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Distinctive role of Stat3 and Erk-1/2 activation in mediating interferon- γ inhibition of TGF- β_1 action

Myrto Giannopoulou¹, Steven C. Iszkula¹, Chunsun Dai¹, Xiaoyue Tan¹, Junwei Yang², George K. Michalopoulos¹, and Youhua Liu¹

¹ Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania

² Department of Medicine, Nanjing Medical University, Nanjing, Jiangsu, China

Abstract

Interferon- γ (IFN- γ) is a multi-functional cytokine that elicits antifibrotic activity in a variety of organs. In this study, we investigated the potential role and mechanism of IFN- γ in modulating the fibrogenic action of transforming growth factor (TGF)- β_1 in tubular epithelial cells. Incubation of human proximal tubular epithelial (HKC) cells with IFN- γ inhibited TGF- β_1 -mediated α -smooth muscle actin (α -SMA) expression. IFN- γ also abolished TGF- β_1 -induced fibronectin and plasminogen activator inhibitor-1 (PAI-1) expression. To explore the mechanisms by which IFN- γ inhibits TGF- β_1 action, the signaling pathways that are critical for mediating the antifibrotic activity of IFN- γ were studied. Stimulation of HKC cells with IFN- γ triggered a sustained activation of Erk-1/2 and signal transducer and activator of transcription-3 (Stat3). Blockade of Erk-1/2 activation with an Mek1 inhibitor abolished the inhibitory effect of IFN- γ on α -SMA expression, whereas inhibition of Stat3 activation had no influence. Constitutive activation of Erk-1/2 by ectopic expression of activated Mek1 mimicked IFN- γ and suppressed TGF- β_1 -mediated α -SMA expression. Interestingly, inhibition of Stat3 activation abolished the ability of IFN- γ to attenuate TGF- β_1 -mediated PAI-1 and fibronectin expression in HKC cells. These findings indicate that IFN- γ is capable of antagonizing the fibrogenic actions of TGF- β_1 in renal tubular epithelial cells. The antifibrotic action of IFN- γ appears to be mediated through a coordinated activation of both Erk-1/2 and Stat3 signal pathways in a mutually independent fashion.

Keywords

transforming growth factor- β ; signal transducer and activator of transcription-3; renal fibrosis

THE IMPORTANCE OF TUBULOINTERSTITIAL lesions in the progressive loss of renal function in chronic kidney diseases has been widely recognized (6,28). Despite this, the role of tubular epithelial cells (TECs), which constitute the vast majority of renal parenchyma, in renal fibrogenesis remains incompletely understood (6). Evidence indicates that renal TECs are not innocent bystanders in the evolution of chronic interstitial fibrosis. Rather, they appear to play a decisive role in the fibrogenic process. This view is strengthened by many observations that under pathological circumstances tubular cells predominantly express the

Address for reprint requests and other correspondence: Y. Liu, Dept. of Pathology, Univ. of Pittsburgh, S-405 Biomedical Science Tower, 200 Lothrop St., Pittsburgh, PA 15261 (e-mail: liuy@upmc.edu.).

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receptors for profibrotic cytokine transforming growth factor (TGF)- β_1 and readily respond to its stimulation (2,34). Renal TECs contribute to the fibrotic lesions by numerous mechanisms. They can play a pivotal role in recruiting inflammatory cells or activating kidney resident cells that are closely adjacent to them, presumably via the secretion of proinflammatory and fibrogenic cytokines. Furthermore, TEC apoptosis after injurious stimuli could lead to tubular atrophy and structural collapse (4), processes that likely promote the accumulation of fibrous components. More importantly, ample studies have shown that TECs may undergo a phenotypic conversion known as the epithelial-to-mesenchymal transition (EMT) (8,11,13,34), thereby directly contributing to the generation of the matrix-producing effector cells in the fibrotic kidney.

TECs respond to the stimulation of fibrogenic cues to express α -smooth muscle actin (α -SMA) and produce interstitial matrix components. This process is regulated by many factors in different ways (13). TGF- β_1 is believed to be the most potent cytokine that initiates this process, whereas hepatocyte growth factor (HGF) and bone morphogenic protein-7 suppress this process by antagonizing TGF- β_1 action (15,33,35). Intriguingly, blockade of tubular EMT with HGF or bone morphogenic protein-7 leads to prevention and regression of renal interstitial fibrosis in animal models (14,33,35). In this context, identification of additional agents that can effectively antagonize the fibrogenic action of TGF- β_1 may hold promise for designing a rational strategy for therapeutic interventions in renal fibrosis.

Interferon- γ (IFN- γ) is a multiple functional cytokine that is mainly produced by activated T cells and natural killer cells. However, its biological activities are not limited to the immune modulation. IFN- γ is known to exert antiproliferative and cytostatic actions in various cell types (10). It also suppresses collagen synthesis in human dermal fibroblasts (5) and inhibits myofibroblastic activation from hepatic stellate cells (1,23,25). In cultured human renal interstitial fibroblasts, IFN- γ has been demonstrated to strongly inhibit α -SMA and fibronectin expression (27). Several in vivo studies have confirmed the antifibrotic effect of IFN- γ in animal models (20,29) and in patients with systemic sclerosis or idiopathic pulmonary fibrosis (22,36). Administration of IFN- γ resulted in significant reduction of ECM deposition and hepatic fibrosis induced by carbon tetrachloride or pig serum (24,29). In a rat remnant kidney model, IFN- γ treatment also leads to amelioration of proteinuria and preservation of the creatinine clearance and inhibits myofibroblastic activation and fibrotic lesions.

The present study was undertaken to examine the potential effects of IFN- γ on the fibrogenic response of tubular epithelial cells induced by TGF- β_1 and to investigate the underlying mechanism that accounts for its action.

MATERIALS AND METHODS

Antibodies and reagents

The mouse monoclonal anti- α -SMA (clone 1A4), rabbit polyclonal anti-Erk-1/2, and mouse monoclonal anti- α -tubulin were purchased from Sigma (St. Louis, MO). The anti-phospho-Erk-1/2 and anti-phospho-signal transducer and activator of transcription-3 (Stat3) antibodies were obtained from Cell Signaling Technology (Beverly, MA). Antibodies against fibronectin, plasminogen activator inhibitor (PAI)-1, and actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Recombinant human TGF- β_1 and human IFN- γ were purchased from R&D Systems (Minneapolis, MN). Recombinant human HGF protein was provided by Genentech (South San Francisco, CA). Cell culture media, FBS, and supplements were obtained from Invitrogen (Carlsbad, CA). Specific Mek1 inhibitor PD-98059 and cell-permeable Stat3 inhibitor peptide (PpYLKTK-mts; cat. No. 573096)

were purchased from Calbiochem (La Jolla, CA). All other chemicals were of analytic grade and were obtained from Sigma or Fisher (Pittsburgh, PA) unless otherwise indicated.

Cell culture and treatment

Human proximal tubular epithelial HKC cells (clone 8) were obtained from Dr. L. Racusen of the Johns Hopkins University and maintained in DMEM/F-12 medium supplemented with 10% FBS, as described previously (33). The HKC cells were seeded on six-well culture plates in complete medium containing 10% FBS for 16 h and then switched to serum-free medium after being washed twice with medium. Recombinant human TGF- β_1 was added to the culture at a final concentration of 0.1 nM unless otherwise indicated. Recombinant human IFN- γ was also added in the same time at the indicated concentrations. The cells were typically incubated for 72 h following addition of cytokines, except when indicated otherwise, before being harvested and subjected to Western blot analysis or immunofluorescence staining, respectively.

Western blot analysis

Cells were lysed with SDS sample buffer (62.5 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 50 mM dithio-threitol, and 0.1% bromophenol blue). Samples were heated at 100°C for 5–10 min before being loaded and separated on 10% SDS-polyacrylamide gels. After the proteins were electrottransferred to Hybond-P polyvinylidene difluoride membranes (Amersham Biosciences, Piscataway, NJ), nonspecific binding to the membrane was blocked for 1 h at room temperature with 5% Carnation nonfat milk in TBST buffer (20 mM Tris-HCl, 150 mM NaCl, and 0.1% Tween 20). The membranes were then incubated for 16 h at 4°C with various primary antibodies in blocking buffer containing 5% milk at the dilutions specified by the manufacturers. Following extensive washing in TBST buffer, the membranes were incubated with horseradish peroxidase-conjugated secondary antibody for 1 h at room temperature in 5% nonfat milk dissolved in TBST. Membranes were then washed with TBST buffer, and the signals were visualized using the SuperSignal West Pico Chemiluminescent Substrate kit (Pierce Biotechnology, Rockford, IL).

Immunofluorescence staining

Indirect immunofluorescence staining was performed using an established procedure (12,33). Briefly, cells cultured on coverslips were washed with cold PBS twice and fixed with cold methanol:acetone (1:1) for 10 min at -20°C. Following extensive washing with PBS containing 0.5% BSA three times, the cells were blocked with 20% normal donkey serum in PBS buffer for 30 min at room temperature and then incubated with the specific primary antibodies described above. To visualize the primary antibodies, cells were stained with cyanine Cy2-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories). As a negative control, the primary antibody was replaced with nonimmune IgG, and no staining occurred. Cells were double stained with 4',6-diamidino-2-phenylindole, HCl to visualize the nuclei. Stained cells were mounted with Vectashield mounting medium (Vector Laboratories, Burlingame, CA) and viewed under a Nikon Eclipse E600 Epi-fluorescence microscope equipped with a digital camera (Melville, NY).

Adenovirus infection

HKC cells were seeded on six-well culture plates to 90% confluence in complete medium. After an overnight incubation, the cultures were washed twice and switched to serum-free medium. The recombinant adenovirus harboring constitutively active Mek1 (Ad.Mek1-CA) was kindly provided by Dr. Sakae Tanaka of the University of Tokyo Faculty of Medicine (Tokyo, Japan) (19). The adenovirus containing β -galactosidase (Ad.LacZ) was provided by Dr. A. Gambotto at the Vector Core Facility of the University of Pittsburgh (Pittsburgh,

PA). The adenovirus was added to the cultures at a concentration of 2×10^7 particles/ml. Cells were incubated for 48 and 72 h, and then cell lysates were collected for Western blot analysis.

RESULTS

IFN- γ blocks TGF- β_1 -mediated α -SMA expression in tubular epithelial cells

Figure 1 shows that IFN- γ blocked α -SMA expression induced by TGF- β_1 in human proximal tubular epithelial HKC cells. When incubated with 0.1 nM TGF- β_1 , α -SMA expression in HKC cells was markedly induced after 3 days of incubation, as previously reported (34). Simultaneous incubation of HKC cells with IFN- γ largely abolished TGF- β_1 -mediated α -SMA expression. IFN- γ exerted its inhibitory effect at the concentration of 0.2 nM. Higher concentration of IFN- γ did not result in further inhibition of TGF- β_1 -mediated α -SMA expression. Treatment with IFN- γ alone also slightly inhibited α -SMA expression under basal conditions in HKC cells. As a positive control (33), HGF completely abolished TGF- β_1 -induced α -SMA expression (Fig. 1A). Similar results were obtained when indirect immunofluorescence staining was used (Fig. 1, B–D). TGF- β_1 treatment induced the formation of α -SMA-positive cytoplasmic microfilaments in HKC cells (Fig. 1C), which was abrogated by coinubation with 1 nM IFN- γ (Fig. 1D).

IFN- γ abolishes TGF- β_1 -mediated fibronectin and PAI-1 expression

We next examined the effects of IFN- γ on fibronectin expression in tubular epithelial cells. TGF- β_1 induced a substantial increase in fibronectin expression in HKC cells, and IFN- γ significantly inhibited its expression (Fig. 2A). Immunofluorescence staining revealed that the fibronectin protein stimulated by TGF- β_1 was properly assembled and deposited (Fig. 2C), and IFN- γ at 1 nM significantly abolished the TGF- β_1 -mediated fibronectin deposition (Fig. 2D). IFN- γ also inhibited PAI-1 expression induced by TGF- β_1 in tubular epithelial cells. As shown in Fig. 2A, IFN- γ at a concentration as low as 0.2 nM substantially abrogated the TGF- β_1 -mediated PAI-1 induction. Similar to the α -SMA and fibronectin expression, higher IFN- γ concentrations did not cause a further reduction in PAI-1 expression in HKC cells.

IFN- γ activates both Erk-1/2 and Stat3 signal pathways in tubular epithelial cells

To identify the signal pathways that are responsible for mediating IFN- γ inhibition of TGF- β_1 action, we first examined the signals that were activated by IFN- γ in tubular epithelial cells. As shown in Figure 3, incubation of tubular epithelial cells with IFN- γ markedly activated the phosphorylation of Erk-1/2, members of the MAP kinase family. Kinetic studies revealed that Erk-1/2 activation started as early as 10 min after IFN- γ treatment, was sustained, and reached a second wave of activation at 12 h. Quantitative determination revealed an ~20-fold induction of phosphorylated Erk-1/2 at 24 and 48 h following IFN- γ stimulation in HKC cells (Fig. 3B).

Besides Erk-1/2 activation, we found that IFN- γ also stimulated the activation of the signal transducer and activator of transcription (Stat)-3, as detected by using phospho-specific Stat3 antibody. Shown in Fig. 3C, a sustained activation of Stat3 in HKC cells was observed at different time points after IFN- γ stimulation throughout the experimental period, ranging from 10 min to 48 h.

Erk-1/2, but not Stat3, activation is both necessary and sufficient for mediating IFN- γ suppression of α -SMA expression

To define which signal pathway is necessary for mediating the inhibitory effect of IFN- γ on TGF- β_1 action, we examined the effects of blockade of different signaling with specific

inhibitors in HKC cells. As shown in Fig. 4, blockade of Erk-1/2 activation with the upstream kinase Mek1 inhibitor PD-98059 largely abolished IFN- γ activity, which led to a substantial restoration of α -SMA expression induced by TGF- β_1 . However, treatment of HKC cells with specific Stat3 inhibitor did not affect the inhibitory effect of IFN- γ on α -SMA expression (Fig. 4B). These results indicate that IFN- γ suppression of the TGF- β_1 -mediated α -SMA expression is mediated by an Erk-1/2-dependent, Stat3-independent pathway.

To provide further evidence of a critical role for Erk-1/2 activation in mediating IFN- γ action, we sought to determine whether Erk-1/2 activation is sufficient for inhibiting TGF- β_1 -induced α -SMA expression. To this end, HKC cells were infected with an adenoviral vector (Ad-caMek1) containing constitutively activated Mek1, the upstream kinase of Erk-1/2. An adenoviral vector (Ad-LacZ) containing the β -galactosidase gene was used as a negative control. As shown in Fig. 4C, infection of HKC cells with Ad-caMek1 specifically induced Erk-1/2 phosphorylation at different time points compared with the Ad-LacZ controls. Interestingly, constitutive activation of Erk-1/2 in HKC cells after infection with Ad-caMek1 completely blocked TGF- β_1 -mediated α -SMA expression (Fig. 4D), suggesting that Erk-1/2 activation can mimic IFN- γ to block TGF- β_1 -mediated α -SMA expression in tubular epithelial cells.

IFN- γ inhibition of PAI-1 and fibronectin expression is dependent on activation of Stat3

Although ablation of Stat3 activation did not affect IFN- γ suppression of α -SMA expression (Fig. 4B), we found that Stat3 signaling is required for IFN- γ to inhibit PAI-1 and fibronectin induction in tubular epithelial cells. As shown in Fig. 5B, inhibition of Stat3 signaling largely abolished the inhibitory effect of IFN- γ and restored PAI-1 expression induced by TGF- β_1 . Similarly, suppression of Stat3 activation also restored TGF- β_1 -mediated fibronectin expression in tubular epithelial cells (Fig. 5C).

Figure 5 also demonstrated that blockade of Erk-1/2 signaling with an Mek1 inhibitor did not influence PAI-1 and fibronectin expression (Fig. 5, A and C), suggesting that IFN- γ inhibition of their expression is independent of Erk-1/2 activation. In accordance with this, constitutive activation of Erk-1/2 signaling through an adenoviral vector did not abolish PAI-1 expression in HKC cells (Fig. 5D). These results suggest that IFN- γ inhibits TGF- β_1 -mediated PAI-1 and fibronectin expression by a Stat3-dependent, Erk-1/2-independent mechanism.

DISCUSSION

TECs have recently emerged as the major matrix-producing cells in the fibrotic kidney after chronic injury (11,13). On stimulation by profibrotic cytokines such as TGF- β_1 , TECs undergo an EMT and become fibrogenic cells that express α -SMA and produce interstitial matrix components such as fibronectin (33,34). In view of the fact that TECs constitute the vast majority of renal parenchyma, the process of EMT would give rise to a virtually inexhaustible supply of the fibrogenic cells in the diseased kidney, thereby having a pivotal impact on the pathogenesis of renal interstitial fibrosis. In this context, inhibition of tubular EMT may be of vital importance in preventing renal fibrogenesis. The results presented in this study demonstrate that IFN- γ inhibits the fibrogenic response of TECs, as evidenced by downregulating TGF- β_1 -mediated α -SMA, fibronectin, and PAI-1 expression. Furthermore, we have found that the antifibrotic effect of IFN- γ is mediated by both Stat3 and the Erk branch of the MAP kinase family. Interestingly, either Stat3 or Erk-1/2 activation is only responsible for IFN- γ suppression of a subset of fibrogenic gene expression, suggesting an independent, distinctive role for each signal pathway in mediating the action of IFN- γ (Fig.

5E). These studies provide significant insights into the molecular mechanisms by which IFN- γ inhibits the TGF- β_1 -mediated fibrogenic responses of TECs.

IFN- γ is generally regarded as a proinflammatory cytokine that plays a critical role in modulating immune responses (7). The expression of IFN- γ is often induced in various chronic kidney diseases, including diabetic nephropathy (18,21). The antifibrotic activity of IFN- γ as shown in this study suggests that increased IFN- γ after injury may not only have an implication in inflammation but also suppresses the fibrogenic effects of TGF- β_1 , which is also upregulated in response to injurious stimuli. The reciprocal interaction between IFN- γ and TGF- β_1 establishes a sophisticated mechanism for fine-tuning the effect of these factors during tissue repair and fibrogenesis after injury. IFN- γ and TGF- β_1 are known to exert an opposite action on inflammation, as TGF- β_1 is an anti-inflammatory cytokine (30). In this context, it is also not surprising to find out that IFN- γ antagonizes the fibrogenic action of TGF- β_1 in kidney TECs. In accordance with this, the inhibitory effects of IFN- γ on matrix production have also been documented in other cell types, including renal interstitial fibroblasts (27).

Compared with HGF, IFN- γ cannot completely inhibit the fibrogenic responses initiated by TGF- β_1 . Increases in IFN- γ concentration from 0.2 to 2 nM did not result in further inhibition of α -SMA, fibronectin, and PAI-1 expression. Therefore, it appears that a low dose of IFN- γ (0.2 nM) is able to render the maximal inhibition of TGF- β action. These observations suggest that a high concentration of IFN- γ may not be necessary in designing clinical utilization for blocking renal fibrosis. At this stage, it remains unknown why IFN- γ at higher concentrations failed to completely inhibit TGF- β_1 action. One possibility could be that the magnitude of the signals triggered by IFN- γ is not strong enough to totally counteract the fibrogenic action of TGF- β_1 in TECs. Another scenario is that the fibrogenic responses of TGF- β_1 in TECs may be mediated by multiple pathways that work in concert, and IFN- γ may not directly antagonize all of this fibrogenic signaling.

One of the novel findings in the present study is that IFN- γ apparently inhibits the fibrogenic action of TGF- β_1 by a coordinated activation of independently regulated Erk-1/2 and Stat3 pathways. Erk-1/2 activation is both necessary and sufficient for mediating IFN- γ inhibition of α -SMA expression but not for downregulating fibronectin and PAI-1. Vice versa, Stat3 activation is responsible for reducing fibronectin and PAI-1 expression but not for α -SMA inhibition. The distinctive role of Erk-1/2 and Stat3 signal pathways highlights that IFN- γ -mediated inhibition of fibrogenic responses in TECs requires a coordinated activation and participation of diverse signals. These observations also imply that the induction of α -SMA, fibronectin, and PAI-1 expression by TGF- β_1 may not be controlled by a common pathway in TECs.

The exact mechanisms by which IFN- γ inhibits the fibrogenic responses of TECs remain unclear. Erk-1/2 signaling has been previously shown to inhibit TGF- β /Smad signaling through multiple mechanisms (2,17,26), which include blocking Smad nuclear translocation (31), upregulating the Smad transcriptional corepressor TGIF via protein stabilization (3), or inducing SnoN expression (32). However, although Erk-1/2 activation is implicated in suppressing α -SMA expression, it fails to downregulate fibronectin and PAI-1 expression. As TGF- β -mediated fibronectin expression is also dependent on intact Smad signaling in HKC cells (32), this suggests that the action of Erk-1/2 may be operated at the stage downstream of Smad signaling. On the other hand, Stat3 is a transcription factor that can interact with other transcription regulators such as Smad, thereby modulating the *trans*-activation of many TGF- β target genes. It has been reported that inhibition of Stat3 by PIAS3, a member of the protein inhibitor of activated Stat (PIAS) family, activates Smad transcriptional responses (16). PIAS3 can form a ternary complex with Smad3 and general

transcriptional coactivator p300/CBP and activate Smad transcriptional activity. Consistent with this, a recent study shows that the hyperactivation of Stat3 in gp130 mutant mice desensitizes TGF- β /Smad signaling through transcriptional induction of inhibitory Smad7, thereby providing a novel link for cross talk between Stat and Smad signaling (9). Therefore, it appears that activation of Stat3 by IFN- γ would lead to an inhibition of TGF- β /Smad-mediated gene transcription. It remains, however, ambiguous why Stat3 activation is only responsible for the inhibition of a subset of fibrogenic gene expression in TECs.

The ability of IFN- γ to suppress the fibrogenic response of TECs underscores its potential to be utilized as a therapeutic agent for the treatment of renal fibrotic diseases. Consistent with this notion, administration of IFN- γ ameliorates proteinuria, improves creatinine clearance, and inhibits myofibroblastic activation and fibrotic lesions in a rat remnant kidney model (20). However, in light of its proinflammatory activity, one should be cautious so that the antifibrotic role of IFN- γ is balanced against its perceived role in triggering inflammation. It is possible that the effects of IFN- γ on fibrotic processes may depend on the types and etiologies of different renal diseases. Clearly, more studies are warranted to evaluate the protective role of IFN- γ in fibrotic diseases and to better understand the molecular mechanism by which IFN- γ exerts its antifibrotic action.

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Fig. 1.

Interferon- γ (IFN- γ) abolishes transforming growth factor (TGF- β_1)-mediated α -SMA muscle actin (SMA) expression in tubular epithelial cells. **A**: Western blot analysis shows that IFN- γ inhibits α -SMA expression induced by TGF- β_1 . HKC cells were treated with TGF- β_1 (0.1 nM) and increasing amounts of IFN- γ as indicated. Whole cell lysates were immunoblotted with antibodies against α -SMA and actin, respectively. Hepatocyte growth factor (HGF; 0.25 nM) was used as a positive control. Shown are representative blots from 2 independent experiments. **B–D**: immunofluorescence staining demonstrates α -SMA expression after various treatments. **B**: control. **C**: TGF- β_1 (0.1 nM) alone. **D**: TGF- β_1 plus IFN- γ (1 nM). Nuclear staining with 4',6-diamidino-2-phenylindole is also shown. Scale bar = 10 μ m.

Fig. 2.

IFN- γ inhibits TGF- β 1-mediated fibronectin and plasminogen activator inhibitor-1 (PAI-1) expression in tubular epithelial cells. **A**: Western blot analysis shows that IFN- γ inhibited fibronectin and PAI-1 expression induced by TGF- β 1. HKC cells were treated with TGF- β 1 or TGF- β 1 plus increasing amounts of IFN- γ as indicated. Whole cell lysates were immunoblotted with antibodies against fibronectin, PAI-1, and α -tubulin. Shown are representative blots from 3 independent experiments. **B–D**: immunofluorescence staining demonstrates fibronectin expression after various treatments. **B**: control. **C**: TGF- β 1 (0.1 nM) alone. **D**: TGF- β 1 plus IFN- γ (1 nM). Scale bar = 10 μ m.

Fig. 3. IFN- γ induces sustained Erk-1/2 and Stat3 activation in tubular epithelial cells. *A* and *C*: HKC cells were treated with IFN- γ (0.5 nM) for various periods of time as indicated. Cell lysates were immunoblotted with antibodies against p-Erk-1/2 and total Erk-1/2 (*A*) or p-Stat3 and actin (*C*), respectively. *B* and *D*: graphic presentation of relative abundances of p-Erk-1/2 or p-Stat3 (fold-induction over controls) is given after normalization with total Erk-1/2 or actin, respectively.

Fig. 4.

Erk-1/2, but not Stat3, signaling is both necessary and sufficient for mediating IFN- γ inhibition of α -SMA expression. *A* and *B*: activation of Erk-1/2, but not Stat3, is required for mediating IFN- γ inhibition of α -SMA induction by TGF- β_1 . HKC cells were pretreated with Mek1 inhibitor PD-98059 (10 μ M; *A*) or Stat3 inhibitor (25 μ M; *B*) for 30 min, followed by treatment with TGF- β_1 (0.1 nM) or/and IFN- γ (1 nM) for 48 h as indicated. Whole cell lysates were immunoblotted with antibodies against α -SMA and actin, respectively. *C* and *D*: constitutive activation of Erk-1/2 is sufficient for inhibiting TGF- β_1 -mediated α -SMA induction. HKC cells were infected with adenoviral vector harboring constitutively active Mek1 (Ad.ca-Mek1) or control LacZ adenoviral vector (Ad.LacZ), followed by treatment with TGF- β_1 (0.1 nM) as indicated. Constitutive activation of Erk-1/2 after adenovirus infection was assessed by immunoblotting with antibodies against either phospho-specific or total Erk-1/2, respectively (*C*). Whole cell lysates were immunoblotted with antibodies against α -SMA and actin (*D*), respectively. Shown are the representative blots for two independent experiments.

Fig. 5.

IFN- γ inhibition of TGF- β ₁-mediated PAI-1 and fibronectin expression is dependent on the activation of Stat3, but not Erk-1/2. *A–C*: HKC cells were pretreated with Stat3 inhibitor (25 μ M) or Mek1 inhibitor PD-98059 (1 μ M) for 30 min, followed by treatment with TGF- β ₁ (0.1 nM) or/and IFN- γ (1 nM) for 48 h as indicated. Whole cell lysates were immunoblotted with specific antibodies as indicated. Shown are the representative blots from 2 independent experiments. *D*: constitutive activation of Erk-1/2 does not abolish TGF- β ₁-mediated PAI-1 induction. HKC cells were infected with adenoviral vector harboring constitutively active Mek1 (Ad.ca-Mek1) or control LacZ adenoviral vector (Ad.LacZ), followed by treatment with TGF- β ₁ (0.1 nM) as indicated. *E*: diagram depicts the signal pathways triggered by IFN- γ leading to inhibition of fibrosis-related genes.