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Short Communication

Accredited translational medicine centre
Human renal fibrotic disease: Translational research at the Center for Cell Biology and Cancer Research (CCBCR), Albany Medical College, Albany, NY

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ABSTRACT

Translational studies conducted in the Center for Cell Biology & Cancer Research at the Albany Medical College integrate the discovery of basic mechanisms underlying the development of human fibrotic disease with *in vivo* interventional strategies and tissue repair outcomes in animal models. This structured research program is expected to lead to the clinical adaptation of novel therapies specifically directed to the control of pathologically-relevant profibrotic genes in several organ systems. Perhaps the most mature, clinically-relevant, multidisciplinary effort focuses on molecular events underlying the pathophysiology of the renal fibrotic response to tissue injury. This program involves a network of collaborating urological surgeons, nephrologists, graduate students, pathologists, residents, transplant surgeons, basic scientists and molecular biologists and is built on a highly-collaborative framework that fosters translational interactions. This cooperative enterprise resulted in a new appreciation for the complexity of the TGF-β1-activated pathways leading to fibrotic gene expression in an *in vivo* model of renal injury that mimics obstructive uropathy in humans. Moreover, we have uncovered new, translationally-relevant and therapeutically-accessible, molecular targets. These are the focus of current pre-clinical studies with the goal being to assess their potential utility in the therapy of human renal fibrotic disease.

Focal points:

- Interstitial fibrosis is a progressive disorder that frequently results in organ failure; current treatments are limited and largely ineffective.
- The majority of fibrotic diseases are irreversible and eventually fatal.
- Regardless of etiology, elevated tissue TGF-β1 levels and transcription of TGF-β1-responsive genes are linked to the activation of profibrotic signaling pathways.
- Plasminogen activator inhibitor-1(PAI-1) is a major causative factor in several clinically significant fibrotic syndromes.
- Translational research in the Center for Cell Biology & Cancer Research at Albany Medical College focuses on molecular events underlying transcriptional control of the profibrotic PAI-1 gene using an animal model of obstructive uropathy.
- The need for novel targeted approaches for the treatment of fibrosis highlights the clinical potential in the current probe of molecular mechanisms underlying TGF-β1-regulated PAI-1 gene control.

Abbreviations: Albany Medical College, (AMC); Ataxia Telangiectasia Mutated, (ATM); Center for Cell Biology & Cancer Research, (CCBCR); Connective Tissue Growth Factor, (CTGF); Epidermal Growth Factor Receptor, (EGFR); Mitogen-Activated Protein Kinase, (MAPK); NADPH Oxidase, (NOX); Plasminogen Activator Inhibitor-1, (PAI-1); Reactive Oxygen Species, (ROS); Serine Protease Inhibitor Clade E Member 1, (SERPINE1); Transforming Growth Factor-β, (TGF-β)

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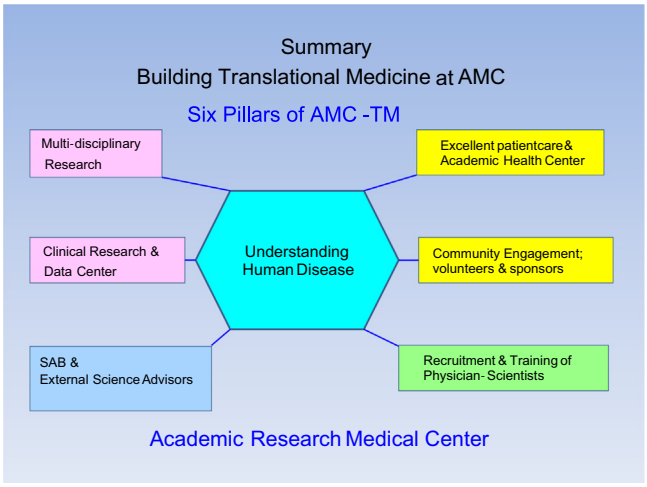

Fig. 1. Translational medicine (TM) model at AMC. SAB=Scientific Advisory Board.

1. Translational research in the Center for Cell Biology and Cancer Research (CCBCR) at Albany Medical College

Translational medicine (TM), as defined by the European Society for Translational Medicine, is an interdisciplinary branch of the biomedical field supported by three main pillars: benchside, bedside and community. The goal of TM is to combine advances, expertise and techniques within these pillars to leverage and promote enhancements in prevention, diagnosis and therapies [1].

Our program, although relatively small in size, attempts to integrate institutional basic and clinical research focusing on areas in which key questions can be addressed by multidisciplinary teams of basic and clinical scientists. The success of this program relies largely on the development and implementation of specific "tools" including the "Innovation Suite" which is coupled to a software application that facilitates linkage among biomedical investigators. Outlined below is the most prominent translational effort in the Center geared to the discovery of pathways and genes amenable to diagnosis, outcomes predication and therapy of human fibrotic disease.

1.1. The renal fibrosis program in the Center for Cell Biology and Cancer Research

Studies conducted in the Center for Cell Biology and Cancer Research (CCBCR) at the Albany Medical College (AMC) integrate the discovery of basic molecular events underlying the etiology and progression of human fibrosis with in vivo interventional strategies and tissue repair outcomes assessments. This effort, it is expected, will provide the rationale for the clinical adaptation of new and focused therapies directed to the control of pathologically-relevant profibrotic genes. Perhaps the most mature, clinically-relevant, multidisciplinary effort focuses on mechanisms underlying the renal fibrotic response to tissue injury. This program involves an extensive network of collaborating urological surgeons, nephrologists, graduate students, residents, basic scientists and molecular biologists and is built on a highly-collaborative framework that fosters translational interactions (Fig. 1).

The Center's faculty is comprised of an interdisciplinary group of scientists with expertise in cell biology, molecular biology, biochemistry and physiology. This diversity is reflected in the combined use of cellular, molecular genetic and mouse modeling approaches to clinically-important issues in human pathophysiology. There is an excellent research infrastructure and a visiting investigator program with an emphasis on both basic and translational science. Graduate students and postdoctoral fellows are

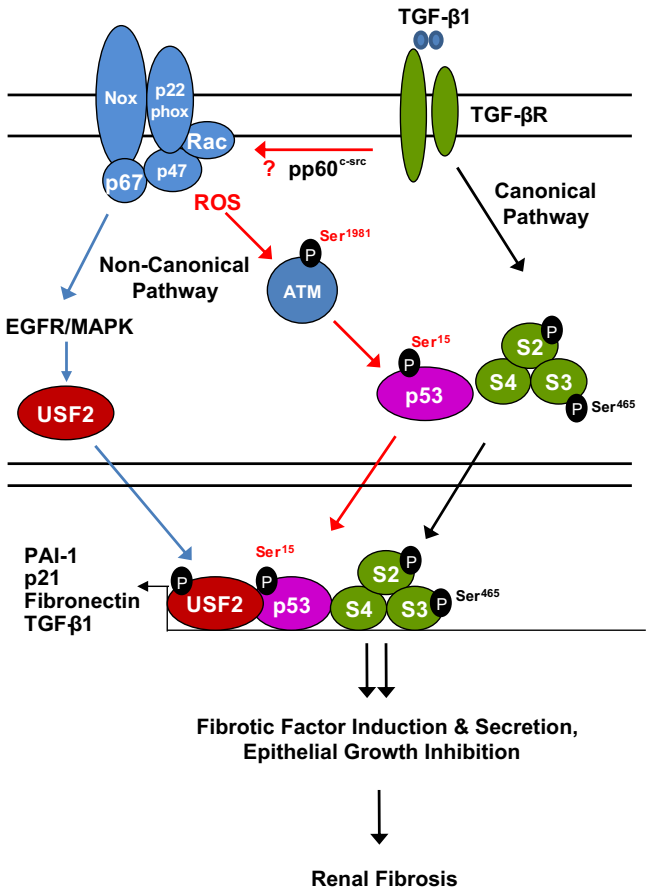

Fig. 2. Model of fibrotic gene regulation. TGF-β1 ligand-binding initiates signaling events resulting in the activation of SMAD2/3/4 (canonical pathway). In a collateral (non-canonical) pathway, TGF-β utilizes the $p47^{phox}$ and $p22^{phox}$-dependent NADPH oxidases to generate ROS necessary for ATM activation as well as its downstream substrate p53, p53 and SMAD, in turn, form a complex that binds to TGF-β target gene promoters (e.g., PAI-1), likely in cooperation with members of the upstream stimulatory factor family (e.g., USF2) of helix-loop-helix transcription factors to stimulate optimal gene expression. Other ROS-dependent non-SMAD elements, including c-src/EGFR/MAPK, function to recruit additional transcriptional elements as part of a highly-interactive canonical and non-canonical expression control network (adapted from [5]).

exposed to a highly-interactive, clinically-relevant, training environment focused on clarifying cell function in normal and disease states. The translational nature of the research facilitates the application of new findings not only to the field of tissue fibrosis but also to a spectrum of human diseases including cancer, atherosclerosis, arthritis and diabetes.

The Center has been granted 'Standard Translational Medicine Centre Accreditation' and Accreditation as an 'Advanced Translational Medicine Centre for Renal Fibrosis Research' by the European Society for the Translational Medicine (EUSTM).

1.2. Interstitial fibrosis

Interstitial fibrosis is a common and progressive disorder that frequently culminates in organ failure; current treatments are limited and largely ineffective. The majority of fibrotic diseases are progressive, irreversible and eventually fatal. Extent of tissue involvement is a predictive end-stage pathophysiologic hallmark of chronic vascular, renal, hepatic and pulmonary disease, particularly in the clinical setting of diabetes, hypertension, ischemia, obesity, metabolic syndrome, and chronic injury. Regardless of etiology, elevated TGF-β1 levels (the predominant driver of the fibrotic response) and transcription of TGF-β1-responsive genes

are linked to the activation of profibrotic signaling pathways. Plasminogen activator inhibitor-1 (PAI-1; SERPINE1), a member of the serine protease inhibitor family and a prominent negative regulator of the plasmin-based pericellular proteolytic cascade, is a major causative factor in several clinically significant fibrotic syndromes and healing anomalies. Increased PAI-1 expression, due to increased TGF-β1 in the injured tissue, initiates and perpetuates the fibrotic cycle. The need for novel targeted approaches for the treatment of fibrosis highlights the clinical potential in the current probe of molecular mechanisms underlying TGF-β1-regulated PAI-1 gene control [2–4].

The collective research efforts in the CCBCR resulted in the identification of a novel pathway of TGF-β1-initiated fibrotic gene expression in an in vivo model of renal injury that mimics obstructive uropathy in humans [2–5]. While TGF-β1-induced SMAD2/3 signaling is a critical event in the progression of chronic kidney disease, the role of non-SMAD mechanisms in the orchestration of fibrotic gene changes remain largely unexplored yet constitute likely targets that could be exploited for clinical gain. TGF-β1/SMAD3 pathway activation in renal fibrosis (induced by ureteral ligation) correlated with epidermal growth factor receptor^{Y845} (EGFR^{Y845}) and p53^{Ser15} phosphorylation followed by induction of the fibrotic disease causative target genes PAI-1 and connective tissue growth factor (CTGF) prompting an investigation of mechanistic involvement of EGFR and p53 in profibrotic signaling. TGF-β1, PAI-1, CTGF, p53 and EGFR were found to be co-expressed in the obstructed kidney localizing predominantly to the tubular and interstitial compartments and TGF-β1, activated EGFR, p53 and SMAD2/3. Genetic deficiency of either EGFR or p53 or functional blockade of EGFR kinase activity or p53 signaling with AG1478 or pifithrin-α, respectively, effectively inhibited PAI-1and CTGF induction as did SMAD3 knockdown or pretreatment with the SMAD3 inhibitor SIS3. These results provided the first insight as to pharmacologic interventions with clinical potential in the therapy of renal fibrosis that focused on specific pathways involved in controls on disease-critical genes. Reactive oxygen species (ROS)-dependent mechanisms initiated by TGF-β1 were similarly determined to be critical for EGFR^{Y845} and p53^{Ser15} phosphorylation and target gene expression. The p22^{Phox} subunit of NADPH oxidase was elevated in the fibrotic kidney with an expression pattern similar to p53 and EGFR. This has important translational implications as several inhibitors of ROS generation that block TGF-β1-induced PAI-1 transcription are already in clinical trials.

These data (summarized in Fig. 2) highlight the extensive cross-talk among SMAD2/3, EGFR and p53 pathways essential for expression of TGF-β1-induced fibrotic genes. The additional clinical impact of this work resulted from the subsequent discovery of the involvement of ATM, an upstream activator of p53, in the TGF-β1 pathway related to renal fibrosis. ATM activation (pATM^{Ser1981}) increased 4-fold in the tubulointerstitial region of the obstructed kidney in mice correlating with SMAD3 and p53^{Ser15} phosphorylation and elevated levels of the p22^{phox} subunit of the NADP(H) oxidases and increases in the fibrotic markers PAI-1 and fibronectin. ATM is rapidly phosphorylated at Ser^{1981} by TGF-β1 stimulation. Stable silencing or pharmacological inhibition of ATM significantly attenuated TGF-β1-induced p53 activation and subsequent PAI-1, fibronectin, CTGF and p21 expression in renal tubular epithelial cells and fibroblasts. ATM or p53 depletion in renal epithelial cells, moreover, bypassed TGF-β1-mediated cytostasis while stable silencing of NADPH oxidase (NOX) subunits, p22^{phox} and p47^{phox} blocked TGF-β1-induced pATM^{Ser1981} and target gene induction via p53-dependent mechanisms.

TGF-β1, thus, promotes NOX-dependent ATM activation leading to p53-mediated fibrotic gene reprogramming and growth arrest in renal proximal tubular cells. TGF-β1/ATM-initiated paracrine factor secretion by dysfunctional renal epithelium also promotes interstitial fibroblast growth, suggesting a role of tubular ATM in mediating epithelial-mesenchymal crosstalk highlighting the translational benefit of targeting the NOX/ATM/p53 axis in renal fibrosis [5].

Collectively, this work represents a close and productive collaboration between basic and clinical scientists in the Center for Cell Biology and Cancer Research at Albany Medical College. This effort has resulted in the identification of translationally-relevant, pharmacologically-manipulatable, targets with potential utility in the therapy of human renal fibrotic disease.

Clinical application of this work has been extended across three additional related lines of human fibrosis. In collaboration with Urological Surgery there is an effort to apply these findings to transitional cell bladder cancer as well as renal and prostate malignancies to determine whether the biological responses identified in the renal injury model might provide universal biomarkers of disease progression or response to conventional therapy at other organ sites. A second line of research involves investigators with expertise in Vascular Surgery to obtain human specimens and develop mouse models to assess the role of this defined pathway (Fig. 2) in vaso-occlusive disease. Finally, interstitial lung disease offers a unique human model to validate our tissue and animal data in a prominent pathophysiologic context. Our pulmonary division operates a large Scleroderma clinic providing an opportunity to not only collect patient samples but to correlate findings throughout the natural progression of disease and during the initiation of novel therapies to delay lung fibrosis. Current collaborations with pulmonary research centers in the US and Canada provide opportunities and model systems that are likely to result in clarification of the general applicability of these findings in the broader context of human fibrotic disorders.

Executive Summary

- Interstitial fibrosis is a progressive disease that culminates in organ failure.
- Extent of tissue involvement is a predictive end-stage pathophysiologic biomarker of chronic vascular, renal, hepatic and pulmonary fibrosis.
- Elevated TGF-β1 levels activate profibrotic signaling pathways leading to the transcription of TGF-β1-responsive genes.
- The TGF-β1 target gene plasminogen activator inhibitor-1(PAI-1) is a major causative factor in a number of clinically significant fibrotic syndromes.
- Translational research in the Center for Cell Biology & Cancer Research at Albany Medical College focuses on molecular events underlying renal fibrosis and transcriptional activation of the PAI-1 gene using an animal model of obstructive uropathy.
- This combined, and highly-mechanistic, effort by basic and clinical scientists has uncovered a complex interaction by canonical and non-canonical factors in the TGF-β1 signaling pathway required for expression of pro-fibrotic effectors.
- Results of this ongoing translational effort highlight the clinical potential in the current probe of the molecular complexity of TGF-β1-regulated gene control.

Conflict of Interest

None Declared.

Ethical approval

None Declared.

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