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Induction of renal fibrotic genes by TGF-β1 requires EGFR activation, p53 and reactive oxygen species

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ABSTRACT

While transforming growth factor-β (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 remains largely unexplored. 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 and induction of disease causative target genes plasminogen activator inhibitor-1 (PAI-1) and connective tissue growth factor (CTGF) prompting an investigation of mechanistic involvement of EGFR and tumor suppressor p53 in profibrotic signaling. TGF-β1, PAI-1, CTGF, p53 and EGFR were co-expressed in the obstructed kidney localizing predominantly to the tubular and interstitial compartments. Indeed, TGF-β1 activated EGFR and p53 as well as SMAD2/3. Genetic deficiency of either EGFR or p53 or functional blockade with AG1478 or Pifithrin-α, respectively, effectively inhibited PAI-1and CTGF induction and morphological transformation of renal fibroblasts as did SMAD3 knockdown or pretreatment with the SMAD3 inhibitor SIS3. Reactive oxygen species (ROS)-dependent mechanisms initiated by TGF-β1 were critical for EGFR^{Y845} and p53^{Ser15} phosphorylation and target gene expression. The p22^{Phox} subunit of NADPH oxidase was also elevated in the fibrotic kidney with an expression pattern similar to p53 and EGFR. EGF stimulation alone initiated, albeit delayed, c-terminal SMAD3 phosphorylation (that required the TGF-β1 receptor) and rapid ERK2 activation both of which are necessary for PAI-1 and CTGF induction in renal fibroblasts. These data highlight the extensive cross-talk among SMAD2/3, EGFR and p53 pathways essential for expression of TGF-β1-induced fibrotic target genes.

1. Introduction

Excess deposition of ECM, leading to the progressive decline in renal function, is a common pathologic hallmark of CKD [1,2]. TGF-β1 is a potent inducer of ECM synthesis and fibrosis regardless of etiology (diabetes, hypertension, ischemic injury and obstructive uropathy) [1–6]. PAI-1 and CTGF, two prominent downstream targets of TGF-β1, are major causative factors in CKD etiology and disease progression [7–11]. PAI-1 is the major negative regulator of the plasmin-dependent pericellular proteolytic cascade while CTGF facilitates TGF-β1 binding to its receptor (TGF-βR) and inhibits BMP-7/receptor interactions suppressing, thereby, the negative effect of BMP-7 on TGF-β1/SMAD3 signaling [12,13]. Transgenic overexpression of TGF-β1 in the tubular epithelium is sufficient to induce kidney fibrosis [14] and the attenuation of disease severity in SMAD3$^{-/-}$ mice or upon administration of TGF-β receptor inhibitors further highlighted the pathophysiologic importance of the TGF-β1/SMAD3 axis in the kidney [15,16].

Previous studies implicated the tumor suppressor p53 and the EGFR in the progression of renal disease [17–20]. The mechanistic involvement of such non-canonical mechanisms in the TGF-β1-initiated profibrotic program, including the role(s) of p53 and EGFR in TGF-β1-dependent expression of fibrosis-related genes, however, remains to be clarified. This paper provides evidence that the EGFR and p53 are activated in the UUO model of renal fibrosis, a largely TGF-β1-driven disease, and required for PAI-1, CTGF and fibronectin expression in response to TGF-β1. Generation of ROS, a causative factor in tissue fibrosis [21,22], furthermore, is required for EGFR and p53 activation, consistent with

Abbreviations: ALK, activin-like kinase; α-SMA, α-smooth muscle actin; BMP, bone morphogenic protein; CDK, chronic kidney disease; CTGF, connective tissue growth factor; DAB, 3,3′-diaminobenzidine; DPI, diphenyleneiodonium chloride; ECM, extracellular matrix; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinases; HB-EGF, heparin-binding EGF; IHC, immunohistochemistry; MEFs, (mouse embryo fibroblasts); MEK, mitogen-activated protein kinase kinase; NAC, N-acetyl cysteine; NOX, NADPH oxidase; PAI-1, plasminogen activator inhibitor-1; ROS, reactive oxygen species; TGF-β1, transforming growth factor-β1; UUO, unilateral ureteral obstruction.

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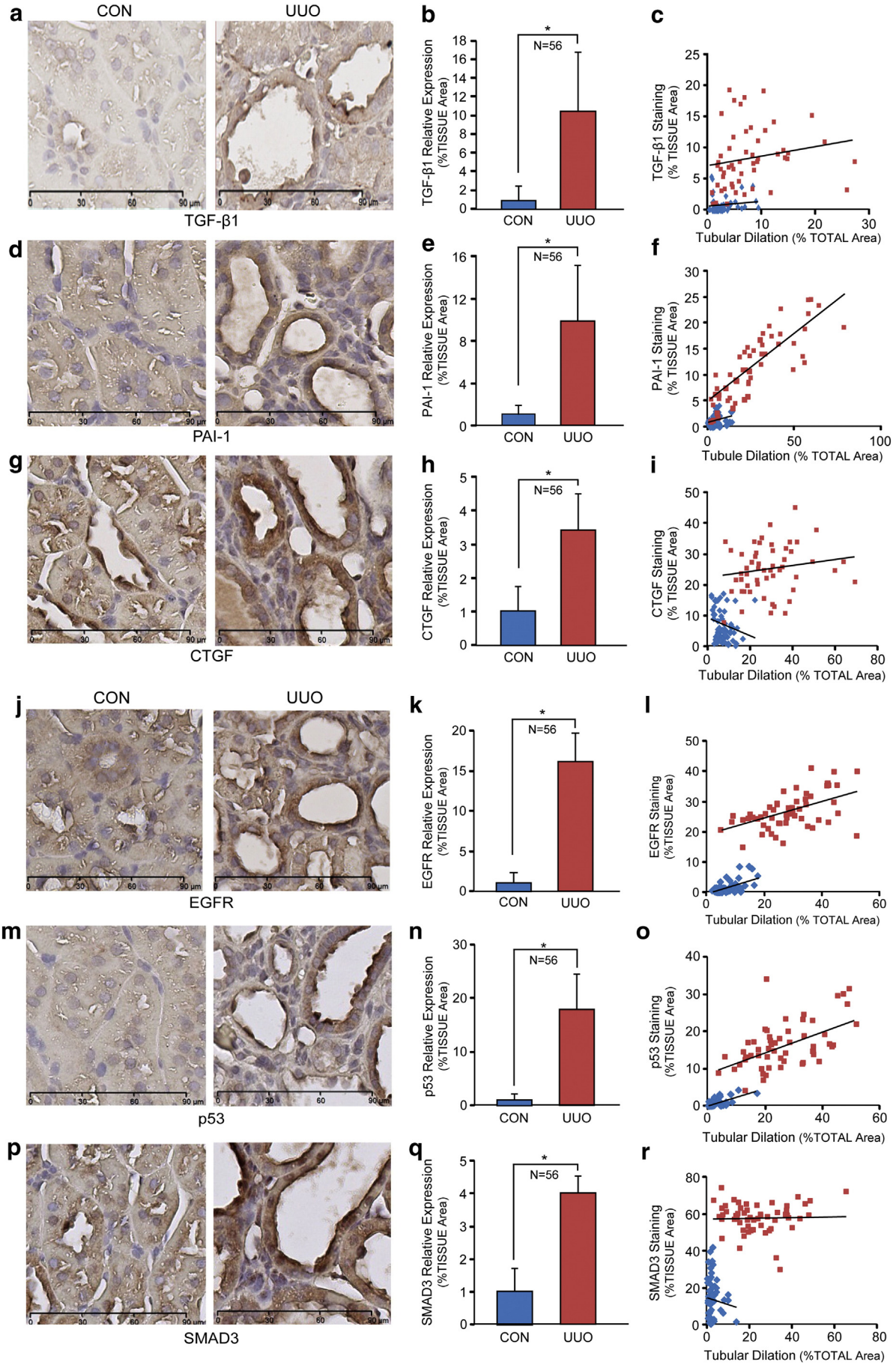
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