCTION

The development of a multicellular organism from a single cell into multiple cells which gets organised into tissues and organs are piloted by processes such as cell proliferation, differentiation, cellular interactions and cell movements. During embryogenesis, the cells undergo phenotypical changes and differentiates into specialised cells for structural and functional adaptations. One of the very early divergence in cellular phenotype are seen with the development of epithelial and mesenchymal cell populations during early embryonic stage. The epithelial tissue gets assembled as a sheet of epithelial cells which abuts each other. The epithelial cells maintain an apico-basal polarity, internal skeletal framework through cytoskeletons and adheres to one another through cell–cell junctions. They line the external and internal surface of organs and act as a barrier to the external environment. The mesenchymal cells, unlike epithelial cells do not possess a rigid framework, they provide support and forms the structural framework of the organism by producing the extra cellular matrix [1].

The epithelial and mesenchymal cellular phenotypes are dynamic and the transition between epithelial and mesenchymal cell population is known as Epithelial Mesenchymal Transition (EMT). It is a transition process where the epithelial cells loose their cell polarity, inter cellular adhesion, shape and turn into more mesenchymal like shape. The resultant more mesenchymal like cell can revert back to epithelial like cell through a reversible process known as Mesenchymal Epithelial Transition (MET). Initially it was described as '*epithelial-mesenchymal transformation'* and later the term '*transformation'* was replaced with *'transition'* indicating the reversibility of the process. While EMT is a crucial normal process in embryogenesis, studies suggest that it plays an important role in pathologies including inflammation, wound healing and cancer. EMT is a determining step in cancer metastasis as tumour cells utilise the process of EMT for invasion, migration and colonisation at distant sites [2].

The transforming growth factor-β (TGF-β) is a pleiotropic inflammatory cytokine belonging to the TGF-β super family of growth and differentiation factors secreted by immune, non-hematopoetic and tumour cells [3]. The prominent family members includes TGF-β isoforms, TGF-β1, TGF-β2, and TGF-β3 and Activins, Nodal and Bone morphogenetic proteins (BMPs), all of which play major roles in physiological process like developmental patterning, tissue differentiation, and maintenance of homeostasis and pathological process like cancer progression [3]. In tumorogenesis, the TGF-β signalling pathway is significant for the driving of EMT process. TGF-β secreted by tumour cells, cancer-associated fibroblasts (CAFs), or immune cells, is capable of inducing new extracellular matrix (ECM) synthesis remodelling by secretion of metalloproteases (MMPs-2, 9) that promote cell invasion. [4]

EPITHELIAL-MESENCHYMAL TRANSITION

The epithelial mesenchymal transition (EMT) is a reversible process of transition of epithelial cells into a quasi- mesenchymal state (Figure 1). However, the plasticity of this transition is revealed by the conversion of mesenchymal cells to epithelial cells by a process known as mesenchymal-epithelial transition [5]. The classification of EMT was proposed by an expert panel meeting on EMT in Poland in 2007, followed by a further meeting in Cold spring harbour Laboratories in 2008. EMT is classified according to the three different biological

settings namely EMT Type 1, 2 and 3. EMT1 occurs in embryogenesis, type 2 in wound healing and tissue regeneration and type-3 in cancer progression. A variety of biomarkers are used to demonstrate all three subtypes of EMT which are shown in Table 1. [6].

Studies suggest that activation of EMT process is critical for the acquisition of malignant phenotypes by epithelial cancer cells. EMT plays major role in cancer progression, tumour invasion and distant metastasis. Various epithelial mesenchymal transition transcription factors such as Zinc-finger E-box binding homeobox factors ZEB1 and ZEB2, SNAIL (SNAI1), SLUG (SNAI2) and basic helix-loop-helix factors TWIST1 and TWIST2 induces the expression of genes that promotes the transition of epithelial cells into mesenchymal cells and suppresses the genes responsible for the maintenance of epithelial state [7]. E-cadherin, epithelial cell adhesion molecules (E-CAM), occludins, claudins and $\alpha_6\beta_4$ integrins are down regulated which leads to the dissolution of epithelial cell and apico-basal polarity. Snail, Zeb, and Twist (E-cadherin repressors) pathways induces E-cadherin silencing by hyper methylation of E-cadherin promoter region. This leads to weakening of adhering junctions and loss of epithelial integrity. The tight junction integrity is lost by down-regulating molecules like claudins and occludins. Subsequently desmosomes and tight junctions are disrupted. As the transition progresses the junctional proteins are transcriptionally reversed resulting in permanent loss of epithelial junctions which in turn affects the apico-basal polarity of the epithelial cells due to disruption of hemidesmosomes and α_6 β_4 integrins. There is simultaneous up-regulation of mesenchymal characteristics induced by proteins such as N-cadherin, Vimentin, Fibronectin, β_1 and β_3 integrins. Additionally the activation of proteinases (MMPs-2, 9) facilitates basement degradation and cell migration. [7]

Transforming growth factor-β (TGF-β)

The members of the Transforming growth factor (TGF) super family encompasses multifunctional growth and differentiation factors which play major roles in physiological cellular functions such as growth, differentiation, and apoptosis and in pathological conditions such as inflammation and tumerogenisis [3]. The term Transforming growth factor is derived from the transforming ability of the cytokines to induce anchorage-independent growth on cells irrespective of the action of Epidermal growth factor (EGF). In mammals they are encoded by 33 genes. The TGF super family is classified into several subgroups based on their structure and sequence of the encoded polypeptides. Three TGF-β isoforms, TGF-β1, TGF-β2, and TGF-β3 were recognised in mammals. TGF-β is a 25-kd disulphide-linked dimeric pleotropic cytokine secreted by immune, nonhemotopoietic and tumour cells. It is synthesised as a large precursor molecule consisting of 390 amino acid chain and it consist of three segments: an amino terminal segment, a carboxy terminal segment and a proterminal segment (Figure 2a). The prosegments are known as latency-associated peptides (LAPs) and they vary in length from 150 to 450 residues. These prosegments remain noncovalently linked to the mature TGF-β dimer in complexes called “small latent complexes” and makes it unable to bind to the receptors (Figure 2b). The prosegment of TGF-β1 contains a proline-rich loop, or latency lasso, which surrounds a TGFβ1 monomer like a straitjacket. Activation of TGF ligand is brought about by proteolytic cleavage of proteinases (MMP-9, MMP-2), interaction with $\alpha_4\beta_6$ or by the pH changes in local microenvironment [8]. Thus, tumour cells are well equipped to activate TGF-β locally by secretion of MMPs (MMP-9, MMP-2) [8].

TGF-β SIGNALING PATHWAYS IN EMT

EMT is initiated by numerous pathways such as resident fibroblast induced hypoxic pathways and inflammatory cytokine induced pathways (TGF-β, MMP-2, MCP-1 and type Ⅱ collagen) out of which the TGF-β induced signalling pathways remains the most prominent [9]. The TGF-β induced signalling pathways are inducted in all three types of EMT including the physiological embryogenesis process and pathological process such as wound healing and tumorogenisis. The TGF-β can activate cell signalling pathways via canonical pathway also known as SMAD (SMA from 'Caenorhabditis elegans Sma gene' and MAD from 'Mothers against decapentaplegic') related proteins pathway and the non-canonical pathway known as non SMAD pathway. TGF-β is secreted as an extracellular inactive dimeric protein. Upon secretion of proteases (MMPs-9, 2) the inactive precursor molecule undergo cleavage and structural transformation as active ligands which binds to its transmembrane receptors [10]. TGF-β signalling pathway is intervened by three TGF ligands namely TGF-β1, β2, β3 via acting on their receptors namely TGF-β Type-1 (TGFβR1) and Type-2 receptors (TGFβR2). Hyaluronan is found to be a cofactor which aids the TGF-β ligands to bind to its receptors [11].

C**anonical pathway (SMAD) pathway**

In the canonical pathway (SMAD) pathway the TGF-β ligands binds with the Type-2 receptor monomers which induces the dimerization of the Type-2 receptor monomers. This dimerized Type-2 receptors will recruit the Type 1 receptors to their proximity and exhibits kinase activity and phosphorylates the Type 1 monomers at serine residues and a hetromeric tetra complex of both Type-1 and Type-2 receptors along with the signalling ligands are formed. This activates the Type-1 receptor. This activated hetromeric tetra receptor complex attracts regulatory SMADs (SMAD 2 and SMAD 3) which are cytosolic receptors responsible for down streaming of pathway [12]. The regulatory SMADs (R-SMADS 2 and 3) are phosphorylated directly by type1 receptor, which enables them to bind to SMAD4 which is a co regulatory SMAD and SMAD anchor for receptor activation (SARA) [13] .This activated complex gets transferred to the nucleus where it binds to the DNA and regulates gene expression. The down streaming of the signalling pathway is co-piloted by Co-Smad and regulated by the inhibitory activity of inhibitory SMAD (SMAD7). SMAD7 acts as a negative regulator of TGFβR1 by recruiting SMURF2 (SMAD ubiquitination regulatory factor 2), an E3 ubiquitin ligase, to the receptor, and mediates its degradation, and thereby attenuates the signalling. This negative feedback loop prevents prolonged pathway activation [14]. (Figure 3 a)

N**on-canonical pathway (non-SMAD) pathway**

The non-canonical pathway, also known as non-SMAD pathway activates the Erk/MAPK pathway and the phosphoinositide 3 kinase (PI3K)/Akt pathway. These non-Smad pathways work independently or together with Smad complexes (Figure 3). In the non-canonical pathway, the activated TGF-β receptor complex conveys signals through a number of factors such as tumor necrosis factor (TNF), receptor associated factor 4 (TRAF4), TRAF6, TGFβ-activated kinase 1 (TAK1), p38 mitogen-activated protein kinase (p38 MAPK), p42/p44

MAPK, phosphoinositide 3-kinase PI3K/AKT, extracellular signal regulated kinase (ERK), JUN N-terminal kinase (JNK), or nuclear factor-kB (NF-kB) to reinforce or attenuate downstream cellular responses. The TGF-β pathway cross talks with several pathways associated with tumor progression such as TNF-alpha pathway, Notch pathway, Hedgehog pathways, WNT pathway, Hippo pathway and Ras pathway [15]. (Figure 3 b)

TGF –β signalling pathways controls the gene expression of several extracellular matrix components [16]. They up regulate the production of proteases and paves ways for cell signalling, cell migration and tumour metastasis. The TGF –β signalling pathway down regulates the epithelial cell surface molecule such as E-cadherin thereby accelerating the EMT process. TGF-β also down regulates epithelial cell polarity proteins such as Crumbs3 (CRB3) and RhoA small GTPase, by inducing Snail1, culminating in the loss of tight junctions [17]. The TGF-β Type 2 receptor phosphorylates Par6, a polarity adaptor protein, which in turn binds to Smad ubiquitylation regulatory factor 1 (Smurf1) and degrades RhoA. The canonical and non-canonical signalling pathways also induces the expression of the salt-inducible kinase 1 (SIK1) which along with Smad7 recruits Smurf 2 to the TGFβ type I receptor and promotes lysosomal degradation [13]. SIK1 promotes phosphorylation and proteosomal degradation of Par3 and facilitates the TGFβ-induced EMT [17]. TGFβ also induces SOX5 expression, which binds to Twist-1 promoter and activates its expression to induce the EMT [18].

Dual role of TGF-β signalling in tumorogenesis

Studies in knockout mice suggests that TGF-β acts a tumour suppressor in the initial stages of tumorogenesis. However, in later stages they promote cellular proliferation and migration by initiating the process of EMT [19]. The role of balance effect of the TGF-β induced signalling pathways in normal and tumour cells is of utmost importance as they may serve as therapeutic targets in epithelial malignancies. Studies have shown that TGF-β signalling arrests the cell cycle in G1 phase, up regulates cyclin-dependent kinase (CDK) inhibitors and down regulates Myc expression for inhibition of cell growth. Smad3/Smad4 complexes interact with forkhead box O (FoxO) to increase the expression of CDK inhibitors, namely p15 and p21. In the presence of co-repressors, Smad3/Smad4 complex interacts with a cell cycle regulator gene (Myc promoter)[20]. Smads induces apoptosis in epithelial cells through the activation of P53, Bcl-2-like protein 11 and death-associated protein kinase, and the repression of Akt. TGFβRI also induces apoptosis in normal epithelial cells through TGF-β-activated kinase 1 (TAK1)-p38/c-Jun N-terminal kinase, independent of Smads [19].

Among the various proposed mechanisms of its dual role, the imbalance between the canonical (SMAD) and the non-canonical pathways (non-SMAD) has been suggested as major playback mechanism (Figure 4) [20]. Oncogenic Ras down regulates Smad4 and the resultant low levels of Smad4 activate Ras-dependent ERK signalling, leading to the progression of undifferentiated carcinoma in keratinocytes [21]. Another possible mechanism is the suppression of SMAD2 by Disabled homolog 2 (DAB2), a putative tumour suppressor gene. Hannigan *et al* reported that in oral squamous cell carcinoma the epigenetically down regulated DAB2 negatively regulates Smad2 and its downstream pathway [22]. Therefore, down regulation of DAB2 leads to the conversion of TGF-β as a tumour promoter. Additionally, the suppressive effect of Smad7 on the canonical TGF-β signalling pathway is greater than that on non-canonical pathways involving TAK1 signalling, such as TAK1-NF-

κB signalling, which favours malignant progression. The TGF-β-mediated growth inhibition and progression is currently an important focus of carcinogenesis research [23-25].

ROLE OF TGF-β IN LUNG CANCER

TGF-β signalling plays critical roles in the lung epithelium and mesenchyme, and is required for epithelial-mesenchymal interactions to achieve lung alveologenesis via SNAI2/ZEB pathway. The alveolar epithelium includes basal, ciliated and neuro endocrinal cells and the alveoli is lined by type 1 and type 2 epithelial cells [26]. TGF-β induces EMT process in alveolar epithelial cells to configure a mesenchymal phenotype, which is a crucial process in lung differentiation and development. TGF-β induced EMT-related transcriptional repressors (SNAI1/SNAI2 and ZEB1/ZEB2) inhibits the action of NKX2-1, a homeodomain transcription factor which is essential for lung epithelial cell differentiation [26]. TGF-β promotes transdifferentiation of surfactant protein C (SPC)-positive type II alveolar epithelial cells to type I alveolar epithelial cells that express podoplanin. It also promotes transdifferentiation of lung fibroblasts to myofibroblasts by up regulating α-smooth muscle actin (α-SMA), and down regulating TBX4, a T-box family transcription factor unique to lung fibroblasts. TGF- β signalling induced EMT via canonical pathway by phosphorylation of SMAD 2 and induction of SMAD 7 was observed in human small cell lung carcinoma cells and non-small cell lung carcinoma which includes histological subtypes such as adenocarcinoma, squamous cell carcinoma [27]. In lung carcinoma, the TGF-β pathway cross talks with other pathways such as oncogenic KRAS pathways. Oncogenic KRAS significantly enhances TGF-β-mediated SNAI1 expression in cancer cells harbouring constitutively active KRAS mutations. [28]

ROLE OF TGF-β IN BREAST CANCER

Studies suggests that decreased TGFβR2 is associated with an increased risk of developing invasive breast cancer, and that TGFβR2 is a marker of poor prognosis in breast cancers [29-31]. In the TGF-β signalling pathways, TGFβR2 is critical for transcription. Tumour cells are less sensitive to TGF-β-mediated growth inhibitory responses upon TGFβR2 down-regulation [32]. Epigenetic reprogramming of cells has been attributed to the development of a wide range of cancers, including breast cancer [33]. An intricate relationship between epigenetic regulators and TGF-β signaling has been established in breast cancers. TGF-β induced SNAI2 promotes EMT by repressing miR-203. Although miR203 targeted SNAI2, SNAI2 induced by TGF-β could directly bind to the miR-203 promoter to inhibit its transcription. SNAI2 and miR-203 formed a double negative feedback loop to inhibit each other's expression, thereby controlling EMT. [34]

ROLE OF TGF-β IN ORAL SQUAMOUS CELL CARCINOMA

Oral squamous cell carcinoma (OSCC) accounts for 90% of head and neck cancers and the common risk factors include tobacco, areca nut and human papilloma virus infection [35]. Evidence suggests that deregulation of TGF-β signaling is of great importance in OSCC [36]. Expression of Smad4 and Smad2 is frequently lost in OSCC, while increased TGF-β1 expression has been reported [36]. In the initial stages of OSCC TGF-β acts a tumour suppressor while in later stages it acts a tumour promoter. In keratinocytes TGF-β up regulates the expression of cyclic AMP-dependent transcription factor-3, which serves as a cofactor for binding of Smad3/Smad4 complexes to the ID1 promoter [36]. This in turn down regulates the IDI leading to cell differentiation. Over expression of TGF-β1 was reported

which results in hyperproliferation of cells at the head and neck epithelium enhancing inflammation and angiogenesis. The TGF-β1 promotes cell proliferation by the formation of an extracellular microenvironment that favours tumour formation [36]. Loss of TGFβRI or TGFβR2 partly subverts TGF-β1-induced cell cycle arrest, and this effect along with the increased production of TGF-β1 may result in its accumulation in the extracellular microenvironment. The loss of TGFβRI or TGFβR2 has also been proven to lead to increased cell proliferation and inhibit the apoptosis of head and neck squamous cell carcinomas [37]. In OSCC, TGF-β signalling has been implicated in EMT through Snail and up regulation of MMP-9 levels [36]. Yu *et al* has demonstrated that the TGF-β1 expression in HNSCC was correlated with decreased E-cadherin level through the phosphorylation of Smad2/3 and subsequent involvement of Smad4, which bound to the Snail promoter and also via non smad pathways such as extracellular signal-regulated kinase (ERK) [38]. In addition, MMP-9 degradation of the extracellular matrix components and basement membrane is regulated by TGF-β1 through Smad2/3 and myosin light chain kinase in OSCC leading to tumour invasion and metastasis. [36]

CONCLUSION

TGF-β is a multifunctional inflammatory cytokine secreted by immune and tumour cells and it regulates cell proliferation, differentiation and apoptosis by the TGF-β mediated pathways. TGF-β plays dual role in tumourogenesis as a tumour suppressor in the initial stage of oncogenesis and as a promoter in later stages. TGF-β signalling is reported to balance the proliferation and apoptosis in the epithelium and defective TGF-β signalling in tumour cells leads to the resistance in TGF-β-induced growth inhibition and undergo EMT process. The factors responsible for controlling the molecular and cellular consequences of TGF-β signalling has to be identified. Several epigenetic regulators have been shown to directly control certain components of TGF-β signalling, which might plausibly offer a route to successfully targeting this pathway. With the advent of novel target based therapeutics in human cancers, further researches in the context of cancer cell lines are required.

References:

1. Lee JM, Dedhar S, Kalluri R, Thompson EW. The epithelial-mesenchymal transition: new insights in signaling, development, and disease. J Cell Biol. 2006; 172 (7):973-81.

2. Gos M, Miłoszewska J, Przybyszewska M. Epithelial-mesenchymal transition in cancer progression. Postepy Biochem. 2009; 55(2):121-8.

3. Morikawa M, Derynck R, Miyazono K. TGF-β and the TGF-β Family: Context-Dependent Roles in Cell and Tissue Physiology. Cold Spring Harb Perspect Biol. 2016; 8 (5):a021873.

4. Kahata K, Dadras MS, Moustakas A. TGF-β Family Signaling in Epithelial Differentiation and Epithelial-Mesenchymal Transition. Cold Spring Harb Perspect Biol. 2018;10 (1):a022194

5. Ribatti D, Tamma R, Annese T. Epithelial-Mesenchymal Transition in Cancer: A Historical Overview. Transl Oncol. 2020; 13 (6):100773.

6. Jayanthi P, Varun BR, Selvaraj J. Epithelial-mesenchymal transition in oral squamous cell carcinoma: An insight into molecular mechanisms and clinical implications. J Oral Maxillofac Pathol. 2020;24 (1):189.

7. Georgakopoulos-Soares I, Chartoumpekis DV, Kyriazopoulou V, Zaravinos A. EMT Factors and Metabolic Pathways in Cancer. Front Oncol. 2020; 10: 499.

8. Hinck AP, Mueller TD, Springer TA. Structural Biology and Evolution of the TGF-β Family. Cold Spring Harb Perspect Bio*l*. 2016; 8(12):a022103.

9. Zumchenko E, Chang X, Michailidi C, Kagohara L, Ravi R, Paz, K et al. The TGFβ-miR200-MIG6 pathway orchestrates the EMT-associated kinase switch that induces resistance to EGFR inhibitors. Cancer Res. 2014; 74:3995–4005.

10. Zi Z, Chapnick DA, Liu X. Dynamics of TGF-β/Smad signaling. FEBS Lett. 2012; 586(14):1921-1928.

11. Shi Y and Massagué J. Mechanisms of TGF-beta signalling from cell membrane to the nucleus. Cell 2003; 113 (6): 685-700.

12. Liu S, Chen S, Zeng J. TGFβ signalling: A complex role in tumorigenesis (Review). Mol Med Rep. 2018; 17 (1):699-704.

13. Seng XH and Derynck R. Specificity and versatility in TGF-beta signalling through Smads. Annu Rev Cell Dev Biol 21: 659-693.

14. Singha PK, Pandeswara S, Geng H, Lan R, Venkatachalam MA, Saikumar P. TGF-β induced TMEPAI/PMEPA1 inhibits canonical Smad signaling through R-Smad sequestration and promotes non-canonical PI3K/Akt signaling by reducing PTEN in triple negative breast cancer. Genes Cancer. 2014; 5 (9-10):320-36.

UNDER PEER REVIEW

15. Martin M, Herceg Z. From hepatitis to hepatocellular carcinoma: a proposed model for cross-talk between inflammation and epigenetic mechanisms. Genome Med. 2012; 4(1):8.

16. Saed GM, Zhang W, Diamond MP. Effect of hypoxia on stimulatory effect of TGF-beta 1 on MMP-2 and MMP-9 activities in mouse fibroblasts. J Soc Gynecol Investig. 2000;7(6):348-54.

17. Xu J, Lamouille S, Derynck R. TGF-beta-induced epithelial to mesenchymal transition. Cell Res. 2009;19(2):156-72.

18. Tsubakihara Y, Moustakas A. Epithelial-Mesenchymal Transition and Metastasis under the Control of Transforming Growth Factor β. Int J Mol Sci. 2018; 19(11):3672.

19. Lebrun JJ. The Dual Role of TGFβ in Human Cancer: From Tumor Suppression to Cancer Metastasis. ISRN Mol Biol. 2012;24:381428.

20. Kim SI, Choi ME. TGF-β-activated kinase-1: New insights into the mechanism of TGF-β signaling and kidney disease. Kidney Res Clin Pract. 2012;31(2):94-105.

21. Iglesias M, Frontelo P, Gamallo C, Quintanilla M. Blockade of Smad4 in transformed keratinocytes containing a Ras oncogene leads to hyperactivation of the Ras-dependent Erk signalling pathway associated with progression to undifferentiated carcinomas. Oncogene. 2000; 19 (36):4134-45.

22. Xu S, Zhu J, Wu Z. Loss of Dab2 expression in breast cancer cells impairs their ability to deplete TGF-β and induce Tregs development via TGF-β. PLoS One. 2014; 9 (3):e91709.

23. Tian M, Schiemann WP. The TGF-beta paradox in human cancer: an update. Future Oncol. 2009; 5(2):259-271.

24. Freudlsperger C, Bian Y, Contag Wise S, Burnett J, Coupar J, Yang X et al. TGF-β and NF-κB signal pathway cross-talk is mediated through TAK1 and SMAD7 in a subset of head and neck cancers. Oncogene. 2013; 32(12):1549-59.

25. Katz LH, Li Y, Chen J-S, Munoz NM, Majumdar A, Chen J et al. Targeting TGF-β signaling in cancer. Expert Opin Ther Targets. 2013;17(7):743-760.

UNDER PEER REVIEW

26. Saito A, Horie M, Nagase T. TGF-β Signaling in Lung Health and Disease. Int J Mol Sci. 2018;19(8):2460.

27. Gu L, Zhu YJ, Yang X, Guo ZJ, Xu WB, Tian XL. Effect of TGF-beta/Smad signaling pathway on lung myofibroblast differentiation. Acta Pharmacol Sin. 2007; 28(3):382-91.

28. Hao Y, Baker D, Ten Dijke P. TGF-β-Mediated Epithelial-Mesenchymal Transition and Cancer Metastasis. Int J Mol Sci. 2019; 20(11):2767.

29. Momenimovahed Z, Salehiniya H. Epidemiological characteristics of and risk factors for breast cancer in the world. Breast Cancer (Dove Med Press). 2019; 11:151-164.

30. Sharma GN, Dave R, Sanadya J, Sharma P, Sharma KK. Various types and management of breast cancer: an overview. J Adv Pharm Technol Res. 2010; 1(2):109-126.

31. Suriyamurthy S, Baker D, Ten Dijke P, Iyengar PV. Epigenetic Reprogramming of TGF-β Signaling in Breast Cancer. Cancers (Basel). 2019 May 24; 11(5):726.

32. Yu L, Liu X, Cui K, Di Y, Xin L, Sun X et al. SND1 Acts Downstream of TGFβ1 and Upstream of Smurf1 to Promote Breast Cancer Metastasis. Cancer Res. 2015; 75 (7):1275–86.

33. de Herreros, AG, Peiro S, Nassour M, Savagner P. Snail family regulation and epithelial mesenchymal transitions in breast cancer progression. J. Mammary Gland Biol. Neoplasia 2010; 15:135–47.

34. Ding X, Park SI, McCauley LK, Wang C-Y. Signaling between transforming growth factor β (TGF-β) and transcription factor SNAI2 represses expression of microRNA miR-203 to promote epithelial-mesenchymal transition and tumor metastasis. J. Biol. Chem. 2013; 288 (15): 10241–53.

35. Bugshan A, Farooq I. Oral squamous cell carcinoma: metastasis, potentially associated malignant disorders, etiology and recent advancements in diagnosis. F1000Res. 2020; 9:229.

36. Pang X, Tang YL, Liang XH. Transforming growth factor-β signaling in head and neck squamous cell carcinoma: Insights into cellular responses. Oncol Lett. 2018 ; 16(4):4799-806.

UNDER PEER REVIEW

37. Lu S-L, Herrington H, Reh D, Weber S, Bornstein S, Wang D et al. Loss of transforming growth factor-beta type II receptor promotes metastatic head-and-neck squamous cell carcinoma. Genes Dev 2006; 20 (10): 1331-42.

38. Smith A, Teknos TN, Pan Q. Epithelial to mesenchymal transition in head and neck squamous cell carcinoma. Oral Oncol 2013; 49 (4): 287-92.

Table 1: Biomarkers of EMT

CATEGORY /FUNCTION	**Biomarker**	**EMT associated change**
Cell surface protein molecules	E-cadherin, Desmoplakin, Z0-1,occludins,Claudins	Down regulated
Transcriptional factors	Snail, Slug (Snail 2),Twist, LEF-1,ZEB,NF-kB	Up regulated
Cytoskeletal molecules	β-catenin, Cytokeratins	Down regulated
	Vimentin, ά-smooth muscle actin	Up regulated
Extracellular matrix proteins	Collagen I and II, fibronectin	Up regulated
	Collagen Ⅳ	Down regulated

UNDER PEER REVIEW

Figure 1: Epithelial- Mesenchymal Transition

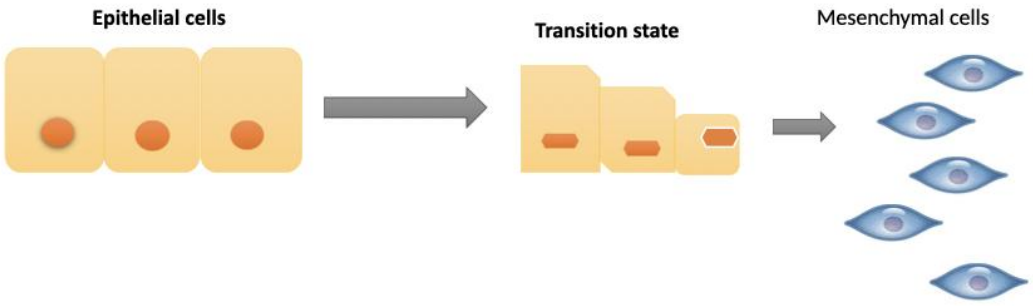


Figure 2a: PROSEGMENT TGF--β monomer

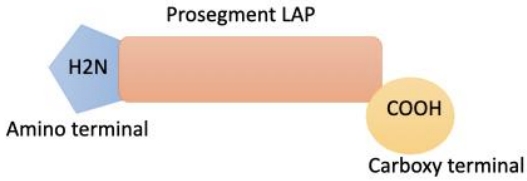


Figure 2b: Small Latency Complex

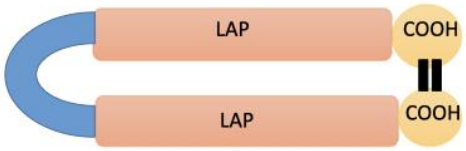
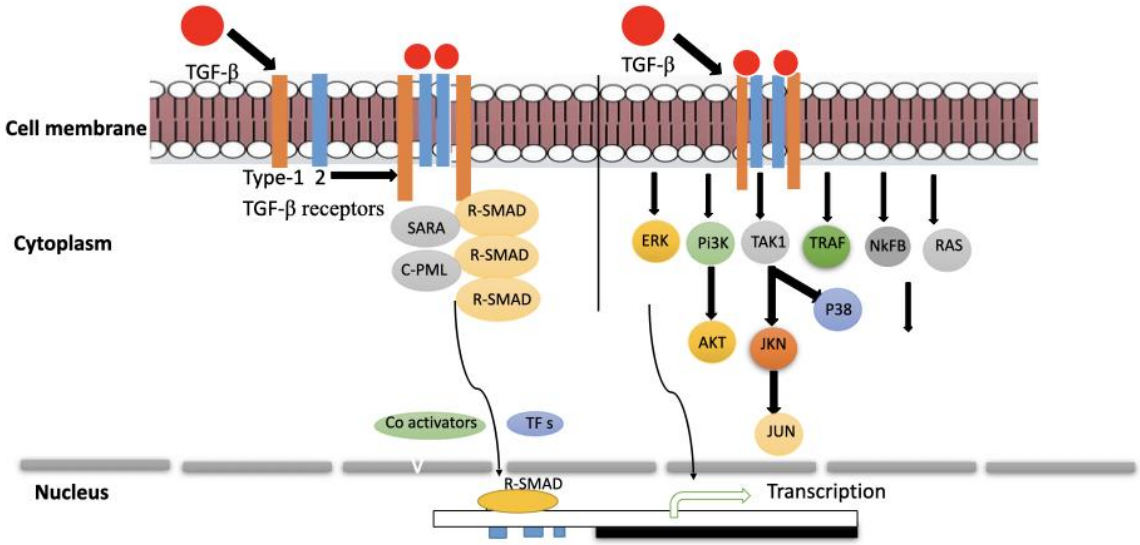


FIGURE 3a: **CANONICAL (SMAD) PATHWAY**

FIGURE 3b: **NONCANONICAL(Non-SMAD) PATHWAY**



UNDER PEER RE