Fibrosis: is it a coactivator disease?

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1. ABSTRACT

Fibrosis is an abnormal fibroblast-activation-associated pathological manifestation in injured organs where excessive non-physiological synthesis and accumulation of extracellular matrix (ECM) proteins by activated/differentiated fibroblasts disrupts tissue homeostasis. Like other eukaryotic genes, expression of ECM protein genes not only depends on its gene sequences in the regulatory region but also influenced by non-genetic factors called epigenetic regulators including acetyltransferases, deacetylases, methyltransferases and microRNAs. The acetyltransferase p300 (ATp300), a transcriptional coactivator, is a major player in the epigenetic regulation of genes whose products are involved in cellular growth, proliferation, apoptosis and essential for embryonic development. ATp300 acetylates specific lysine residues in histones and transcription factors (KAT) and as a transcriptional coactivator it forms a bridge between upstream regulatory element binding protein complex and basal transcriptional machinery. Abnormal coactivator activity-associated diseases are known as coactivator diseases. Abnormalities in ATp300 activities in adults are associated with numerous diseases. Here, we review the significant roles of ATp300 in epigenetic regulation of collagen synthesis and deposition in extracellular spaces, matrix remodeling and tissue fibrogenesis. The present day understanding on the distinct role of acetyltransferases, deacetylases, and deacetylase inhibitors on epigenetic regulation of matrix remodeling and fibrosis has also been discussed.

2. INTRODUCTION

Fibrosis is a common end-stage pathological manifestation of tissue injury related diseases and loss of tissue homeostasis in multiple organs due to excessive accumulation of matrix proteins in the extracellular spaces by abnormally activated fibroblasts. Extracellular matrix (ECM) plays a significant role in tissue homeostasis which is maintained by the rate of ECM protein synthesis and degradation by proteolytic activities including uPA/tPA/Plasmin/ MMP system. Abnormality in ECM protein synthesis and or proteolytic activities disrupts tissue homeostasis and leads to an abnormal matrix remodeling, the salient feature fibrosis, in different organs (1-9). Fibrosis is associated with numerous diseases including hypertension-induced-cardiac hypertrophy, myocardial infarction, heart failure, liver failure, renal failure, lung diseases, systemic sclerosis, and atherosclerosis (10-17). Fibrosis is characterized by initial vascular injury, mononuclear cell infiltration, secretion of cytokines, migration, proliferation and activation of fibroblasts, epithelial-to-mesenchymal transition (EMT)/endothelial-to-mesenchymal transition (EndMT), myofibroblast differentiation, synthesis of excessive non-physiological levels of collagen and other extracellular matrix proteins. The pleiotropic cytokine, TGF-β plays a pivotal role in the processes of inflammation and fibrogenesis. TGF-β-activated resident fibroblasts or EMT/EndMT-derived fibroblast-like cells differentiate to myofibroblasts which produce elevated levels of collagen, the major extracellular matrix proteins in fibrotic tissues (3,5, 11-23).

Epigenetics play a significant role in organogenesis during embryonic development as well as in the development of diseases in adults (24-27). The major enzymatic systems including acetyltransferases, deacetylases, methyltransferase and microRNAs are involved in epigenetic regulation of genes whose products are involved in the maintenance of tissue homeostasis. Abnormality in this epigenetic regulation causes diseases including asthma, lupus, cardiovascular diseases, cancers, diabetes, and Rubenstein Taybi syndrome (28-37). Histone/Lysine/Factor acetyltransferase (HAT/KAT/FAT) and histone/lysine deacetylases (HDAC/KDAC) maintain the balance of acetylated and deacetylated states of histones in chromatin (38) as well as transcription factors in the transcriptional complex, thereby controlling the magnitude of gene expression at the level of chromatin organization as well as transcription (39,40). The transcriptional coactivator ATp300, a nuclear phosphoprotein, with its intrinsic acetyltransferase activity plays a crucial role in epigenetic regulation of gene expression. In 1994, Eckner and colleagues (41) first identified ATp300 as an adenovirus E1A-associated factor and encoded by a unique gene located at 22q13.2. The length of ATp300 gene is ~8.77 Kb, and codes for a polypeptide of 2414 amino acids in length. ATp300 lacks DNA binding property and consists of several functional domains, namely cysteine-histidine domains CH1, CH2 and CH3, lysine acetyltransferase (HAT/KAT/FAT) domain, KIX domain and C-terminal glutamine rich domain. These domains play an important role in protein-protein interaction and acetyltransferase activity of ATp300 (39,41,42). which acetylates specific lysine residues in histones as well as transcription factors (43-46). In order to control the acetyltransferase activity of ATp300, numerous synthetic and natural small molecule inhibitors of acetyltransferase activity have been discovered and tested *in vitro*. The efficacies of these small molecules in therapeutic approaches *in vivo* are now under investigation (47). Acetyltransferase activity of ATp300 as well as its interaction with transcription factors, in a context-dependent manner, largely depends on its posttranslational modifications by phosphorylation, acetylation, methylation and sumoylation (42,48-54).

As ATp300 interacts with numerous transcription factors *in vitro*, one of the most controversial issues is what determines the gene specific action of ATp300. Numerous studies established that specificity of ATp300 activity in the context of a particular gene expression is determined by its levels, posttranslational modifications including site specific phosphorylation by a variety of kinases, auto- and trans-acetylation, subcellular localization and post-translational modifications including phosphorylation-dephosphorylation of its interacting transcription factors and signal transducers (48-56). Here, we review the molecular mechanism by which the gene expression and synthesis of extracellular matrix proteins is epigenetically regulated by coactivator ATp300 and its significance in tissue fibrosis. The roles of HDAC and HDACi in epigenetic regulation of matrix remodeling and fibrosis have also been discussed.

3. MATRIX PROTEIN SYNTHESIS IS POSITIVELY REGULATED BY ATp300

Under normal physiological conditions, fibroblasts synthesize and secrete very low levels of extracellular matrix proteins including collagens, elastin, fibrillins, fibronectin, and laminin to maintain the tissue homeostasis. Along with other cell types, fibroblasts are also known to produce proteases such as matrix metaloproteinases, as well as protease inhibitors including plasminogen activator inhibitor-1 (PAI-1) and tissue inhibitor of matrix metalloproteinase (TIMPs). A balance between synthesis of matrix proteins and their degradation is necessary to maintain the tissue homeostasis (2,7,8). In different tissues including, skin, lungs, and heart, more than 70 % of the total collagens are Type I collagen, a heterotrimer of two collagen 1α1 and one collagen 1α2 polypeptide chains. In response to injury and during wound healing, cytokine activated or differentiated fibroblasts produce excess extracellular matrix proteins to heal the wounds. However, sustained activation of fibroblasts, excessive non-physiological accumulation of extracellular matrix proteins, and a decreased rate of degradation of matrix protein by MMPs as a result of increased PAI-1 or TIMPs leads to loss of tissue homeostasis, the pathological manifestation of tissue fibrosis (7, 8, Figure 1 A and B).

Transforming growth factor-beta (TGF-β), a pleiotropic cytokine, plays crucial role in wound healing and is implicated in pathogenesis of fibrosis (3,5,12). TGF-β transmits its signal from cell surface to nucleus via activation of its receptors (TβRI and TβRII). Receptor activated downstream signaling molecules Smad2/3 and coactivators ATp300/CBP activate transcription from Smad-binding element driven reporters (57-60). Upon ligand binding, TβRII transphosphorylates TβRI and activates its kinase activity. TβRI kinase-phosphorylated Smad2/3 heterodimerize with Smad4, translocate to nucleus, bind to TGF-β response element, and interact with Sp1 to activate collagen gene expression in fibroblasts (61-63). For Smad-dependent TGF-β-induced collagen synthesis in fibroblasts, the presence of coactivator ATp300 in the transcriptional complex is required (64). Elevated levels of ATp300 in fibroblasts lead to increased synthesis of collagen. Most importantly, in the presence of excess Smad3, the stimulation of collagen synthesis by ATp300 is significantly higher compared to stimulation by ATp300 or Smad3 alone (64,65). Furthermore, TGF-β induces the physical interaction of phosphorylated Smad2/3 with ATp300, indicating physical and functional interaction of cellular Smad2/3 with ATp300 is an important event in TGF-β-induced collagen synthesis by human dermal fibroblasts. Additionally, lysine acetyltransferase (HAT/KAT/FAT) deleted p300 fails to stimulate TGF-β-induced collagen synthesis suggesting intrinsic acetyltransferase activity of ATp300 is required for maximal stimulation of profibrotic responses exerted by TGF-β (64-66). The significant role of ATp300 in TGF-β-induced collagen synthesis has been further confirmed in mouse mesangial cells by Kanamaru and colleagues (67). Taken together, these results indicate that ATp300
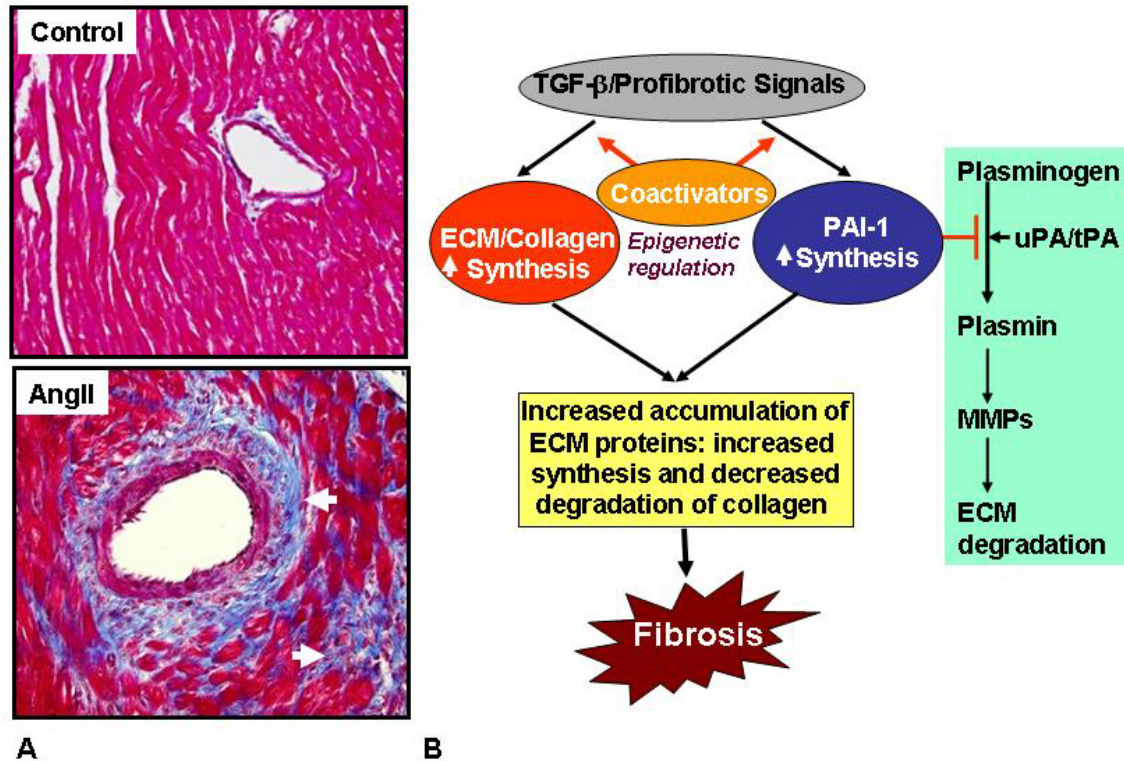
Figure 1. Fibrosis in myocardial tissues derived from Angiotensin II–induced hypertensive heart. A. Histology images showing excessive collagen deposition in fibrotic heart by Masson's Trichrome staining. Please note: presence of perivascular and interstitial fibrosis (white arrows). B. Dual roles of TGF-β on profibrotic signaling and fibrosis. TGF-β induces collagen synthesis as well as synthesis of PAI-1 via induction of coactivators. PAI-1 inhibits plasminogen to plasmin conversion and thus blocks plasmin-dependent activation of MMPs and proteolytic degradation of ECM proteins. Increased accumulation of matrix proteins in extracellular spaces is due to increased synthesis and decreased degradation of ECM proteins.

promotes elevated collagen synthesis by mesenchymal cells and potentiates Smad-dependent TGF-β-induced collagen synthesis.

4. CELLULAR ATp300 IS ESSENTIAL FOR MATRIX PROTEIN SYNTHESIS IN RESPONSE TO PROFIBROTIC SIGNALS: LOSS-OF-FUNCTION ANALYSIS

ATp300 stimulates TGF-β-induced collagen gene transcription in a dose dependent manner indicating the cellular levels of ATp300 sensitize fibroblasts to TGF-β-induced profibrotic responses. This statement has been further supported by studying TGF-β-induced profibrotic signaling in p300 depleted fibroblasts (68). ATp300 in dermal fibroblasts was depleted using ATp300 specific ribozyme (small RNA molecule) which specifically cleaves ATp300 mRNA but not ATp300 related CREB binding protein (CBP) mRNA and thus depletes ATp300 protein but not CBP (69). TGF-β induces cellular ATp300 mRNA and protein levels and stimulates the synthesis of Type I collagen in fibroblasts. However, TGF-β fails to stimulate Type I collagen synthesis in ribozyme-mediated ATp300 depleted fibroblasts signifying cellular ATp300 is essential for collagen synthesis in response to profibrotic signaling.

This has been further supported by the observation that overexpressed Smad-interacting domain of ATp300 blunts TGF-β-induced collagen synthesis in fibroblasts (Ghosh AK unpublished observation). In contrast to Type I collagen, the TGF-β-induced α-SMA and PAI-1 levels remain unaltered in the absence of cellular ATp300 indicating influence of ATp300-deficiency is gene specific. Failure of TGF-β to induce collagen synthesis in ATp300 deficient cells is not due to altered expression or activation of R-Smads and I-Smad or ATp300 related CBP further suggesting cellular ATp300 is essential for TGF-β-induced collagen synthesis by activated fibroblasts (68). Furthermore, results of this study indicate that ATp300 and its closely related protein CBP are functionally distinct in response to TGF-β-induced collagen synthesis by fibroblasts. It is important to investigate the role of CBP and ATp300-CBP associated factor (PCAF) in the regulation of α-SMA and PAI-1 and its implication in fibrosis.

5. DISRUPTION OF SMAD-ATp300 INTERACTION: SIGNIFICANCE IN FIBROSIS THERAPY

Type I collagen, the major ECM protein in a variety of tissues, is regulated by a wide range of proinflammatory, anti-inflammatory, profibrotic and anti-

fibrotic cytokines. IFN-γ negatively regulates collagen gene transcription and its synthesis in fibroblasts via activation of different transcription factors including STAT1α (65), class II transactivator (CIITA) (70), the RFX5 complex (71), Y-box binding protein-YB-1 (72), and C/EBP-β (73), thus acting as an antifibrotic agent. IFN-γ not only inhibits basal collagen synthesis, but it also antagonizes TGF-β-induced and overexpressed Smad-induced collagen synthesis without affecting Smad2/3 phosphorylation, nuclear translocation and DNA binding (65). While IFN-γ inhibits collagen synthesis via activation of the JAK-STAT pathway, TGF-β stimulates collagen synthesis via activation of TβR1 kinase-Smad pathway. When fibroblasts are exposed to TGF-β and IFN-γ, both IFN-γ-activated phospho-STAT-1α and TGF-β activated phospho-Smad2/3 compete for ATp300. In this competition, phospho-STAT-1α sequesters cellular ATp300 from phospho-Smad2/3 complex and thus blocks TGF-β-induced collagen synthesis. IFN-γ failed to antagonize TGF-β-induced collagen gene transcription in JAK1 deficient cells. Furthermore, in the presence of elevated ATp300, IFN-γ fails to block TGF-β-induced collagen synthesis suggesting ATp300 plays pivotal role in elevated synthesis of matrix protein collagen (65). Interestingly, like IFN-γ, IFN-α also suppresses collagen gene transcription *in vitro* in hepatic stellate cells and blocks CCL₄-induced hepatic fibrosis *in vivo* in mice (74). This study demonstrates that like IFN-γ, IFN-α also antagonizes the Smad-dependent TGF-β-induced collagen gene transcription via induction of interaction between STAT-1α and ATp300 (74). Another study using lung fibroblasts indicated that IFN-γ antagonized TGF-β-induced collagen synthesis via sequestration of ATp300 by IFN-γ-activated STAT-1α from TGF-β-induced AP1 transcriptional complex (75). Other investigators showed that ATp300 sequestration by IFN-γ-induced transcription factor YB1 from TGF-β-induced pSmad2/3 containing complex leads to abrogation of induced collagen synthesis (72,76), suggesting ATp300 plays a pivotal role in integration of profibrotic and antifibrotic signaling via interaction with corresponding signal transducers, Smad or AP1 and STAT-1α or YB1 (65,72,75,76). Together, these results indicate that ATp300 plays an important role in both IFN-α and IFN-γ-mediated antagonistic action on TGF-β-induced collagen synthesis, the major matrix protein in fibrotic tissues.

ATp300 also integrates antagonistic action of TNF-α on TGF-β-induced collagen synthesis in fibroblasts where TNF-α-activated cJun/JunB sequesters ATp300 from TGF-β-induced Smad3 containing transcriptional complex from a TGF-β-responsive reporter construct. Furthermore, excess ATp300 prevents TNF-α mediated suppression (77). The significant role of ATp300 in integration of IFN-γ/TNF-α and TGF-β signaling has been further supported by an elegant study of Feinberg *et al.* (78) in macrophages. This study demonstrates that Kruppel-like factor 4 (KLF4), a zinc finger family transcription factor, is induced by IFN-γ or TNF-α and is suppressed by TGF-β in macrophages. KLF4 inhibits TGF-β-induced expression of the PAI-1 gene, a major profibrotic marker and a potent inhibitor of cellular fibrinolytic system; it induces the expression of macrophage activation marker iNOS via interaction and sequestration of cellular limiting ATp300 from TGF-β-induced Smad complex on the PAI-1 gene and recruitment of ATp300 to transcriptional complex on iNOS gene promoter (78). Additionally, KLF4 deficiency is associated with increased Smad3/TGF-β-induced PAI-1 promoter activity in macrophages. Therefore, these results further signify the pivotal role of ATp300 in controlling intracellular signals involves in matrix remodeling. It is important to mention that HAT-deleted p300 is unable to block antagonistic effects of IFN-γ or TNF-α on TGF-β signaling indicating that not only Smad-ATp300 interaction but HAT/KAT/FAT activity of ATp300 is also required for these signal integrations (65, 77).

ATp300 is also involved in negative regulation of TGF-β-induced collagen synthesis by cellular repressors of TGF-β-induced profibrotic signaling including p53 and PPAR-γ. The tumor suppressor protein, guardian of the genome or gatekeeper of cell cycle, p53 is involved in multiple cellular events including cellular growth, proliferation, differentiation and apoptosis in a cell type and context-dependent manners (79). As a transcriptional regulator, p53 can activate or repress the target gene expression in a gene specific manner. In general, p53 mediated activation and repression of target gene expression depends on its direct interaction with regulatory DNA elements and via protein-protein interaction in the transcriptional complex of the target genes respectively (80 and references therein). The transcriptional activity of p53 depends on its protein level, and posttranslational modification including site specific phosphorylation, lysine acetylation and ubiquitination (81). Abnormalities in p53 activity due to mutation (a most frequently mutated gene in the genome), abnormal posttranslational modification, or stability, are associated with different types of cancers (82). p53 is known to be involved in TGF-β-induced suppression of cellular proliferation (83).

Cellular p53 is a modulator of TGF-β-induced Type I collagen synthesis (80). p53 also inhibits fibronectin synthesis (84,85). While excess p53 blocks TGF-β-induced Type I collagen synthesis, lack of cellular p53 is associated with significantly elevated basal as well as TGF-β-induced collagen synthesis compared to wildtype controls. Furthermore, excess p53 blocks Smad3-induced collagen gene transcription suggesting cellular p53 controls the magnitude of Smad-dependent TGF-β stimulation of collagen synthesis by fibroblasts. The p53 mediated suppression of TGF-β-induced collagen gene transcription is not due to altered expression or activation of R-Smad2/3, I-Smad7, Co-Smad4, or coactivator ATp300. Suppression is due to excess p53-mediated disruption of complex formation between TGF-β-induced Smad3 and ATp300 signifying that ATp300 plays an important role in p53-mediated suppression of TGF-β-induced collagen synthesis. The significance of ATp300 in p53-mediated suppression has been further evidenced by the observation that in the presence of excess ATp300, p53 fails to block TGF-β-induced collagen synthesis by fibroblasts. The modulation

of profibrotic TGF-β responses by p53 is gene specific, because excess p53 stimulates basal as well as TGF-β-induced PAI-1 gene expression (80). Interestingly, cellular ATp300 is not required for TGF-β-mediated stimulation of PAI-1 expression in human dermal fibroblasts (Ghosh, AK unpublished data). Possibly TGF-β-induced upregulation of PAI-1 depends on different cellular acetyltransferases such as ATp300 related CBP, pCAF/GCN5 in a cell type dependent manner (86-89). For example, Das *et al.* (87) demonstrated that TGF-β-induced PI3K/Akt plays an important role in induction of PAI-1 and inhibition of PI3K/Akt blocks TGF-β-induced Smad3 acetylation. It also disrupts TGF-β-induced Smad3-CBP interaction and decrease PAI-1 expression. These results further suggest that ATp300 related CBP plays a significant role in profibrotic PAI-1 gene expression. On the other hand, Feinberg *et al.* (**78**) reported that KLF4 blocks TGF-β-induced PAI-1 gene expression in macrophages via disruption of Smad3-ATp300 complex formation and overexpressed ATp300 reverses the inhibitory effect of KLF4. The TGF-β-induced Smad phosphorylation, nuclear translocation and DNA binding are unaltered in the presence of excess KLF4 suggesting ATp300 plays an important role in KLF4-mediated suppression of TGF-β-induced PAI-1 expression and profibrotic signaling (78). In contrast to KLF4, elevated KLF5 plays an important role in cardiac hypertrophy and vascular fibrosis. Haplo-dose of KLF5 is associated with decreased cardiac hypertrophy and vascular fibrosis. Importantly, ATp300 physically interacts with KLF5, activates its transcriptional activity, and is involved in cardiovascular tissue remodeling via activation of PDGF, PAI-1 and other genes (89).

ATp300 plays a significant role in PPAR-γ-mediated target gene expression. PPAR-γ is a nuclear hormone receptor and expresses low to high levels in a variety of cells including adipocytes, hepatic stellate cells, pancreatic stellate cells, monocytes and fibroblasts. PPAR-γ is involved in adipogenesis, insulin sensitivity, cellular proliferation and anti-inflammation. Abnormality in its expression or function is associated with numerous diseases such as diabetes, obesity, cardiovascular disorders like atherosclerosis, abnormal wound healing and matrix remodeling (90, 91). In early 2000, investigators demonstrated that activation of PPAR-γ with naturally occurring ligands like 15d-PGJ$_2$ and synthetic ligands like TZD derivatives, blocks myofibroblast differentiation and elevated collagen synthesis in rat mesangial cells, hepatic stellate cells, pancreatic stellate cells and human dermal fibroblasts. These results revealed the potential role of PPAR-γ as a modulator of matrix remodeling. Additionally, treatment of rodent models of fibrosis with PPAR-γ ligands prevented the development of glomerulosclerosis and pancreatic fibrosis (92-100). In recent years, several studies have been undertaken to understand the precise molecular mechanism by which ligand-activated PPAR-γ imparts its negative influence on collagen synthesis by activated fibroblasts or other mesenchymal cells. For example, treatment of fibroblasts with PPAR-γ synthetic pharmacologic agonists including TZD derivative troglitazone and naturally occurring 15d-PGJ$_2$ prevent TGF-β-induced collagen synthesis. The PPAR-γ ligand mediated suppression of TGF-β-induced and Smad3-induced collagen gene expression in human dermal fibroblasts can be blocked with PPAR-γ antagonist GW9662, suggesting ligand-mediated suppression of collagen synthesis is PPAR-γ dependent (100). Later Zhang *et al.* (101) confirmed these observations using dermal fibroblasts derived from keloid patients and healthy individuals. Furthermore, administration of rosiglitazone blocks bleomycin-induced lung, skin and liver fibrosis in a PPAR-γ-dependent manner (102-105). Interestingly, the levels of PPAR-γ in fibrotic tissues derived from systemic sclerosis patients are significantly lower compared to healthy controls (106,107) indicating decreased levels of PPAR-γ may be responsible for elevated collagen synthesis thus contributing to tissue fibrogenesis.

The physiological significance of cellular PPAR-γ in profibrotic signaling and as a repressor of collagen synthesis has been further evidenced by the observations that i) PPAR-γ deficiency in mouse embryonic fibroblasts is associated with elevated TGF-β levels, increased TβR1 expression, constitutively activated phospho-Smad2/3 and increased Smad-ATp300 interaction; ii) constitutively active Smad-dependent TGF-β signaling is associated with elevated collagen synthesis in PPAR-γ-deficient cells (108). Although, PPAR-γ blocks Smad-dependent TGF-β-induced collagen gene expression in fibroblasts, TGF-β-induced Smad activation remains unaltered in the presence of PPAR-γ ligands suggesting the inhibitory effect of PPAR-γ ligands is further downstream of TGF-β signaling. Transcriptional coactivator ATp300 plays a significant role in ligand-activated PPAR-γ-mediated suppression of TGF-β-induced collagen synthesis, evidenced by the following observations: i) Smad2/3 and Smad7 expression, TGF-β-induced phosphorylation of Smad2/3, nuclear translocation, and binding to Smad binding element remain unaltered in the presence of activated PPAR-γ compared to controls; ii) ligand activated PPAR-γ disrupts TGF-β-induced interaction of phospho-Smad2/3 with ATp300 and reduces histone acetylation on the collagen promoter as evidenced by protein-protein interaction assay in a cell free system, transcriptional complex formation on Smad binding element in a cell free system and transcriptional complex formation on chromatin (collagen gene) in live cells; iii) while overexpressed wildtype PPAR-γ abrogates TGF-β-induced collagen gene expression, dominant negative PPAR-γ which cannot interact with coactivators, fails to block stimulation of collagen synthesis; iv) ligand-induced PPAR-γ interacts with ATp300 and finally, v) overexpressed ATp300 rescued PPAR-γ-induced suppression of TGF-β-induced collagen gene expression in dermal fibroblasts (100,108,109) (Figure 2). Similarly, PPAR-γ ligand abrogates TGF-β-induced expression of
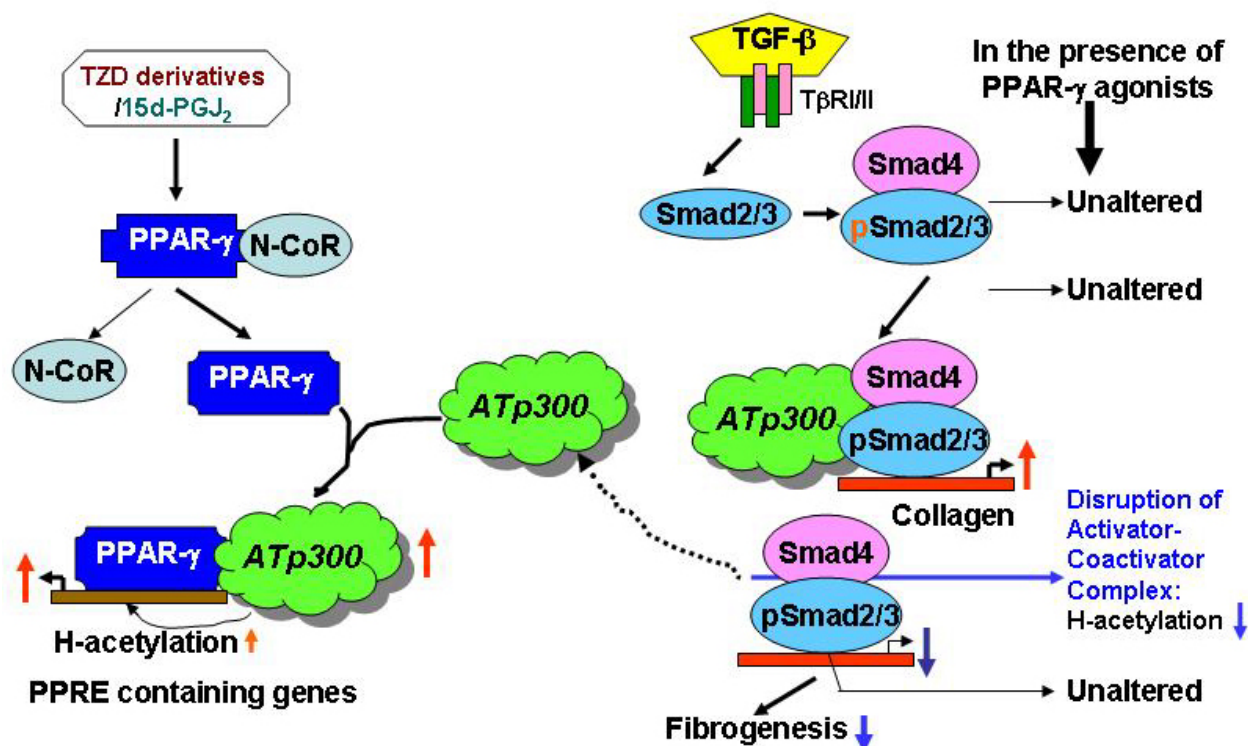
Figure 2. PPAR-γ-mediated suppression of TGF-β-induced profibrotic signaling: Role of coactivator ATp300: PPAR-γ ligands TZD derivatives or 15d-PGJ$_2$ activates PPAR-γ via dissociation of corepressors (N-COR/HDAC). While activated PPAR-γ interacts with coactivator ATp300 and stimulates expression of PPAR-γ response element containing genes, it disrupts TGF-β-induced ATp300-Smad complex formation on collagen promoter. In the absence of ATp300, TGF-β-induced Smad containing transcriptional complex fails to stimulate collagen synthesis and fibrogenesis [This model is based on data obtained from *in vitro* studies].

CTGF, a potential profibrotic factor, via disruption of Smad-ATp300 complex formation in rat hepatocytes and caffeine potentiates the inhibitory effect of PPAR-γ ligand via induction of PPAR-γ expression (110). In hepatic stellate cells, PPAR-γ suppresses collagen gene transcription via blocking ATp300-facilitated NF1 binding to collagen promoter further signifying involvement of ATp300 in the PPAR-γ-mediated antifibrotic effect (111). These studies collectively suggest that lowering of ATp300 interaction with functional transcriptional complex on collagen gene promoter may be an ideal therapeutic approach to downregulate the magnitude of collagen synthesis and ultimately to control fibrogenesis.

The critical role of ATp300 in the regulation of extracellular matrix proteins and tissue remodeling has been further documented by other studies. For example, elevated levels of cAMP, which activates PKA and its substrate nuclear CREB via phosphorylation, antagonizes TGF-β-induced Type I collagen synthesis and other profibrotic genes including PAI-1, CTGF and TIMP. PKA activated CREB strongly associates with transcriptional coactivators ATp300 related CBP and interacts with CRE of several cAMP induced target genes (112 and references therein). TGF-β induces transcription from Smad-driven reporter constructs and Bt$_2$cAMP blocks Smad-dependent

TGF-β-induced transcription. Furthermore, overexpressed ATp300 reverses the inhibitory effect of cAMP on TGF-β-induced profibrotic signaling, indicating cAMP antagonizes the TGF-β-induced profibrotic genes, and perhaps via sequestration of cellular ATp300 from TGF-β-induced Smad complex by PKA activated CREB (113). Involvement of ATp300 in cardiac fibrosis is documented by the observation that prostacyclin receptor prevents AngiotensinII-induced cardiac fibrosis (114). Prostacyclin, an eicosanoid derived from endothelium, prevents cardiac fibrosis via activation of G protein coupled receptor and induction of cellular cAMP levels. The levels of TGF-β-activated Smad and MAPK are unaltered in prostacyclin receptor-activated cardiac fibroblasts. The protacyclin receptor-mediated suppression of TGF-β-induced collagen synthesis is associated with cAMP activation and phosphorylation of CREB which sequesters ATp300 from Smad containing complex and abrogates collagen synthesis. Furthermore, inhibition of acetyltransferase activity of ATp300 with garcinol inhibits collagen synthesis by cardiac fibroblasts *in vitro* (114). Taken together, these studies suggest that i) ATp300 is an essential factor in tissue matrix remodeling via interaction with a variety of transcription factors depending on tissues and profibrotic inducers, and ii) lysine acetyltransferase activity of ATp300 is required for its maximal influence on profibrotic
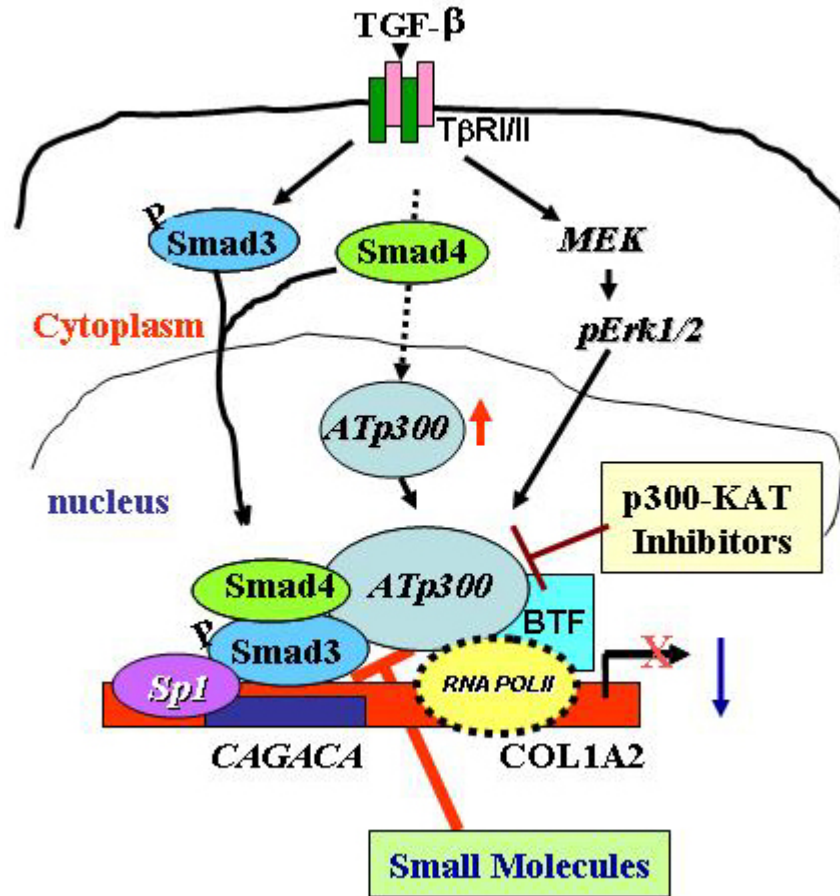
Figure 3. Molecular basis of coactivator ATp300 contribution in fibrogenesis. TGF-β induces ATp300. Acetyltransferase activity of ATp300 and increased interaction of ATp300 with activated Smads contribute to increased profibrotic signaling and elevated collagen synthesis *in vitro*. TGF-β also transduces its signal via activation of MAPK pathway. In fibrotic tissues, the levels of ATp300 and its interaction with phosphorylated-Smad2/3 are elevated. Reduction of the elevated acetyltransferase activity of ATp300 or disruption of ATp300-Smad interaction *in vivo* using small molecules may ameliorate fibrogenesis.

responses and increased collagen gene expression, a natural TGF-β target gene and a major matrix protein, as has been originally proposed in human dermal fibroblasts (64). Therefore, disruption of ATp300 interaction with profibrotic factors may be an ideal approach to control tissue fibrogenesis (Figure 3).

6. EVIDENCE FOR POTENTIAL LINK OF ATp300 WITH TISSUE FIBROGENESIS

TGF-β, the most potent and essential profibrotic cytokine, not only activates its signal transducers Smads but also stimulates its coactivator ATp300. TGF-β fails to stimulate the expression of its profibrotic target gene collagen in the absence of ATp300 (64,68). Furthermore, the elevated levels of TβRI and II, constitutively active Smads and increased TGF-β signaling are implicated in fibrosis (18,115). These observations raised the obvious question: what are the levels of ATp300, an essential acetyltransferase and coactivator for TGF-β-induced collagen synthesis, in fibrotic tissues? Measurement of

ATp300 protein levels in explanted fibroblasts derived from patients with tissues fibrosis revealed that the levels of ATp300 are significantly elevated in fibroblasts derived from fibrotic tissues compared to healthy controls. The elevation of ATp300 is associated with increased collagen synthesis, indicating an excellent correlation of elevated ATp300 with tissue fibrogenesis (68,116). The significance of ATp300-Smad interaction in fibrogenesis has also been evidenced by the observations of constitutive interaction of ATp300 with Smad3 and impaired interaction of ATp300 with negative regulator of TGF-β signaling c-Ski/SnoN in fibroblasts derived from fibrotic skin with elevated levels of collagen (116,117). Furthermore, the levels of ATp300 are significantly elevated in fibrotic tissues derived from murine models of tissue fibrosis including skin, lung and kidney (68,118,119). Collectively, these results establish a potential link between coactivator ATp300 and tissue fibrosis. Therefore, it is reasonable to propose that fibrosis is a coactivator disease or epigenetic disease, where ATp300 plays a major role.

7. MECHANISMS OF EPIGENETIC REGULATION OF MATRIX PROTEIN SYNTHESIS BY HAT, HDAC AND HDACi ARE DISTINCT

The expression of eukaryotic gene is mostly regulated at the levels of chromatin organization, transcription and post-transcription (120). Two key enzyme families involved in chromatin organization are lysine acetyltransferases (HATs/KATs/FATs) and lysine deacetylases (HDACs/KDACs), which acetylate and deacetylate lysine residues in histones as well as transcription factors respectively. An elegant study by Wang *et al.* (120) on genome-wide distribution of HATs and HDACs revealed that i) both HATs and HDACs transiently bind to regulatory and structural regions of genes and are involved in the cycling of acetylation and deacetylation of genes, and ii) dynamic acetylation and deacetylation of histones in chromatin maintain the underexpressed state of genes keeping the promoter ready for expression based on external, or physiological or pathological signals (120). Therefore, balance of HATs and HDACs in a particular cellular state controls the expression of target genes (120). In this section, we discuss the influence of acetylation-deacetylation imbalance on TGF-β regulation of collagen gene expression and its significance in epigenetic regulation of matrix remodeling as well as fibrosis. In the previous sections, substantial evidences have been provided showing the pivotal role of ATp300 in induced collagen gene expression, its elevated levels in fibrotic tissues and potential link of ATp300 to fibrogenesis (64-68,80,109,116,121). TGF-β induces ATp300 which in turn acetylates histones and activates collagen gene expression (109). In contrast, ectopically expressed lysine deacetylase (class I, HDAC1) blocks TGF-β-induced collagen gene expression in fibroblasts (Ghosh AK unpublished data). Furthermore, class II HDACs reduce stimuli-induced cardiac hypertrophy and fibrosis (122), suggesting the balance of HAT/KAT/FAT and HDAC/KDAC levels control the expression of collagen gene. Based on these observations, one can anticipate that inhibition of HDACs using HDAC inhibitor (HDACi) will increase cellular lysine acetylation and collagen synthesis. To the contrary, treatment of fibroblasts with HDACi leads to abrogation of TGF-β-induced collagen synthesis *in vitro* and HDACi attenuates induced fibrosis in multiple organs. Moreover, both general HDACi (TSA) and class I HDAC-specific inhibitor (SK-7041), blunt AngiotensinII- or Aortic banding-induced cardiac hypertrophy and cardiac fibrosis. Therefore, HDACi are considered as potent antifibrotic compounds (122-133). As both overexpressed HDAC and HDACi treatment abrogate TGF-β-induced collagen synthesis, and overexpressed ATp300 activates collagen synthesis, it is reasonable to interpret that the molecular mechanisms of inhibition of profibrotic responses by HDAC and HDACi are quite different, and the impacts of increased acetylation by elevated ATp300 versus HDACi on profibrotic signal-induced collagen synthesis are distinct.

What have we learned about the molecular basis of HDACi-mediated suppression of profibrotic signals and collagen gene expression? Treatment of fibroblasts with HDACi such as Trichostatin A (TSA) inhibits collagen synthesis via activation of inhibitors of TGF-β signaling including inhibitory Smad (Smad7) and TG-element binding inhibitory factor (TGIF) in rat skin fibroblasts (125). Another study demonstrated that treatment of human dermal fibroblasts with TSA caused i) significantly decreased collagen synthesis, ii) significant increase in H4 acetylation, iii) modest decrease in Smad2 phosphorylation, iv) unaltered activation of Smad3 and its nuclear translocation compared to controls, and v) decreased levels of Sp1, its interaction with TGF-β-induced Smad complex in the presence and absence of Smad-binding element. These results strongly suggest that HDACi TSA-mediated inhibition of TGF-β-induced collagen synthesis in human dermal fibroblasts is not due to alteration of Smad activation but due to suppression of Sp1 transcription factor (126), which is known to interact with activated Smad complex and plays significant role in TGF-β-induced collagen gene expression (63). This conclusion was further supported by the observations that TSA failed to block TGF-β-induced PAI-1 synthesis and Smad-dependent reporter constructs in fibroblasts (126). Interestingly, both Smad-dependent reporter constructs and TGF-β-induced expression of PAI-1 are Sp1 independent (63), and overexpressed Sp1 reversed the TSA-induced inhibition of collagen gene transcription in human dermal fibroblasts (126). Recently, Sanders *et al.* (131) reported that HDAC inhibitor TSA activates a suppressor of fibrosis, Thy-1, in lung fibroblasts and blocks profibrotic activity. Thy-1, a cell surface glycoprotein, is present in normal lung fibroblasts and absent in fibroblasts derived from idiopathic pulmonary fibrosis.

Collectively, these findings suggest that HDACi-induced increased acetylation may activate expression of inhibitors of TGF-β-induced profibrotic responses such as Smad7, or TGIF or Thy-1, or activate expression of inhibitors of activators (Sp1) which are responsible for abrogation of induced collagen synthesis. In a separate study, Kaimori *et al.* (134) demonstrated that HDACi TSA attenuates TGF-β-induced profibrotic responses in hepatocytes during epithelial-to-mesenchymal transition (EMT). This occurs via modulation of Sp1-Smad-ATp300 complex formation on the collagen gene promoter and through activation of Fli1, a potent antifibrotic factor (135), as evidenced by the observations that i) TSA suppresses TGF-β-induced nuclear translocation of Smad3/4, its DNA binding and interaction with Sp1, ii) the levels of ATp300 and its TGF-β-induced nuclear localization are significantly decreased in TSA treated hepatocytes, iii) TSA blocks TGF-β-induced interaction of pSmad3 and ATp300, and iv) TSA activates the levels and nuclear accumulation of Fli-1, a member of Ets transcription family and antifibrotic factor, in hepatocytes in the presence and absence of TGF-β. Based on these observations, Kaimori and colleagues (134) concluded that TGF-β-induced EMT and elevated collagen synthesis are associated with increased nuclear accumulation of ATp300 and its interaction with Smad-Sp1 complex. Additionally, TSA blocks TGF-β-induced activation of ATp300 and acetylation of Fli1 (less stable) and thus increases the levels of antifibrotic Fli1 (134 and references therein). Recently, Diao *et al.* (133) showed that
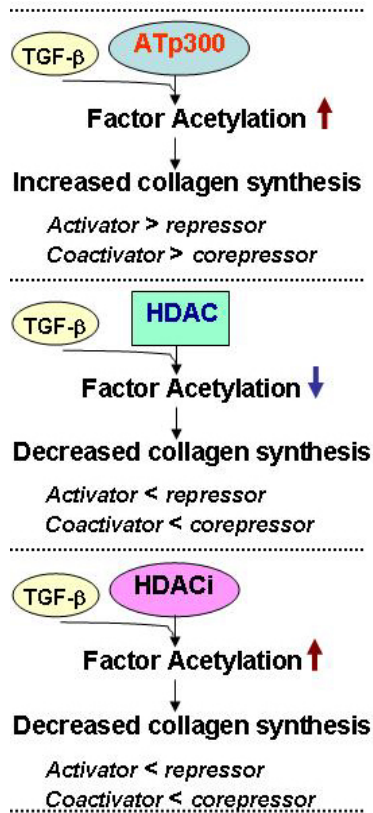
Figure 4. Possible molecular mechanisms of collagen gene regulation by HAT/KAT/FAT, HDAC and HDACi. While elevated ATp300 causes upregulation of ECM synthesis, both overexpressed HDAC and HDACi abrogate profibrotic responses and TGF-β-induced ECM protein synthesis. Effects of HAT, HDAC and HDACi on profibrotic responses and epigenetic regulation of matrix remodeling are distinct. The relative ratios of activators to repressors and coactivators to corepressors are altered differentially by HAT, HDAC and HDACi which determine the expression levels of extracellular matrix proteins in a particular tissue.

high concentration of TSA induces apoptosis of fibroblasts derived from keloids. These results collectively suggest that molecular mechanisms by which HDACi TSA exerts its antifibrotic influence are tissue and cell type specific. While the influence of HDACi TSA on increased lysine acetylation is global and non-specific (136), elevated ATp300-mediated acetylation of lysine residues in histones and transcription factors is limited and specific, and thus epigenetic regulation of profibrogenic genes by HDACi and elevated ATp300 are distinct (Figure 4).

8. SUMMARY AND PERSPECTIVE

This review article provided a comprehensive picture of the critical role of ATp300 in the epigenetic regulation of Type I collagen, and its implication in matrix remodeling and fibrosis. Numerous studies indicate a pivotal role of ATp300 in epigenetic regulation of collagen synthesis by activated resident or EMT/EndMT- derived fibroblasts. The elevated levels of ATp300 in the fibrotic tissues further strengthen the bridge between epigenetic regulator ATp300 and tissue fibrosis. Tissue fibrosis, associated with numerous diseases, is one of the major threats of morbidity and mortality. At present there is no effective therapy for preventing or reversing fibrosis and establishing physiological tissue homeostasis. As coactivator ATp300 is essential for TGF-β-induced elevated collagen synthesis *in vitro,* and the levels and physical interaction of pSmad2/3 and ATp300 are significantly elevated in fibrotic tissues, we propose that fibrosis may be a coactivator disease. Therefore, ATp300 may be an ideal target for reduction of excess matrix deposition and fibrosis *in vivo*. As ATp300 performs numerous physiological roles, complete depletion of p300 activity will not be an ideal approach to control fibrosis. There are two different directions currently being investigated in the author's laboratory. First: specific suppression of acetyltransferase activity of ATp300 using specific pharmacological or natural inhibitors; Second: disruption of induced complex formation between Smad2/3 and AT p300 using small molecules, miRNA and small peptides. Furthermore, the gene-specific and tissue-specific role of p300 related CBP and p300/CBP associated factor (PCAF) and their implications in epigenetic regulation of fibrosis in different organs also have to be investigated.

9. ACKNOWLEDGEMENTS

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