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ROLE OF SMADS IN RESPIRATORY DISEASE PATHOGENESIS

RUOLO DEGLI SMADS NELLA PATOGENESI DELLE MALATTIE RESPIRATORIE

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Abstract.
Transforming Growth Factor beta (TGFβ) cytokine plays an important role in normal pulmonary morphogenesis and function as well as in the pathogenesis of lung diseases. The principal signaling pathway downstream to activate TGFβ is the Smad pathway. Even though many studies have focused on Smads' structural features and pathway, less is known about the possible relationship between protein and mRNA expression of Smads and lung diseases. This review will focus on Smads and sum up what is know about their role in some respiratory diseases: COPD, asthma and fibrosis.

KEYWORDS: TGFβ, Smads, Lung disease, COPD, pulmonary fibrosis.

Riassunto.
La citochina TGFβ (Transforming Growth Factor beta) gioca un ruolo importante nella normale morfogenesi e funzione polmonare nonchè nella patogenesi delle malattie respiratorie. Il principale pathway di trasduzione del segnale indotto dal TGFβ attivato è quello degli Smads. Anche se molti studi si sono soffermati sull'analisi delle caratteristiche strutturali e sui meccanismi alla base del pathway, poco si sa circa la possibile correlazione tra l'espressione proteica e trascrizionale degli Smads e le patologie polmonari. Questa review analizzerà le caratteristiche degli Smads e riassumerà le informazioni riguardanti il loro ruolo nelle patologie respiratorie: asma, COPD e fibrosi.

PAROLE CHIAVE: TGFβ, Smads, malattie polmonari, COPD, fibrosi polmonare.

Introduction

COPD (Chronic Obstructive Pulmonary Disease) is characterised by a slow, progressive and partially reversible airflow limitation, which is associated with an abnormal inflammatory response of the lung to noxious particles or gases. The pathological hallmarks of COPD are typical of the principal lung diseases: mucus hypersecretion and submucosal gland hyperplasia (chronic bronchitis), collapsed airways and destruction of airways parenchyma (emphysema), tightening of the muscles around the airways (chronic asthma) followed by tissue damage and inflammation of small airways and fibrosis (1). TGFβ appears to be directly involved in the development and maintenance of pulmonary diseases. TGFβ superfamily consists of secreted growth factors regulating different cellular process such as cell growth, development, differentiation, proliferation, motility, adhesion and apoptosis (Table 1) (2-4). Members of this family are secreted as latent forms, due to the presence of a propeptide, or in trapped form by binding to occluding factors. The active form is a dimer able to initiate signal by binding to a specific pair of membrane serine/threonine kinases receptors, type I and type II receptor. Destruction of the TGFβ signaling system has been implicated in embryonic anomalies (5-8), cancer and tumorigenesis (4,9), autoimmune diseases (10-13), atherosclerosis (14-16), hypertension (17), osteoporo-

Tab. 1: *TGFβ regulatory effects on target cells.*

Endothelium	Epithelium	Fibroblasts
Migration Morphogenesis[55] Growth control[56]	Cell cycle arrest Apoptosis [55, 57] Adhesion ECM production[58] Cytochine production Growth control[56] Epithelial-mesenchymal transition (EMT)[59]	ECM production[58,60] Proliferation[61,62,63] Cytokine secretion Anchorage-independent growth[64] Growth arrets[56,65]

sis (18), fibrotic disease (9-19) and hereditary hemorrhagic telangiectasia (20-21). These pathological states suggest a possible involvement of Smads/TGFβ signaling in development and maintenance of these pathological conditions.

Basic features of Smads

The name "Smad" was coined in reference to its sequence similarity to the *Sma* and *Mad* proteins. Eight Smad proteins are encoded in the human and mouse genome, four in *Drosophila* and three in *C. Elegans* (22). Only five of the mammalian Smads (Receptor-regulated Smads or R-Smads: Smad1, Smad2, Smad3, Smad5 and Smad8) act as substrates for the TGFβ family's receptors; specifically Smad1, 5, and 8 serve principally as substrates for the BMP (Bone Morphogenetic Protein) and anti-Muellerian receptors, Smads2 and 3 for the TGFβ, activin, and nodal receptors. Co-Smad Smad4 serves as a common partner for all R-Smads while Smad6 and 7 are inhibitory Smads (I-Smads) that serve as decoys interfering with Smad-receptor or Smad-Smad interactions (23). Functional studies, together with the X-ray crystal structure analysis, showed that these ~500 amino acids proteins consist of two conserved globular domains (*MH1 and MH2 domains*) coupled by a flexible *linker region* rich of binding sites for Smurf (Smad ubiquitination-related factors) ubiquitin ligase, of phosphorylation sites for several classes of protein kinases and, in Smad4, a nuclear export signal (NES) involved in nucleus-cytoplasmatic translocation (23). The MH1 domain is conserved in all R-Smads and in Co-Smad, but not in I-Smads and functions as a DNA-binding site, while the MH2 domain is conserved in Smad proteins and involved in Smad-Smad interaction and in R-Smad activation/phosphorylation. Binding of TGFβ to the type I receptor triggers phosphorylation of its cytoplasmatic GS domain by the type II receptor, thus creating a repeated pS-X-pS motif, that serves as a docking site for the R-Smads. The latter are presented to the activated type I receptor by the anchor protein SARA (Smad Anchor for Receptor Activation). In the basal state, Smads form homoligomers and remain in an inactive conformation until both activated R-Smads and Smad4 form homotrimeric complexes (24,25). In this state R-Smads decrease their affinity for SARA, and the R-Smad/Smad4 complex is translocated into the nucleus, where can directly bind DNA with DNA promoters or interact with transcription factors or co-factors (26,27) (Figure 1). Smads activation and downstream targets activation can be regulated in both cytoplasmatic and nuclear compartments by different mechanisms including inhibitory Smads activation (28), ubiquination (29), acetylation (30), sumoylation (31) and, as recently reported, dephosphorylation by PPM subfamily phosphatases (PPM1A, PPM1B and SCP1) (32,33).

TGFβ and lung disease: role of Smads

Injury of lung tissue leads to induction of TGFβ that limits some inflammatory reactions. It is also involved in mediating fibrotic tissue remodelling, by increasing the production and decreasing the degradation of connective tissue, and acts mediating the normal tissue repair (34). In several studies, TGFβ has been shown to be a marker of activity of tissue repair and remodelling; acute, as well as chronic, lung diseases showed an increase of TGFβ protein and mRNA expression during the phase of tissue remodelling (35-36). Disruption of the TGFβ signaling system has been shown to be involved in different pulmonary diseases such as COPD and pulmonary fibrosis.

Fibrosis

The direct involvement of TGFβ in fibrosis has been observed during several studies on fibrotic diseases. The prominent hypothesis of fibrosis development is that it is caused by chronic inflammation in response to an unknown etiologic agent, leading to tissue destruction, ongoing wound healing responses, and fibrosis (37). TGFβ is a critical element of progression from inflammation to chronic fibrosis. The pro-fibrotic effects of TGFβ are numerous, including induction of myofibroblasts, increase of matrix synthesis, and inhibition of col-

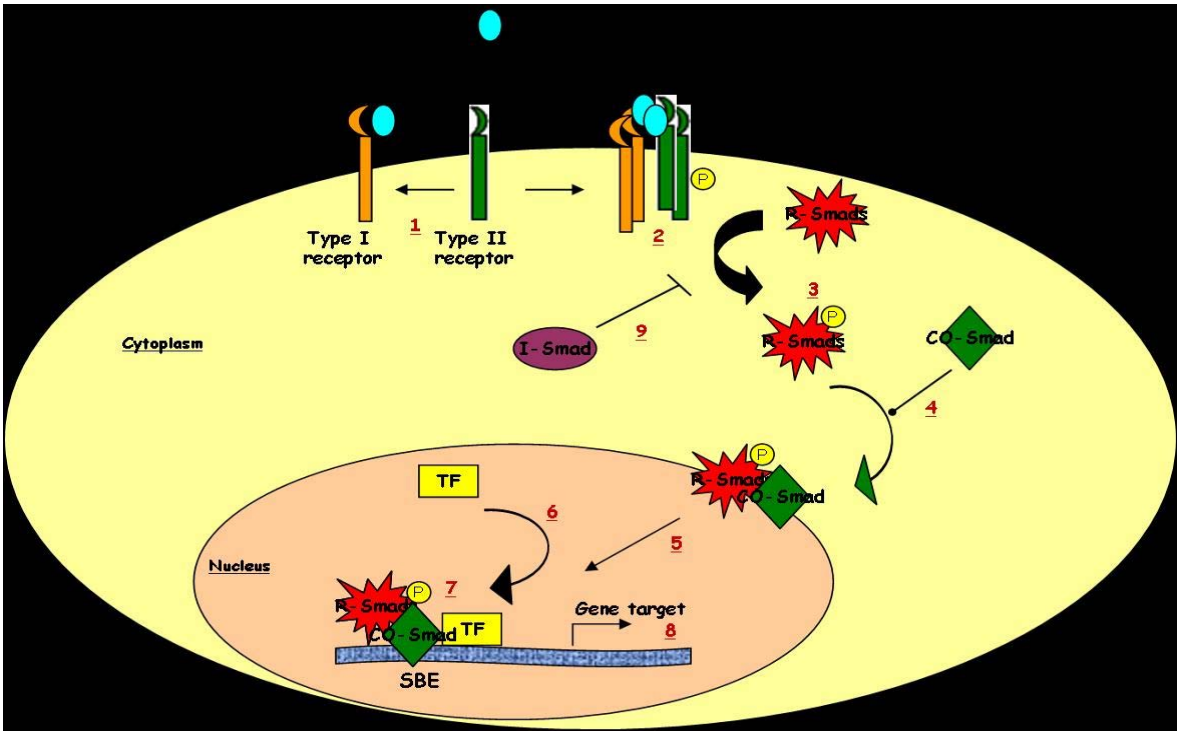

Fig. 1*: Molecular mechanism of Smads pathway.* ***1)*** *Binding of TGFβ to the type I and type II receptors, induces formation of multimeric receptors.* ***3-4)*** *Activated R-Smads dissociate from the receptor/SARA complex and form an oligomeric complex with Co-Smad Smad4.* ***5-6-7)*** *The complex R-Smad/Co-Smad traslocates to the nucleus where it can interact with transcription factors or directly with DNA in Smad Binding Elements (SBE).* ***8)*** *Gene target transcription is activated.* ***9)*** *I-Smads (Smad7) inhibits signals competing with R-Smads for interacting with type I receptor and prevending phosphorylation.*

lagen breakdown. Most of these effects are mediated through the Smad signaling pathway. Smad3 is principally related to fibrotic phenotype. Smad3 pathway is involved in pathogenic mechanisms mediating tissue destruction (lack of repair) and fibrogenesis (excessive repair); Smad3 null mice are protected from progressive fibrosis mediated by overexpression of TGFβ1 (38), do not develop lung fibrosis induced by bleomycin (39), and are protected against radiation-induced fibrosis of the skin (39). In a study by Gauldie and colleagues (41), administration of active TGFβ to mice deficient in Smad3, blocked the ability of TGFβ to induce matrix gene expression, enzyme inhibitors' gene expression and matrix accumulation, thus not progressing to scar formation or fibrosis. This indicates that TGFβ and Smad signalling pathway, specifically Smad3, are required to initiate fibrosis and that mechanisms inducing expression of this growth factor are prominent in this disease. Loss of Smad3 was shown to confer resistance to fibrosis and resulted in reduced inflammatory cell infiltrates, reduced autoinduction of TGF-β (important to sustain the process) and reduced elaboration of collagen. Also, the key cellular mechanism of fibrosis is associated with myofibroblasts transdifferentiation; myofibroblasts are generated from resident mesenchymal cells, endothelial and epithelial cells (epithelial-mesenchymal transition-EMT) (42); TGFβ stimulates myofibroblast transdifferentiation through Smad3-dependent and -independent signals, contributing to the excessive matrix deposition that characterizes obliterative bronchiolitis. A significantly reduced expression of the Smad3 protein was observed in cystic fibrotic epithelial cells of nasal epithelium, and this reduction was apparently sufficient to influence the transmission of TGFβ signals, including anti-inflammatory signals. (43).

COPD and Asthma

Less is known about the direct involvement of Smad proteins in COPD, even though various studies have shown that TGFβ is involved in airways remodelling which characterize this disease. TGFβ1 protein and mRNA expression were increased in the bronchial and alveolar epithelium of COPD patients and correlated with the number of intraepithelial macrophages (44). Elevated levels of TGFβ1 have been observed in bronchial epithelium of smokers with COPD compared with those without COPD (45). Springer et colleagues (46) demonstrated that cigarette smoke down-regulates the inhibi-

tory Smad6 and 7 transcription in bronchial mucosal biopsies from severe COPD patients. A reduced mRNA expression of Smad7 was observed in bronchial biopsies of COPD stage II patients in comparison with controls, but no significant change was observed for Smad3 and 4. The study did not detect Smad2 transcription in the bronchial biopsies tested. In contrast, Zandvoort and colleagues (47) verified Smad2 protein expression, but found no significant differences between controls and COPD. Increased presence of TGFβ1 in the parenchyma may protect against emphysema as demonstrated in a mouse model, where constitutive expression of TGFβ1 prevented emphysema development. Absence of proper Smad3 signaling results in an ineffective repair response to damage in the lung, reduction of suppression of expression of MMPs, and susceptibility to airspace enlargement and emphysema. Disregulation of MMP expression has been shown as a key feature of smoke-exposed human lung fibroblasts in an in vitro model of COPD pathogenesis (48). Moreover Smad3-deficient animals are protected from fibrosis but are more susceptible to emphysema: Smad3 null mice are resistant to bleomycin- and TGFβ-mediated fibrosis, but they develop spontaneous age-related airspace enlargement, consistent with emphysema, with a lack of ability to repair tissue damage appropriately (49). Studies on asthmatic patients showed increased levels of TGFβ1 and its transductor factors in the airways (50). Airway remodelling is one of the hallmark features of asthma. TGFβ appears to be implicated in ECM proteins deposition which characterizes asthma, especially collagen. Concentrations of the active form TGFβ1 are higher in bronchoalveolar lavage fluid (BAL) of patients with severe asthma compared with controls (51). Runyan and colleagues (52) found a cross talk between Smads and PI3K (Phosphoinositide 3-kinase) pathway that enhanced TGFβ-induced collagen type I expression in human mesangial cells. Recently, a different Smad2 activation was observed between asthmatic and non-asthmatic airway smooth muscle cells (ASM) with levels of phosphorylated Smad2 significantly higher in the asthmatic cells in comparison to the non-asthmatic (53). Expression levels of Smad7 in bronchial epithelial cells of asthmatic patients were inversely correlated with basement membrane thickness and airway hyperresponsiveness in asthmatic subjects, suggesting an active remodelling process resulting in a thickened basement membrane (54).

Concluding remarks

In the last ten years the attention of scientists for Smad proteins has increased, in view of their prominent roles in the pathogenesis of lung diseases. Studies on Smads have started to explain their potential role in regulation and inhibition of TGFβ effects. Modifications of Smad pathways or of their receptor systems, with a combination of genetic and environmental factors, could contribute to development of several TGFβ-dependent diseases. All these information will expand the complex network about the TGFβ signaling and thereby the potential role of Smad proteins in human respiratory diseases, potentially helping to design therapeutic strategies that should target TGFβ signaling.

Reference

1. Global Initiative on Obstructive Lung Diseases (GOLD), Workshop Report 2006.
2. Hogan BLM. Bone morphogenetic proteins: Multifunctional regulators of vertebrate development. Genes & Dev. 1996; 10:1580–1594.
3. Mehler, MF, Mabie PC, Zhang D and. Kessler JA. Bone morphogenetic proteins in the nervous system. Trends Neurosci 1997; 2-0:309–317.
4. Dijke PT, Gourmans MJ, Itoh F, Itoh S. Regulation of cell proliferation by smad proteins. J.Cell. Phys. 2002; 191:1-16.
5. Chang H, Huylebroeck D, Verschueren K, Guo Q, Matzuk MM, Zwijsen A. Smad5 knockout mice die at mid-gestation due to multiple embryonic and extraembryonic defects. Development 1999; 126:1631–1642.
6. Chang H, Zwijsen A, Vogel H, Huylebroeck D, Matzuk MM. Smad5 is essential for left-right asymmetry in mice. Dev Biol 2000; 21-9:71–78.
7. Galvin KM, Donovan MJ, Lynch CA, Meyer RI, Paul RJ, Lorenz J, Fairchild-Huntress V, Dixon KL, Dunmore JH, Gimbrone MA, Falb D, Huszar D. A role for Smad6 in development and homostasis of the cardiovascular system. Nat Genet 2000; 24:171–174.
8. Yang X, Castilla LH, Xu X, Li C, Gotay J, Weinstein M, Liu PP, Deng C-X. Angiogenesis defects and mesenchymal apoptosis in mice lacking SMAD5. Development 1999; 126:1571-1580.
9. Blobe GC, Schiemann WP, Lodish HF. Role of transforming growth factor b in human disease. N Engl J Med 2000; 342:1350–1358.
10. Oshima M, Oshima H, Taketo MM. TGF-b receptor type II deficiency results in defects of yolk sac hematopoiesis and vasculogenesis. Dev Biol 1996; 179:297–302.
11. Zhu Y, Richardson JA, Parada LF, Graff JM. Smad3 mutant mice develop metastatic colorectal cancer. Cell 1998; 94:703–714.
12. Yang X, Letterio JJ, Lechleider RJ, Chen

L, Hayman R, Gu H, Roberts AB, Deng C. Targeted disruption of Smad3 results in impaired mucosal immunity and diminished T cell responsiveness to TGF-b. EMBO J 1999; 1-8:1280–1291.
13. Ashcroft GS, Yang X, Glick AB, Weinstein M, Letterio JL, Mizelm DE, Anzano M, Greenwell-Wild T, Wahl SM, Deng C, Roberts AB. Mice lacking Smad3 show accelerated wound healing and an impaired local inflammatory response. Nat Cell Biol 1999; 1:260–266.
14. Grainger DJ, Kemp PR, Metcalfe JC, Liu AC, Lawn RM, Williams NR, Grace AA. The serum concentration of active transforming growth factor-b is severely depressed in advanced atherosclerosis. Nat Med 1995; 1:74–79.
15. Grainger DJ, Witchell CM, Metcalfe JC. Tamoxifen elevates TGF-b and suppresses diet-induced formations of lipid lesions in mouse aorta. Nat Med 1995; 1:1067–1072.
16. Grainger DJ, Heathcote K, Chiano M, Snieder H, Kemp PR, Metcalfe JC, Carter ND, Spector TD. Genetic control of the circulating concentration of transforming growth factor type b1. Hum Mol Genet 1999; 8:93–97.
17. Cambien F, Ricard S, Troesch A, Mallet C, Generenaz L, Evans A, Arveiler D, Luc G, Ruidavets JB, Poirier O. Polymorphisms of the TGFb-1 gene in relation to myocardial infarction and blood pressure—the ECTIM study. Hypertension 1996; 28:881–887.
18. Langdahl BL, Knudsen JY, Jensen HK, Gregersen N, Erisksen EF. A sequence variation (713-8delC) in the TGFb-1 gene has higher prevalence in osteoporotic women than in normal women and is associated with very low bone mass in osteoporotic women and increased bone turnover in both osteoporotic and normal women. Bone 1997; 20:289-294.
19. Beránek M, Kanˇ kova´ K, Benesˇ P, Izakovicˇova´-Holla´ L, Znojil V, Ha´jek D, Vı´kova´ E, Va´cha J. Polymorphism R25P in the gene encoding transforming growth factor-beta (TBF-b1) is a newly identified risk factor for proliferative diabetic retinopathy. Am J Med Genet 2002; 109:278–283.
20. Johnson DW, Berg JN, Baldwin MA. Mutations in the activin receptor like kinase 1 gene in hereditary haemorrhagic telangiectasia type 2. Nat Genet 1996; 13:189–195.
21. Gallione CJ, Klaus DJ, Yeh EY. Mutation and expression analysis of the endoglin gene in hereditary hemorrhagic telangiectasia reveals null alleles. Hum Mutat 1998; 11:286–294.
22. Massague´ J. TGF-β signal transduction. Annu. Rev. Biochem. 1998; 67, 753–791.
23. Massaguè J, Seoane J, wotton D. Smad transcription factors.. Genes and Dev. 2005; 19:2783-2810.
24. Shi Y, Hata A, Lo RS, Massaguè J, Pevletich NP. A structural basis for mutational inactivation of the tumor suppressor Smad4. Nature, 1997; 388:87-93.
25. Zhang Y, Feng X, We R & Derynck R. Receptor associated Mad homologues synergize as effectors of the TGF-β response. Nature 1996; 383:168–172.
26. Xu L, Chen YG and Massagué J. The nuclear import function of Smad2 is masked by SARA and unmasked by TGFβ-dependent phosphorylation. Nat. Cell Biol. 2000; 2:559–562.
27. Xu L, Kang Y, Col S and Massagué J. Smad2 nucleocytoplasmic shuttling by nucleoporins CAN/Nup214 and Nup153 feeds TGFβ signaling complexes in the cytoplasm and nucleus. Mol. Cell 2002; 10:271–282.
28. Nakao A, Afrakhte M, Morén A, Nakayama T, Christian JL, Heuchel R, Itoh S, Kawabata M, Heldin NE, Heldin CH, et al. Identification of Smad7, a TGF_-inducible antagonist of TGF-β signaling. Nature 1997; 389: 631–635.
29. Suzuki C, Murakami G, Fukuchi M, Shimanuki T, Shikauchi Y, Imamura T and Miyazono K. Smurf1 regulates the inhibitory activity of Smad7 by targeting Smad7 to the plasma membrane. J. Biol. Chem. 2002; 277:39919-39925.
30. Tu AW, Tuo K . Acetylation of Smad2 by the Co-activator p300 Regulates Activin and Transforming Growth Factor β Response. The Journal of Biological Chemistry 2007; 29: 2118-7-21196.
31. Song J, Durrin LK, Wilkinson TA, Krontiris TG and Chen Y. Identification of a SUMO-binding motif that recognizes SUMO-modified proteins. Proc. Natl. Acad. Sci. 2004; 101: 143-73–14378.
32. Lin X, Duan YY, Liang Y, Su Y, Wrighton KH, Long J, Hu M, Davis CM, Wang J, Brunicardi FC, Shi Y, Chen YG, Meng A, Feng XH. PPM1A fuction as a smad phosphatase to terminate TGFbeta signalling. Cell 2006; 125 915-928.
33. Lin X, Chen Y, Meng A, Feng X. Termination of TGFb Superfamily signalling through SMAD desphosphorylation- a functional genomic view. Journal of Genetics and Genomics 2007; 34: 1-9.
34. Sporn MB, Roberts AB. Transforming growth factor-β: recent progress and new challenges. J Cell Biol 1992;119:1017–1021.
35. Elssner A, Jaumann F, Dobmann S, et al. Elevated levels of interleukin-8 and transforming growth factor-β in bronchioalveolar lavage fluid from patients with bronchiolitis obliterans syndrome: proinflammatory role of bronchial epithelial cells. Transplantation 2000; 70:362-367.
36. Broekelmann TJ, Limper AH, Colby TV,

et al. Transforming growth factor-β1 is present at sites of extracellular matrix gene expression in human pulmonary fibrosis. Proc Natl Acad Sci 1991; 88:6642-6646.
37. Bonniaud P, Margetts PJ, Ask K, Flanders K, Gauldie J, and Kolb N. TGF-β and Smad3 Signaling Link Inflammation to chronic fibrogenesis1. The Journal of Immunology, 2005; 175: 5390–5395.
38. Bonniaud P, Kolb M, Galt T, Robertson J, Robbins C, Stampfli M, Lavery C, Margetts PJ, Roberts AB and Gauldie J. Smad3 null mice develop airspace enlargement and are resistant to TGF-β-mediated pulmonary fibrosis. J. Immunol. 2004; 173: 2099–2108.
39. Zhao J, Shi W, Wang JY, Chen H, Bringas P Jr, Datto MB, Frederick JP, Wang XF and Warburton D. Smad3 deficiency attenuates bleomycin-induced pulmonary fibrosis in mice. Am. J. Physiol. 2002; 282:L585–L593.
40. Flanders KC, Sullivan CD, Fujii M, Sowers A, Anzano MA, Arabshahi A, Major C, Deng C, Russo A, Mitchell JB, and Roberts AB. Mice lacking Smad3 are protected against cutaneous injury induced by ionizing radiation. Am. J. Pathol. 2002; 160:1057–1068.
41. Gauldie J, Bonniaud P, Sime P, Ask K and Kolb M. TGF-β, Smad3 and the process of progressive fibrosis. Biochemical Society Transactions 2007; 35:661-664 .
42. Zeisberg EM, Tarnavski O, Ziesberg M, Dorfman AL, McMullen JR Gustafsson E et al. endothelial-to-mesenchymal transition contributes to cardiac fibrosis. Nat Med 2007; 13:952-961.
43. Kelley TJ, Elmer HL, Corey DA. Reduced smad3 protein expression and alterated transforming growth factor-b1-mediated signalling in cystic fibrosis epithelial cells. Am. J. Resp. Cell. Mol. Biol. 2001; 25:732-738.
44. De Boer W, Van Shadwicke A, Sont JK et al. Transforming growth factor b1 and recruitment of macrophages and mast cells in the airways in chronic obstructive pulmonary disease. Am J respire Crit Care Med 1998; 158:1951-1957.
45. De Boer W, Sont JK, Van Sa, Stolk J, Van KJ, Hiemstra PS. Monocyte chemoattractant protein 1, interleukin 8 and chronic airways inflammation in COPD. J Pathol 2000; 190:619-626.
46. Springer J, Scholz FR, Peiser C, Groneberg DA, Fischer A. SMAD-signaling in cronich obstrctive pulmonary disease: transcriptional down-regulation of inhibitory SMAD6 and 7 by cigarette smoke. Biol. Chem. 2004; 385:649-653.
47. Zandvoort A, Postma DS, Jonker MR, Noordhoek JA. Vos JTWM, Van Der Geld YM and Timens W. Altered expression of the Smad signalling pathway: implications for COPD pathogenesis. Eur Respir J. 2003; 28: 533–541.
48. La Rocca G, Anzalone R, Magno F, Farina F, Cappello F and Zummo G. Cigarette smoke exposure inhibits extracellular MMP-2 (gelatinase A) activity in human lung fibroblasts. Resp Res 2007; 8:23.
49. Gauldie J, Kolb M, Ask K, Martin G, Bonniaud P and Warburton D. Smad3 Signaling Involved in Pulmonary Fibrosis and Emphysema. Proc Am Thorac Soc 2006; 3: 696–702.
50. Redington AE, Madden J, Frew AJ, Djukanovic R, Roche WR, Holgate ST, Howarth PH. Transforming growth factor-beta 1 in asthma. Measurement in bronchoalveolar lavage fluid. Am J Respir Crit Care Med 1997; 156:642-47.
51. Tillie-Leblond I, Pugin J, Marquette CH, Lamblin C, Saunier F, Brichet A, Wallaert B, Tonnel AB, Gosset P. Balance between proinflammatory cytokines and their inhibitors in bronchial lavage from patients with status asthmaticus. Am. J. Respir Crit Care Med 1999; 159: 487-494.
52. Runyan CE, Schnaper HW, Poncelet AC. The phosphatidylinositol 3-kinase/Akt pathway enhances Smad3-stimulated mesangial cell collagen I expression in response to transforming growth factor-beta1 J Biol Chem 2004; 279:2632-2639.
53. Johnson PRA, Burgess JK, Ge Q, Poniris M, Boustany S, Twigg SM, Black JL. Connective Tissue Growth Factor Induces Extracellular Matrix in Asthmatic Airway Smooth Muscle. Am J Respir Crit Care Med 2006; 173: 32–41.
54. A Nakao , H Sagara, Y Setoguchi, T Okada, K Okumura, H Ogawa and T Fukuda. Expression of Smad7 in bronchial epithelial cells is inversely correlated to basement membrane thickness and airway hyperresponsiveness in patients with asthma. Journal of Allergy and Clinical Immunology 2002; 110:873-878.
55. Siegel PM, Massaguè J. Cytostatic and apoptotic actions of TGF-beta in homeostasis and cancer. Nat rev Cancer. 2003. 3:807-821.
56. Massague J, Gomis RR. The logic TGFbeta signaling. FEBS Lett. 2006. 580:2811-2820 .
57. Ramjaun AR, Tomlinson S, Eddaoudi A, Downward J. Upregulation of two BH3-only proteins, Bmf and Bim, during TGF beta-induced apoptosis. Oncogene. 2007. 26:970-981.
58. Shi-Wen X, Rodriguez-Pascual F, Lamas S, homes a, Howat S, pearson JD, Dashwood MR, Du Bois RM, Denton CP, Black CM, Abraham DJ, Leask A. Constitutive ALK5-indipendent c-Jun N-terminal kinase activation contributes to endothelin-1 overexpression in pulmonary fibrosis: evidence of an autocrine

endothelin loop operating through the endothelin A and B receptors. Moll cell Biol. 2006. 26:5518-5527.
59. Rahimi RA, Leof EB. TGF-β signaling: a tale of two responses. Journal of cellular Biochemistry. 2007. 102:593-608.
60. Holmes A, Abraham DJ, Sa S, Shiwen X, Black CM, Leask A. CTGF and SMADs, manteinance of scleroderma phenotype is independent of SMAD signaling. J Biol Chem. 2001. 276:10594-10601.
61. Strutz F, Zeisberg M, Renziehausen A, Raschke B, Becker V, Van Kooten C, Muller G. TGF-beta 1 induces proliferation in human renal fibroblasts via induction of basic fibrobalst growth factor (FGF-2). Kidney Int. 2001. 59:579-592.
62. Khalil N, Xu YD, O'Connor R, Duronio V. Proliferation of pulmonary interstitial fibroblasts is mediated by transforming growth factor-beta1-induced release of extracellular fibroblast growth factor 2 and phosphorylation of p38 MAPK and JNK. J Biol Chem. 2005. 280:43000-43009.
63. Pelaia G, Gallelli L, D'agostino B, Vatrella A, Cuda G, Fratto D, Renda T, Galderisi U, Piegari E, Crimi N, Rossi F, Caputi M, Costanzo FS, Vancheri C, Maselli R, Marsico SA. Effects of TGF-beta and glucocorticoids on map kinase phosphorylation, IL-6/Il-11 secretion and cell proliferation in primary culture of human lung fibroblasts. J Cell Physiol. 2007. 210:489-497.
64. Anzano MA, Roberts AB, Smith JM, Sporn MB, De Larco JE. sarcoma growth factor from conditioned medium of virally transformed cells is composed of both type alpha and type beta transforming growth factors. Proc Natal Acad Sci USA. 1997. 80:6264-6268.
65. Datto MB, Frederick JP, Pan L, Borton AJ, Zhuang Y, Wang XF. targeted disruption of smad3 reveals an essential role in transforming growth factor beta-mediated signal transduction. Mol Cell Biol. 1999. 19:2495-2504.