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Review Article

The relationship of transforming growth factor-β and lung cancer

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Abstract: As the No.1 killing cancer in the world, the incidence of lung cancer is still on the rise across the globe these years, such a trend is particularly apparent in China. To study the molecular mechanism of the occurrence of lung cancer and develop new anti-cancer drugs is an arduous task confronting medical researchers of the whole world. Multiple signaling pathways are involved in the lung carcinogenesis. Transforming Growth Factor-β family (TGF-β), known as a multi-functional cytokine superfamily, plays extensively regulatory roles in life activities, including morphogenesis, embryonic development, immunoregulation, healing of wound, as well as inflammation and tumor. Here we review the roles of TGF-β signal transduction pathway in lung cancer.

Keywords: Lung cancer, transforming growth factor beta, signaling pathway, Smad

Introduction

Lung cancer remains the leading cause of death by cancer in the world, marked by the fastest growth of morbidity and mortality and is one of the malignant tumors posing the biggest threat to human health and lives [1, 2]. The incidence of lung cancer in China has come close to the level of advanced countries, but the cause of it is unclear yet. Substantial data reveal that the genesis and progression of lung cancer are co-impacted by hereditary and environmental factors, long-term heavy smoking is closely related to the genesis of lung cancer. Based on different morphological characteristics of cancer cell, lung cancer can be mainly divided into two types: non-small cell lung cancer and small cell lung cancer. Non-small cell lung cancer further includes squamous cell carcinoma, lung adenocarcinoma and large cell carcinoma. These two categories of lung cancer differ distinctly in terms of biological characteristics, prognosis and method of treatment. Small cell lung cancer, less common as it is, is one of the most malignant types, accounting for 10-15% of the primary lung cancer, featuring fast spreading of cancer cells, strong invasiveness, and early distant metastasis. It is sensitive to chemotherapy at first, but often ends in poor prognosis and distant metastasis. This kind of lung cancer is, to a great extent, smoking-related. Squamous cell carcinoma (squamous carcinoma) is the most common type of lung cancer, taking up roughly 40-50% of primary lung cancer, often found in aged male, strongly related to smoking. Squamous carcinoma grows at a slow rate and features late metastasis. It has increased chances of surgical resection, and higher five-year survival rate, but is not so sensitive to chemoradiotherapy as small cell undifferentiated carcinoma. Adenocarcinoma of the lung, featuring early onset and relatively larger number of female patients, has little to do with smoking, accounting for 25% of primary lung cancer. Adenocarcinomas features earlier local infiltration and hematogenous metastasis than squamous carcinoma, and its incidence rate is lower than squamous carcinoma and undifferentiated carcinoma. Metastasis occurs later in large

cell carcinoma than in small cell undifferentiated carcinoma, hence gains greater chances of surgical resection [3-5]. If lung cancer is detected in the early phase, it is highly possible to be cured even it recurs. However, at present, a very good lung cancer marker is still absent for being used as early diagnosis and adjuvant therapy indicator.

Transforming growth factor-β superfamily, known as a big class of multifunctional cell factors, is widely involved in the adjustment of life activities such as embryonic development, cell proliferation, differentiation, migration, apoptosis, vascularization, wound healing, and immune system regulation, and is also related to inflammation, rheumatic disease, heart disease, diabetes, etc. At present, an increasing number of researches have indicated that TGF-β signaling pathway is relevant to the progression of tumor [6-12]. In this review,we focus on the TGF-β signaling pathway and its roles in lung cancer.

The TGF-β superfamily and TGF-β signaling pathway

TGF-β belongs to a group of TGF-β family that regulates cell growth and differentiation [10, 11]. By now, more than 30 members of the TGF-β family have been found, all featuring dimer structure and cysteine group structure motifs. In addition to TGF-β, this family also includes activins, inhibins, Müllerian inhibitory substance (MIS), and bone morphogenetic proteins (BMPs), et al [13-16]. The naming of TGF-β is based on that this kind of cell factor can change the phenotype of normal fibroblast, that is, in case epidermal growth factor (EGF) co-exists, it changes the wall-adherent growth characteristic of fibroblast and gains the ability to grow in agar, and loses the inhibiting effect of density reliance during growth [17-22].

TGF-β is a multifunctional polypeptide cytokine and makes up a group of protein family that regulates cell growth, migration, apoptosis and differentiation. Being the basis of the balance, development and maintenance of tissues in normal or morbid state, it can effectively inhibit the proliferation of most types of cells, such as epithelial cells, endothelial cells, hematopoietic cells, leukomonocyte, etc [9, 23, 24]. It is known as a powerful proliferation and tumor suppressor. According to the researches, TGF-β expression in serum and tissues of tumor patients rises notably along with the malignant degree of tumor, indicating a close relationship between TGF-β and malignant transformation of tumor. Therefore, TGF-β plays a dual role in the genesis and progression of tumor. In other words, it is a tumor suppressor in the early stage of tumor, but promotes malignant transformation of tumor in the later stage through autocrine and paracrine. However, the mechanisms of how TGF-β changes from tumor suppressor into tumor promoter and further promotes malignant transformation of tumor are still unknown [25-30].

TGF-β ligands

The mature TGF-β is a 25 kDa homodimer composed of two monomeric subunits stabilized by hydrophobic interactions and strengthened by a disulfide bond [31]. Three subtypes (TGF-β-1, TGF-β-2, and TGF-β-3) have been found in mammalian cells at present; they have highly similarity in their amino acid sequences but are coded by different genes and arise from different chromosomes [10, 14, 32]. Their human gene mapping is located respectively in chromosome 19q3, lq41 and 14q24, all of which contain 7 exons and whose nucleotide sequences are highly homologous. The C-terminuses of coded precursor molecules all have 9 conservative L-cysteine sequences indicating that TGFβ-1, TGFβ-2 and TGFβ-3 are possibly from the same ancestral gene [31].

The mature TGF-β is synthesized and secreted in the form of a latent complex, the effect of which is to block biological reaction of TGF-β till some physiological conditions have changed or it has contacted the target cell. Latent TGF-β is composed of a TGF-β dimer and latency-associated protein (LAP) that remains bound to TGFβ after secretion and the latent TGF-β-binding protein (LTBP) [33-38]. In the Golgi apparatus, LAP binds again with latent TGF-β binding proteins (LTBPs) through disulfide bond into precursor complex, whose function is to increase the secretion and stability of TGF-β-LAP complex, to ensure the correct folding of TGF-β and to deliver the latent TGF-β complex to certain tissues and extracellular matrixes for storage or present it to the cell surface to be activated [37-43]. Cell identification and LTBP binding with extracellular matrix and consequent identification of latent TGF-β complex

are the crucial steps of TGF-β activation. In vivo, the active TGF-β is released from the latent complex by a series of enzymatic process, such as proteolytic cleavage of LAP by plasmin. Thrombospondin-1 is another activator protein of TGF-β, which can coordinate with the enzymatic process regulated by plasmin [34, 37, 38]. In all, the activation of TGF-β is a multistep, tight and orderly process, the activated TGF-β can interact with many extracellular substances and play an extensive biological function.

TGF-β receptors

TGF-β receptors are high-affinity binding proteins of cell membrane surface and TGF-β, belonging to serine/threonine kinases family. Based on their structure and functional features, they are classified into 3 subtypes: TβRI (53 kDa), TβRII (75 kDa) and TβRIII (250~350 kDa) [38, 44-47]. TβRI and TβRII are both glycoproteins. Their cytoplasmic domain contains Ser/Thr protein kinase domain, possessing Ser/Thr protein kinase activity necessary for cellular signal transduction. TβRI contains a characteristic, highly conserved 30 amino acids long GS domain as it is rich in glycine and serine residues in the cytoplasmic part, which needs to be phosphorylated to fully activate TβRI. TβRII, is a constitutively autophosphorylated serine/threonine kinase receptor [10, 11, 48]. TβRIII, a membrane-anchored proteoglycan, also called beta-glycan, is not directly involved in signaling transduction but has the function of regulating TGF-β indirectly. TβRIII promotes the binding of TGF-β to the signaling TβRI and TβRII [38, 45, 47, 49-51]. Endoglin (CD105) is an RGD-containing membrane glycoprotein and also acts as type III receptor for TGF-β. Although endoglin, like beta-glycan is an co-receptor, which not directly involved in intracellular TGF-β signaling due to lack of kinase domain, it can control the process of TGF-β binding to TGF-β receptors and consequently modulate intracellular TGF-β activity. Beta-glycan binds all three isoforms of TGF-β, with higher affinity for TGF-β2; however, endoglin binds TGF-β1, TGF-β3 but not TGF-β2 in the presence of the TβRI and TβRII [38, 51-55].

Smads

Smad proteins consist of 400~500 amino acid residues, and their molecular mass are about (42~60) × 10^3. They are the important regulatory factors of TGF-β signaling pathway because they transmit the signal directly from the receptors to the nucleus to regulate the transcription of target genes [35, 56]. Smad proteins are classified into three types based on their structural and functional properties: (1) receptor-regulated Smads (R-Smads), including Smad1, Smad2, Smad3, Smad5 and Smad8, wherein Smad2 and Smad3 are involved in TGF-β signaling transduction pathway; (2) Co-mediator Smad (Co-Smad), mainly Smad4, which as a key mediator, oligomerizes with phosphorylated R-Smads; and (3) inhibitory Smads (I-Smads), which includes Smad6 and Smad7. I-Smads negatively regulate TGF-β signal transduction pathway through stable interactions with activated receptors to prevent the activation of R-Smads and Co-Smad [13, 15, 34, 39, 57-62].

Regulation of TGF-β signaling pathway

Smad-dependent pathways: The core of TGF-β signaling pathway is made up of three TGF-β receptors (TβRI, TβRII, TβRIII) and three transcription factors (Smad2, Smad3 and Smad4). TGF-β signaling transduction is initiated by binding two receptors, TβRI and TβRII to form a tetrameric cell surface complex composed of two molecules of TβRII and two molecules of TβRI. TGF-β binds first with the extracellular domain of TβRII and activates TβRII phosphorylase kinases simultaneously which is followed by the recruitment of TβRI into the receptor complex and its transphosphorylation by the TβRII kinase domain. Phosphorylation of the type I receptors occurs mainly in the juxtamembrane region of the intracellular GS domain of the receptor. The activated TβRI phosphorylates the motif SSXS of R-Smads carboxyl terminal and thereby activates R-Smads, Smad2 and Smad3, which is the most important step during signaling transduction. The phosphorylation occurs at two C-terminal serines of Smad2 and Smad3 proteins. Two activated Smad proteins then combine with one Smad4 to form trimeric Smad complexes that translocate into the nucleus and regulate target gene transcription through physical interactions with various other transcriptional regulation factors [13, 38, 46, 51, 57, 63-71].

Smad-independent pathways: In addition to Smad signaling pathway, TGF-β also mediates

non-Smad signaling from the TβRII/TβRI complexes, leading to the activation of pathways that are more commonly seen as effectors of receptor tyrosine kinase signaling, including: (1) MAP kinase ERK1/ERK2, p38MAPK and JNK; (2) growth and survival kinase PI3K, AKT/PKB and mTOR5; (3) small GTP binding proteins Ras, RhoA, Rac1 and Cdc42 [51, 72, 73]. As recent researches have indicated, TGF-β pathway can promote the activation of various tyrosine protein kinases in cells, including FAK, Src and Ab1, especially in interstitial cells and dedifferentiated epithelial cells [11, 42, 48, 74]. The important thing is the hyper-activation of the non-classical TGF-β signaling pathway members may disturb the balance of Smad2/3 signaling pathways of normal cells and cause tumorigenesis. However, the effector molecule activation mechanism of TGF-β and these non-classical pathways as well as their effects in the tumorigenesis and its progression have not yet been ascertained [24, 65, 75, 76].

TGF-β signaling and lung cancer

TGF-β signaling molecules play a complex role in carcinogenesis,,having both tumor suppressor and oncogenic activities. A reduction in TGF-β signaling in tumor cells is often accompanied by increased secretion of the ligand, mutational inactivation or dysregulated expression of components in its signaling pathway. Methods for therapeutic targeting of the TGF-β signaling pathway may be translated into developing more effective strategies for cancer therapy [7, 59, 64, 77-80].

TGF-β ligands and lung cancer

TGF-β1 has been the most studied isoform among the three TGF-β ligands. TGF-β1 will be referred to as TGF-β somewhere. Upregulated TGF-β1 expression is the most frequent in tumor cells, which is the focus of most of the researches that have been carried out on the function of TGF-β in human tumors, including lung cancer [78].

The expression of TGF-β isoformas was immunohistochemically examined on tissue specimens from pulmonary adenocarcinoma patients. The TGF-β expression level was related to be a higher 5-year survival rate of patients. So TGF-β1 may be a useful prognostic marker for lung adenocarcinoma [81-84]. TGF-β1 protein was extracted from tissue specimens of non-small cell lung cancer patients, and its level was examined by enzyme-linked immunosorbent assay. The results suggested that TGF-β1 seem to promote not only tumor angiogenesis but also play an important role in tumor progression and metastasis in NSCLC patients. In addition, TGF-β1 may be a significant predictor of survival in patients with lung adenocarcinoma [85].

Serum level of TGF-β1, a secreted protein, is also the focus of most of the studies that have been. Plasma TGF-β1 in blood samples collected from healthy subjects, patients suffering from nonmaligant pulmonary diseases (NMPD) and patients with lung cancer was measured. TGF-β1 plasma level was increased in patients with lung cancer and was partly related to concomitant NMPD [86]. The plasma TGF-β1 level in lung carcinoma patients before and radiotherapy was higher than that of normal subjects, indicating the plasma TGF-β1 may be a tumor marker of lung carcinoma patients for guiding clinical treatment like radiotherapy [87, 88]. Radiation-induced elevations of TGF-β1 during radiation therapy (RT) were measured using ELISA in patients with NSCLC from Michigan and Beijing. The study suggested that radiation-induced elevations of plasma TGF-β1 level was correlate with radiation-induced lung toxicity (RILT). The combination of TGF-β1 and mean lung dose (MLD) may be a predictor of the patients for their risk of RILT [89]. However, in another study, plasma levels of tumor necrosis factor (TNF)-α and TGF-β1 were quantified in NSCLC patients. Although TNF-α and TGF-β1 levels were increased in NSCLC compared with controls, TNF-α and TGF-β1 levels had no correlation with survival and chemotherapy sensitivity. So TNF-α and TGF-β1 seem not to be reliable prognostic markers for in advanced NSCLC [90]. SCLC cell lines do not synthesize TGF-β1 and TGF-β2 nor TβRII. However, NSCLC cell lines express TGF-β1, TGF-β2 and TβRII. SCLC cell lines avoided inhibitory action of autocrine and paracrine TGF-β. the cell lines expressed the TGF-β were sensitive to the inhibitory action of TGF-β [91]. The expression of TGF-β was measured by ELISA in tissue samples of NSCLC. The results showed the expression of TGF-β was significantly higher in pulmonary metastasis. And using immunohistochemical analysis the

tumor infiltrating stromal cells were confirmed to be the major sources of TGF-β. Therefore, TGF-β expression in tumor infiltrating stromal cells may regulate the occurrence of spontaneous pulmonary metastasis in NSCLC [92]. TGF-β is one of the inhibitors produced by cancer cells that modulate antitumor immunity [93]. TGF-β1 level in bronchoalveolar lavage fluid (BALF) of patients with primary lung cancer was investigated by ELISA. A higher level of TGF-β1 in the BALF samples of patients was observed. These were positive associated with the proportion of lymphocytes and negative correlation with the proportion of macrophages as well as the percentage of cytotoxic and activated T lymphocytes. The study suggested that TGF-β is involved in the local response in the pathogenic course of primary lung cancer [94].

TGF-β1 inhibits tumor in the early stage by inhibiting cell proliferation and promoting cell differentiation or apoptosis [95]. Variation of the TGF-β1 gene may lead to changes of TGF-β1 functions. As revealed in the investigation of the correlations between the TGF-β1-509C > T and 869T > C (L10P) polymorphisms and their haplotypes with risk of lung cancer in a Korean population, individuals with -509T alleles were at a much lower risk of adenocarcinomas and small cell carcinoma compared with individuals with -509CC genotype. For the 869T > C polymorphism, the risk of small cell carcinoma was significantly decreased with the combined TC + CC genotype compared with the TT genotype [96]. To analyze the correlation between the single nucleotide polymorphisms (SNPs) of the TGF-β1 gene and the risk of lung cancer in a Korean population, a SNaPshot primer extension assay was conducted. Two polymorphisms in the promoter region of the TGF-β1 gene (T-1572C, C-509T) and one SNP in codon 10 (T + 869C) were revealed and these were associated with smoking history of patients. The patients with heterozygous C-509T and C-509T genotype who smoke heavily showed an increased risk of lung cancer. However the SNPs had no correlation to the tumor stage or response to treatment [97]. Whether the single nucleotide polymorphisms (SNPs) in the TGF-β1 gene is associated with the risk of radiation pneumonitis (RP) in NSCLC patients was investigated using genomic DNA samples from patients with NSCLC treated with radio chemo therapy. And CT/CC genotypes of TGF-β1 rs1982073:T869C was confirmed to be correlated with lower risk of RP [98]. However, the results in a Chinese NSCLC population was not consistent with the one reported by Yuan et al. Probably the single nucleotide polymorphisms in TGF-β1 gene for anticipating RP vary greatly among different ethnic groups [99]. In human lung cancer cells A549, TGF-β1 increased cell migration and β1 integrin expression. TGF-β1 stimulation increased phosphorylation of p85α subunit of phosphatidylinositol 3-kinase (PI3K) and Ser^{473} of Akt which in turn induced IκB kinaseα/β phosphorylation, IκB phosphorylation and p65 Ser^{536} phosphorylation, resulting in the activations of β1 integrins and contributing to the migration of A549 cells [100].

Most deaths of lung cancer are caused by metastasis epithelial-mesenchymal transition (EMT) plays a critical role in the process of cancer metastasis. TGF-β drives cancer progression through its immunosuppressive and proangiogenic roles. More importantly, TGF-β is a potent inducer of EMT [71, 101-107]. TGF-β1 induced human lung adenocarcinoma cell lines A549 and PC-9 to undergo EMT. The cell morphology changed dramatically and cell motility and invasion were promoted accompanied by downregulated epithelial marker E-cadherin and upregulated mesenchymal markers vimentin and fibronectin following TGF-β1 treatment [108]. Fibroblast is an important component of tumor microenvironment [109]. NSCLC cells (A549, NCI-H358) showed significantly increased EMT signaling synergistically treated with IL-6 and TGF-β1 compared to those treated with TGF-β1 alone. Normal human lung fibroblast (NHLF) cells were also synergistically activated by IL-6 and TGF-β1. IL-6 increased TβRI expression on the surface of A549, NCI-H358 and NHLF cells and enhanced TGF-β signaling. *In vivo*, A549 and NHLF cells promoted tumor formation compared with A549 cells alone in mice. Furthermore, the TGF-β receptor inhibitor SB431542 or the anti-human IL-6 receptor neutralizing antibody (IL-6R-Ab) also attenuated the tumor formation enhanced by co-injection of the two cell types. Thus, IL-6 and TGF-β1 may play a contributing role in maintaining the paracrine loop between fibroblasts and NSCLC cells for tumor progression [110]. In A549 lung adenocarcinoma cells, TNF-α stimulated the

phosphorylation of Smad2 linker region to accelerate EMT induced by TGF-β in Smad-dependent manner confirmed by cell morphology, expression of EMT markers, capacity of gelatin lysis and cell invasion. Further study by gene expression profiling identified that the effects of TGF-β and TNF-α were partially mediated by microRNA microRNA and its downstream target HMGN2 [111]. TGF-β1 induces EMT in both cancer stem cells (CSCs) identified as cells positive for CD133 and non-CSC A549 sublines, increasing the expression of mesenchymal markers such as vimentin and Slug, and downregulating levels of epithelial markers such as E-cadherin and cytokeratins as well as improved migration, upregulation of MMP9 and stem cell transcription factor OCT4 [112]. Thereby TGF-β inducing EMT may be a key mechanism to promote lung cancer cells metastasis.

TGF-β receptors and lung cancer

TGF-β receptors also play an important role in tumorigenesis which is frequently associated with reduced expression or inactivation of TGF-β receptors. The mutations or deletions in TβRI in malignancy are sporadically reported. In contrast, the inactivating mutations of TβR II is the important reason of the loss of negative regulation of TGF-β [59, 77, 113].

Abnormal expression of TβRI and TβRII in various tumor cell lines is highly associated with resistance to TGF-β [113]. The full coding sequences and flanking intron sequences of TβRI gene from primary NSCLC tissue samples were investigated for mutations using SSCP and direct sequencing. Two silent mutations located at codon344 (AAT to AAC) and codon 406 (TTA to CTA) and a polymorphism at the 24th base of intron7 (G to A) were found in the TβRI gene. The subjects with homozygous genotype A/A showed increased risk of developing NSCLC than the common wild genotype G/G. The study showed that polymorphism is frequent in the carcinogenesis of NSCLC [114]. The results of the investigation of the TβRII expression in 11 kinds of small-cell lung carcinoma cell lines indicate that the loss of TβRII expression resulting in the inactivation of TGF-β signaling transduction pathway possibly plays a key role in the small-cell lung carcinogenesis [115]. The expression of TβRI and TβRII in NSCLC cell lines was investigated and the results showed that TβRII expression was decreased or loss, but not of TβRI. After overexpression of full-length TβRII in adenocarcinoma cell line NCI-H358 which lacks TβRII and is insensitive to TGF-β, these cells responded to exogenous TGF-β1 with suppressed cell proliferation and accompanied by morphological change. The research suggests the loss of the expression of wild type TβRII is closely associated with the tumorigenesis in human lung cancer cells [116]. Another study by immunohistology technique, positive TβRI and TβRII expressions in specimens from pulmonary adenocarcinoma patients were associated with poorer prognosis. Therefore, TβRI and TβRII play a critical role in lung adenocarcinoma progression [84]. RNA and protein expressions of TβRII are both downregulation in NSCLC tissue samples using semi-quantitative RT-PCR or western blot. Stable expression of TβRII in lung adenocarcinoma cell line VMRC-LCD, which is insensitivity to TGF-β due to lack of TβRII expression, restored TGF-β-mediated activities including Smad2/3/4 complex formation, TGF-β-responsive reporter gene activity, inhibition of cell proliferation, and increased cell apoptosis. Clones of expressing TβRII decreased the colonies formed in soft-agarose and reduced tumorigenicity in athymic nude mice. These results reveal that stable expression of TβRII in TβRII null cells can reverse malignant behavior of lung tumor cells and loss of TβRII expression may be involved in progression of lung cancer [117]. TβRII was among the signature set of transcriptional profiles of lung adenocarcinoma invasiveness identified by microarray analysis of microdissected bronchioloalveolar carcinoma (BAC). Downregultion of TβRII in adenocarcinoma cells increased tumor cell invasiveness and activate p38 mitogen-activated protein kinases. Repression of TβRII may be an early step in lung adenocarcinoma progression toward metastasis [118]. A decreased in TβRII expression and apoptosis and an increased expression of two cell proliferation markers (Ki-67 and MCM2) coupled with the progression of premalignant lesions toward carcinoma in situ were observed in premalignant bronchoepithelial lesions. The study suggested that impaired TGF-β signaling pathway occur early in the pulmonary neoplastic process [119]. Analysis of TβRII expression level in large cell carcinoma (LCC) and non-LCC using immunohistochemistry method revealed an apparent

downregulation of TβRII expression in LCC versus non-LCC which is consistent with the histopathologic classification of these tumors. The results suggested that the defective TβRII expression might promote the carcinogenesis and/or progression of LCC [120]. Deleting the murine homolog of TβRII (Tgfbr2) in a mouse model of mutant K-ras-induced lung carcinoma which normally induces the formation of multifocal tumors of low invasive potential induced a highly invasive phenotype associated with lymph node metastasis and reduced survival. Moreover, tumor stromal cell profiling revealed increased numbers of B and T cells, collagenized extracellular matrix and increased invasion of TGF-β nonresponsive tumor cells. Together, the KrasTgfbr2-/-mouse model of invasive lung carcinoma confirmed the clinical investigations that repression of TβRII may be an important step in progression of lung adenocarcinoma [121]. In lung adenocarcinoma cell line A549, the knockdown of TβRII using RNAi technology inhibited cell proliferation, apoptosis, invasion and metastasis through Smad-dependent pathway and non-dependent pathways including Erk MAPK, PI3K/Akt and p38 MAPK pathways. The expression of MMPs and VEGF were suppressed as well [122]. Reduced TβRII expression in human NSCLC specimens was found to be associated with smoking and more aggressive tumor behavior such as reduced differentiation, increased tumor stage, increased nodal metastasis, and reduced survival. Furthermore, sporadic TβRII deletion in mouse airway epithelial cells promoted lung adenocarcinoma and lung squamous cell carcinoma (SCC) formation [123].

Decreased TβRII expression has been revealed to be related to various mutations of its gene. TβRII gene maps to chromosome 3p22 [124]. Using PCR-SSCP method to test the loss of heterozygosity (LOH) of chromosome 3p of sporadic lung cancers and analyze whether TβRII gene is inactivated on 3p22. According to the results, in replication error (RER) positive tumors, polyadenine tract is a mutational hot spot of TβRII gene while mutations of TβRII gene occur rarely with LOH on chromosome 3p of lung cancer patients [125]. To elucidate the role of TβRII in lung carcinoma progression, the gene-modified human lung adenocarcinoma cell line NCI-H23, which has a frameshift mutation in and low-expression of TβRII and is resistant to the growth inhibition by TGF-β1 in vitro and is transfected with a retroviral vector expressing wild-type TβRII. The over-expression of TβRII restored the responsiveness of cells to exogeneous TGF-β1 with reduced cell proliferation, nuclear translocation of Smad3 and under phosphorylation of the retinoblastoma protein accompanying p21 up-regulation. These results suggest that impairment of TGF-β receptors contributes significantly to lung cancer progression [126]. Microsatellite instability (MSI) refers to changes in base length of SSR in DNA sequence, manifested as increase or loss of SSR caused by replication error (RER) of DNA. In TβRII gene, MIS is a cause of down-regulated TβRII expression. Polynucleotide stretch frame shift mutations of TβRII were analyzed in sporadic primary NSCLC with microsatellite instability. In the RER+ (replication-error-positive) tumors no alternations in stretches of nucleotide inside TβRII gene were found [127]. Sequencing for TβRII poly-A tract was performed in NSCLC cell lines and surgically resected NSCLC tissues to determine the frame shift mutations with MSI of TβRII. The study indicates that MSI is highly correlated with TβRII frame shift mutations in NSCLC [128]. An overall weak expression of TβRII RNA has been found in small cell lung cancer (SCLC). In one cell line which did not express TβRII, a GG to TT base substitution was found which linked exposure to benzo[a]-pyrene, a component of cigarette smoke. This suggested that the mutation in SCLC was caused by cigarette smoking [129]. In the NSCLC cells and tissues, one novel homozygous microdeletion (c .492_507del) in the giant cell carcinoma (GCC) originated cell lines was found, which was confirmed in the corresponding tumor tissues. Moreover, one heterozygous c .492_507del in germ-line of one patient and the other GCC cases and some large cell carcinomas (LCC) was also observed. This microdeletion resulted in a truncated TβRII protein. After restored the wild type TβRII expression, these GCC cells regained the sensitivity to TGFβ. Therefore, TβRII mutations in GCC cells may play an important role in the impairment of TGFβ signal transduction [130].

Epigenetic change of TβRII gene is also one of the reasons that inhibit its expression. The alteration of histone deacetylation as well as the TSA-responsive region and TRE2 motif in the TβRII promoter was involved in the altera-

tion of chromatin structure in lung cancer cell lines. This may be correlation with loss of TβRII expression [131]. Immunohistochemical assay revealed reduced or lost expression of TβRII gene in tissue samples from patients with primary NSCLC. Methylation-specific PCR analysis on the TβRII promoter in the tumor tissues showed aberrant 5'- CpG methylation, whereas most of the paracarcinoma tissues did not. Accordingly, decreased mRNA level of the gene in the tumor tissue samples was agreement to reduced TβRII expression. Therefore, aberrant methylation in TβRII promoter resulted in the down-regulation of the gene at a transcriptional level has explained the defective expression of TβRII may be one of the most important carcinogenesis mechanisms in NSCLC carcinoma [132]. The histone deacetylase (HDAC) inhibitors (HDIs) can restore TGF-β-induced tumor suppressor function in lung cancer cell lines that lack TβRII expression because HDI-responsive element exits in the TβRII promoter (-127/-75). Sp1 and NF-YA recruit the transcriptional repressor Meis1/2 to the TβRII promoter to repress the TβRII promoter activity [133]. The above studies illustrate epigenetic modification for the downregulation of TβRII in lung cancer.

The chemokine CCL5 (RANTES) was identified as a potential downstream regulator of TGF-β signaling pathway that was upregulated in invasive bronchioloalveolar carcinoma and was required for invasion in cells with loss TβRII expression. In TβRII deficient cells, invasion induced by TβRII inhibitors Met RANTES and a CCR5 receptor blocking antibody was abrogated. Immunostaining was conducted in human lung adenocarcinoma specimens the results of which illustrated moderate or high expression of both RANTES and CCR5 was associated with an increased risk for lung adenocarcinoma patients' death [134, 135].

TβRIII is lost in most NSCLC tissues samples and is associated with tumor grade and disease progression. Loss of TβRIII expression in the studied NSCLC specimens was due to loss of heterozygosity at the TβRIII genome DNA sequence. Restoring TβRIII expression in the NSCLC cell line H460 decreased colony formation in soft agar. In A549 cells, restoring TβRIII expression greatly repressed cell migration, invasion and tumorigenicity *in vivo*. On the contrary, knock-down endogenous TβRIII expression enhanced invasion. These results indicated that TβRIII functions as a novel tumor suppressor gene, inhibiting cell migration, invasion and anchorage-independent growth in NSCLC [136].

Smads and lung cancer

The operation of TGF-β signal transduction pathway is well-achieved mainly by the normal expression of its downstream signal Smads protein [38, 137]. As the important regulatory factor of TGF-β/Smads signal transduction pathway, Smad2, Smad3 are phosphorylated by activated TGF-β and are consequently activated, then bind with Smad4 into heterogeneous complex and enter the cell nucleus to where they regulate downstream transcriptional responses under the co-effect of other transcription regulatory factors [15]. According to the research, the inactivated Smad3 and Smad4 can completely inhibit TGF-β-mediated growth inhibition effect. While Smad7 has negative feedback effect in TGF-β/Smads signal transduction pathway, it can closely bind with activated TGF-β1 receptor and competitively block the R-Smads phosphorylation, thereby inhibiting TGF-β signal transduction. Disturbing expression of Smad7 can disturb the immune response of cell to TGF-β, thereby causing malignant changes to the cells [15, 39, 60, 78, 138-144]. Therefore, abnormal Smads protein function will surely lead to the deviation of signal transduction pathway from normal track, and further induce tumorigenesis [56, 77, 145, 146].

The biological and biochemical functions of Smad2 and Smad4 mutants, which were defective in transmitting growth inhibitory signals originating from TGF-β and incapable of activating Smad/hFAST-1-mediated transcription characterized in human lung cancers. Transcriptional activation of plasminogen activator inhibitor type 1 (PAI-1) was impaired in most of the mutants due to the defects in homo- and/or hetero-oligomerization with wild-type Smads. Significant loss of growth inhibition and Smad/hFAST-1-mediated transcriptional activation by all of the mutants suggested that Smad mutations may play a role in lung tumorigenesis [147]. Smad6 is correlation to poor survival in lung carcinoma patients. In

lung cancer cell H1299, knockdown of Smad6 led to up-regulation of plasminogen activator inhibitor-1 and phosphorylation of Smad2/3. Furthermore, the c-Jun NH_2-terminal kinase signal pathway was activated and Rb-1 phosphorylation was reduced, resulting in reduced cell viability and increased apoptosis as well as G0-G1 cell arrest. While in the normal bronchial epithelial cell line Beas2B, these were not observed. These results indicate that Smad6 plays an important role in promoting lung cancer cell growth and survival. So inactivation of Smad6 may facilitate the development of successful therapies targeting TGF-βsignaling for the treatment of lung cancers [148].

Conclusions

TGF-β plays an important role in the genesis and progression of tumor. In normal cells and early stage of tumor, TGF-β is a tumor suppressor; but in the advanced aggressive tumor, it plays the part of a tumor progression prompter. Though the mechanism of how TGF-β changes from cancer inhibitor to cancer promoter and further promotes malignant transformation of tumor is still in the dark, to understand and illustrate the molecular mechanism of activating TGF-β into tumor promoter is crucial to the development of new anti-TGF-β tumor therapy. Due to its unique carcinogenic effect, TGF-β signaling transduction pathway has been considered as an effective target site for tumor treatment. Therefore, we may envisage that it is possible to apply TGF-β more effectively to clinical diagnosis of lung cancer and innovations in therapies along with a deeper understanding of TGF-β and its functions as well as further knowledge of the mechanism of TGF-β signaling pathway's role in malignant change of lung cancer. Current studies have indicated that serum TGF-β1 level of lung cancer patients is possibly related to the progression of disease; it may serve as the biological marker of lung cancer. The author believes that to draw the conclusion on whether serum TGF-β1 is of great clinical significance, longer follow-up of larger number of patients is necessary. In addition, it is very hard to predict whether blocking TGF-β only effectively inhibits its pro-metastasis effect without influencing its tumor-inhibiting effect and makes spontaneous tumor occur in other places of the organism. In light of this, to develop new therapies with focus placed on the downstream signaling regulating component of pro-tumor effect of TGF-β is safer and more effective in the long run. Smoking induces lung cancer and also reduces the effect of surgery, chemotherapy and molecular targeted therapy. To vigorously publicize the hazards of smoking and lunch non-smoking campaigns is currently a feasible measure for prevention and treatment of lung cancer.

Disclosure of conflict of interest

None.

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