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TNF-related weak inducer of apoptosis (TWEAK) promotes kidney fibrosis and Ras-dependent proliferation of cultured renal fibroblast

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Abstract

Tumor necrosis factor-like weak inducer of apoptosis (TWEAK) regulates apoptosis, proliferation and inflammation in renal epithelial cells and plays a role in acute kidney injury. However, there is little information on the chronic effects of TWEAK. We hypothesized that TWEAK may influence renal fibrosis and regulate kidney fibroblast biology, in part, through Ras pathway.

We studied a chronic model of experimental unilateral ureteral obstruction in wild type and TWEAK deficient mice, and a murine model of systemic TWEAK overexpression. TWEAK actions were also explored in cultured renal and embryonic fibroblasts.

TWEAK and TWEAK receptor expression was increased in the obstructed kidneys. The absence of TWEAK decreased early kidney tubular damage, inflammatory infiltrates and myofibroblast number. TWEAK deficient mice had decreased renal fibrosis 21 days after obstruction, as assessed by extracellular matrix staining. In mice without prior underlying kidney disease, systemic overexpression of TWEAK induced kidney inflammation and fibrosis. In cultured fibroblasts, TWEAK induced proliferation through activation of the Ras/ERK pathway. TWEAK also activated nuclear factor κB (NFκB)-dependent inflammatory chemokine production in murine renal fibroblasts.

In conclusion, lack of TWEAK reduces renal fibrosis in a model of persistent kidney insult and overexpression of TWEAK led to renal fibrosis. TWEAK actions on renal fibroblasts may contribute to the in vivo observations, as TWEAK promotes inflammatory activity and proliferation in fibroblast cultures.

Key words: Kidney fibrosis, Fn14, TWEAK, Ras, proliferation, NFκB.

1. Introduction

Tissue injury results in signals that trigger inflammation, which contributes to the repair process. If repair fails or the initial kidney insult persists, interstitial fibrosis ensues [1-3]. Indeed, recovery from acute kidney injuries (AKI) may evolve to residual interstitial fibrosis [4, 5]. In chronic kidney disease (CKD), the degree of interstitial fibrosis correlates well with outcomes [6-9]. Thus, an improved understanding of the molecular and cellular mechanisms promoting kidney fibrosis may help design novel therapeutic strategies. Irreversible unilateral ureteral obstruction (UUO) is a widely used animal model to study the mechanisms of non-toxin and non-immune renal injury and fibrosis [10]. In this model the cause of kidney injury is maintained throughout the study, thus differing from models of self-limited AKI such as transient ischemia-reperfusion. Following ureteral obstruction kidneys develop the main features of CKD, such as tubular cell death, inflammation and fibrosis, in an accelerated fashion.

Renal interstitial fibrosis is characterized by ECM accumulation and an increased number of activated fibroblasts [11]. Fibroblast number closely correlates with severity of tubulointerstitial fibrosis and renal disease progression [12].

Tumor necrosis factor (TNF) superfamily cytokines contribute to renal injury [13] and may promote renal fibroblast apoptosis [14]. Tumor necrosis factor-like weak inducer of apoptosis (TWEAK, TNFSF12) is a TNF superfamily member, originally described as an inducer of apoptosis [15, 16]. TWEAK promotes cell death in some tumor cell lines and monocytes, but may also modulates cell proliferation, inflammation and angiogenesis [17]. TWEAK activates the TWEAK receptor, Fibroblast growth factor-inducible 14 (Fn14) [18]. Both TWEAK and Fn14 are present in the healthy adult kidney and their expression increases in experimental AKI [19-21]. In tubular cells TWEAK induces proliferation, activates Nuclear Factor κB (NF-κB) and promotes the synthesis of inflammatory mediators [21, 22]. In addition, TWEAK induces tubular cell apoptosis in the presence of an inflammatory milieu [20].

TWEAK regulates some ECM-related proteins, namely matrix metalloproteinases (MMP) and tissue inhibitors of MMP (TIMP) in other organ systems [17, 23-26] but there is no information on the role of TWEAK and its receptor in renal fibroblasts.

Increased leukocyte and urinary TWEAK levels and correlation with renal disease activity in human lupus nephritis, a disease characterized by renal scarring if untreated, suggest that TWEAK may contribute to kidney fibrosis [27, 28]. Studies on the effects of TWEAK on kidney fibrosis are relevant for clinical practice since the first human disease in which neutralizing anti-TWEAK antibodies are being

evaluated in an ongoing clinical trial is a kidney disease, lupus nephritis [29]. In order to dissect the effect of TWEAK on kidney fibrosis, we have addressed the hypothesis that chronic exposure to TWEAK contributes to kidney fibrosis in a non-autoimmune model of persistent kidney injury.

We now report that UUO kidneys from TWEAK knockout (KO) mice show decreased myofibroblast number and proliferation, and reduced ECM deposition when compared to UUO kidneys from wild type (WT) mice. Conversely, mice without underlying kidney injury developed kidney fibrosis following systemic TWEAK overexpression. TWEAK direct actions on cultured renal interstitial fibroblasts may underlie the in vivo observations.

2. Material and methods

2.1. Cell lines

TFB is a murine renal interstitial fibroblast cell line originally isolated from SJL mouse kidney and was a generous gift from Eric G. Neilson. These cells have been extensively characterized, and they express the fibroblast specific protein (FSP-1) [14, 30]. Murine embryonic fibroblasts (MEF) were isolated from mice embryos at 10 days post-coitum. Embryos were treated for 30 minutes with 0.25 % EDTA-trypsin (Gibco-BRL, Cheshire, UK) and mechanically homogenized. Before digestion, top of the head (eye and above) and visible innards were removed. The rest of the embryonic tissue is basically fibroblasts at this stage of the embryo development. Immortalized cells were selected by sequent passages (15 to 20 passages) and genotyped by PCR analysis. MEF cells from Fn14 -/- or wild type mice were used as primary culture within the first 5 passages. For cell culture media and reagents see supplementary material.

Cell viability was quantified by the PMS/MTS test in TFBs [31] and by the crystal violet assay in MEFs [32], and apoptosis was quantified by flow cytometry assessment of DNA content of 10,000 cells [33] and characterized by morphology [34]. Protein expression was assessed by flow cytometry [35] in the case of cell surface Fn14 (as detailed in supplementary information) and by Western blot and immunostaining for other proteins. Expression of mRNA was quantified by real time reverse transcription-polymerase chain reaction [36]. Electrophoretic Mobility Shift Assay (EMSA) was carried out to study the transcription factor NF-κB [37]. Details are presented as supplementary material.

2.2. Animal models

Studies were conducted according with the NIH Guide for the Care and Use of Laboratory Animals.

TWEAK KO or wild type (WT) C57BL/6 mice provided by Biogen Idec [22, 38], (12- to 14-week-old) were anesthetized and the left ureter was ligated (n=9/group) or manipulated without ligation (sham, n=5/ group). Peri-operative analgesic was administered to the mice. Animals were sacrificed 48 h, 7, 14 or 21 days after surgery. Kidneys were perfused in situ with cold saline before removal. Half kidney was snap-frozen in liquid nitrogen for RNA and protein studies, and the other half was fixed and paraffin-embedded for histological studies.

Tubular cell death is an early event in UUO, observed from day 1 [39] and was assessed by TUNEL (supplementary material). Inflammatory mediators like chemokines increase from day 1 up to 7-10 days from obstruction and are followed by inflammatory cell infiltration [40-43]. Inflammation and fibrosis was assessed on days 2 through 21 after ureteral obstruction. Histological studies and ELISA and WB from tissue lysates are detailed in supplementary material.

Adenoviral vector expressing soluble TWEAK or control vector expressing green fluorescence protein (GFP) were generated and delivered intravenously as previously described [44]. Vectors were injected in mice without underlying kidney injury. Kidney histology was assessed 7 and 20 days later.

2.3. Statistics

Statistical analysis was performed using SPSS software (SPSS Inc., Chicago, IL). A non-parametric test (U Mann-Whitney) was used for analysis of statistic differences between the groups. A p value <0.05 was considered significant. Results are expressed as mean ± SEM.

3. Results

3.1. Increased kidney Fn14 in experimental UUO

WT and TWEAK KO mice were sacrificed at days 2, 7, 14, and 21 after UUO. Fn14 (Fig. 1.A) and TWEAK (Fig. 1.B) gene expression were upregulated in obstructed kidneys (Fig. 1.B). An increased expression of the receptor that sensitizes to TWEAK actions is the main regulator of the activity of the TWEAK/Fn14 system [16]. Increased Fn14 protein was confirmed in obstructed kidneys by Western blot (Fig 1.C).

3.2. Renal cell apoptosis and interstitial inflammation is delayed in mice lacking TWEAK

The first stages of UUO are characterized by tubular programmed cell death and tubulointerstitial inflammation [39, 45]. In the early stages of UUO, histological tubular injury score (Fig. 2.A) and TUNEL (Fig. 2.B) positive tubular cells were lower in TWEAK KO than in WT kidneys. Kidney

macrophage chemoattractant protein (MCP-1) and RANTES mRNA upregulation was delayed in UUO kidneys from TWEAK KO as compared to WT mice (Supplementary Fig. S1.A). This was associated with lower protein levels as exemplified by lower kidney MCP-1 in TWEAK KO obstructed kidneys at both days 7 and 14 (Supplementary Fig. S1.B). MCP-1 and RANTES recruit macrophages and T cells [21]. Immunohistochemistry showed less interstitial F4/80 macrophages and CD3 lymphocytes in UUO TWEAK KO kidneys than in WT kidneys (Figs. 3.A and 3.B). In the case of CD3, this reduction was observed up to 14 days after obstruction.

3.3. Renal myofibroblast accumulation is delayed in TWEAK KO mice with UUO

The number of myofibroblasts expressing alpha-smooth muscle actin (αSMA) was lower in TWEAK KO than in WT obstructed kidneys at days 7 and 21 after obstruction (Fig. 4). In addition, the number of proliferating αSMA positive myofibroblasts was lower in TWEAK KO as compared to WT obstructed kidneys at day 7 (Fig. 4). However, by day 14 the differences between TWEAK KO and WT obstructed kidneys were attenuated, suggesting that the lack of TWEAK delays the accumulation of myofibroblasts. TWEAK may modulate a first batch of myofibroblast proliferation, while Fn14 and TWEAK expression are high, but at 21 days the reduced myofibroblast numbers in TWEAK-/- mice could reflect the reduced kidney damage. That may explain why the difference in αSMA positive cells observed at 21 days after obstruction in TWEAK KO mice kidneys is not reflected in double αSMA and PCNA positive cells at the same time point.

3.4. Reduced ECM deposition in TWEAK KO mice with UUO

Consistent with the reduced number of αSMA positive cells in TWEAK KO as compared to WT obstructed kidneys 7 days after UUO, collagen type I and fibronectin (FN) mRNA were significantly reduced in TWEAK KO vs. WT obstructed kidneys at this time point, and FN mRNA expression in TWEAK KO kidneys lagged behind that in WT mice (Supplementary Fig. S2). However, although Sirius red-stained interstitial ECM was significantly increased at day 7 in UUO kidneys, no significant differences between WT and TWEAK KO mice were observed. A trend towards lower interstitial ECM deposition was observed in TWEAK KO kidney by day 14, which was statistically significant after 21 days of obstruction (Fig. 5). These results indicate that TWEAK contributes to late-stage ECM accumulation in the progression of kidney fibrosis.

3.5. Overexpression of TWEAK induces structural damage, inflammation and ECM deposition in mouse kidney

TWEAK was overexpressed in mice by infection with an adenoviral vector encoding TWEAK (Adeno-TWEAK), resulting in increased circulating TWEAK levels, as shown previously [44]. For comparison, mice were infected with a control adenoviral vector (Adeno-GFP). Seven and 20 days following adenoviral infection a complete analysis was performed of tubular cell death, inflammatory infiltrates and fibrotic scar. TUNEL (Fig. 6.A) staining and kidney injury molecule 1 (KIM-1) immunodetection (Fig. 6.B) was performed to study the tubular damage, which was increased in TWEAK overexpressing mice. Increase DNA fragmentation was observed at 7 and 20 days after adenovirus infection, and tubular KIM-1 expression increased significantly at 20 days. TWEAK overexpression was also associated to an increased kidney infiltration by F4/80 macrophages (Fig. 6.C) and CD3 lymphocytes (Fig. 6.D). CD3 population increased earlier than F4/80 cells, as observed in the UUO model. Kidneys from mice overexpressing TWEAK showed more myofibroblasts and proliferating myofibroblasts than control mice kidneys, 7 and 20 days after adenovirus injection (Fig. 6.E), along with an increase of interstitial cortex ECM deposition at 20 days (assessed by masson's trichrome and sirius red stainings) (Figs. 6.F and 6.G). At 20 days after infection, fibrotic areas colocalized with KIM-1 and F4/80 immunostainings in mice overexpressing TWEAK (Supplementary Fig. S3).

3.6. TWEAK promotes cultured renal fibroblast proliferation

We studied the biological actions of TWEAK on cultured murine renal interstitial fibroblasts that express αSMA. These renal interstitial fibroblasts expressed Fn14 mRNA (qRT-PCR, not shown) and protein. Fn14 was immunodetected in the lysates (western blot) and on the surface of the TFB cells (flow cytometry) (Supplementary Fig. S4.A).

In serum-deprived cultures, TWEAK dose-dependently increased the number of viable fibroblasts (Fig. 7.A). The effect persisted for up to 72h. An increased number of viable cells may be the result of increased mitosis or decreased cell death. The main effect of TWEAK was to increase the proportion of dividing cells (Fig. 7.B). Nuclear morphology confirmed an increased number of cells undergoing mitosis among cells treated with TWEAK (Supplementary Fig. S4.B). Flow cytometry of DNA content also showed a mild antiapoptotic effect of TWEAK (Supplementary Fig. S4.C).

3.7. The Ras/ERK pathway mediates TWEAK-induced fibroblast proliferation

Ras GTPases have been recently related to renal fibrosis [4, 46]. There is no information concerning the signaling pathways activated by TWEAK/Fn14 in renal fibroblasts. In renal tubular cells TWEAK activates mitogen activated protein kinases (MAPK) such as the extracellular-regulated kinase (ERK) and

p38 [22]. While Ras has not been previously related to TWEAK/Fn14 signaling, MEK/ERK is activated downstream of Ras in different cell systems [47, 48].

The ERK inhibitor PD98059 prevented TWEAK-induced proliferation in renal fibroblasts (Fig. 7.C). Cyclin D1 regulates the transition from the G_1/G_0 phase (quiescence) to the S phase (synthesis) in cell cycle progression. TWEAK increased cyclin D1 protein expression after 8 hours of stimulation, which correlated with the increase in proliferation observed at 24 hours. TWEAK-induced cyclin D1 expression was prevented by PD98059, indicating ERK activation dependency (Supplementary Fig. S5.A). By contrast, the p38 inhibitor SB203580 did not prevent TWEAK-induced proliferation (Fig. 7.C). TWEAK activation of NF-κB has been implicated in cardiac fibroblasts proliferation [49]. However, neither parthenolide nor BAY11-7082, both of them NF-κB inhibitors, prevented TWEAK-induced renal fibroblast proliferation (Supplementary Fig. S5.B).

In renal fibroblasts TWEAK activated Ras within 1 min of stimulation (Fig. 7.D). This fast activation of Ras GTPases by TWEAK is consistent with the known biology of the Ras system. In order to confirm the receptor specificity of this novel TWEAK action, we compared primary cultured MEF cells from Fn14-/- mice to MEF cells from wild type mice, which express Fn14 (Supplementary Fig. S5.C). TWEAK activated Ras in wild type MEF cells after 1 min of TWEAK stimulation but not in MEF cells lacking Fn14 (Supplementary Fig. S5.C). Activation (phosphorylation) of ERK was observed after 15 minutes and 24 h of TWEAK stimulation. Activation of ERK1/2 was blocked by pretreatment with farnesylthiosalicylic acid (FTS), a Ras inhibitor (Supplementary Fig. S5.D). These results provide the first evidence of a TWEAK/Ras relationship.

Preincubation with the Ras inhibitor FTS prevented TWEAK-induced proliferation in renal fibroblasts (Fig. 7.E). To further explore the role of Ras in TWEAK-induced fibroblast proliferation, mouse embryonic fibroblasts (MEFs) deficient in two Ras isoforms, N-Ras and H-Ras (*NH Ras -/-:N-ras*$^{-/-}$/*H-ras*$^{-/-}$) were studied. *N-ras*$^{+/-}$ and *H-ras*$^{+/-}$ MEFs (*NH Ras +/-*) were used as controls because they express completely functional Ras isoforms [46]. Contrary to control MEFs, cell viability was not increased in MEFs lacking Ras in response to TWEAK stimulation for 24h (Fig. 7.F).

These observations support the involvement of Ras/ERK in TWEAK-induced fibroblast proliferation.

3.8. TWEAK reduces collagen type I and FN protein levels in cultured renal fibroblasts and MEFs

In addition to promoting renal fibroblast proliferation in vitro, TWEAK decreased collagen type I and FN proteins following 72 h incubation in TFB cells and MEFs (Figs. 8 and Supplementary Figs. S6).

In MEFs, TWEAK decreased cell-associated collagen type I (Fig. 8.B), and cell-associated and supernatant fibronectin (Figs. 8.C). By contrast, TWEAK failed to decrease ECM proteins in MEFs lacking N- and H-Ras isoforms (Figs. 8.A to 8.C).

In TFB cells TWEAK decreased cell-associated and secreted type I collagen and FN proteins (Supplementary Figs. S6). TWEAK induces p38 phosphorylation at 15 minutes and 24 h in TFB cells, and this was again blocked by preincubation with FTS (Supplementary Fig. S6.A). The p38 inhibitor SB203580 prevented the TWEAK-induced reduction in collagen type I and FN proteins, suggesting the involvement of MAPK p38 (Supplementary Figs. S6.B to S6.D). These results support a role for Ras and p38 in the regulation of ECM production in response to TWEAK. MAPK p38 has been previously related to Ras signaling [50, 51].

3.9. TWEAK activates NF-κB in renal fibroblasts

TWEAK is a proinflammatory cytokine in tubular cells [21]. In renal fibroblasts TWEAK induced NF-κB RelA/p65 translocation to the nucleus (Fig. 9.A) and nuclear NF-κB DNA binding activity (Fig. 9.B). TWEAK also increased MCP-1 and RANTES mRNA (Fig. 9.C). Parthenolide, an inhibitor of NF-κB, prevented NF-κB activation and chemokine mRNA expression (Figs. 9.A to 9.C). This suggests that TWEAK may contribute to renal inflammation through direct effects on both proximal tubular cells [21] and renal interstitial fibroblasts.

4. Discussion

The main finding of this study is that TWEAK deficiency delays the accumulation of myofibroblasts and results in reduced interstitial fibrosis in a model of persistent kidney insult, UUO. In addition, chronic TWEAK overexpression induces interstitial fibrosis in mice without prior underlying kidney injury. TWEAK actions on renal fibroblasts may contribute to the in vivo observations, as TWEAK promotes inflammatory activity and proliferation in renal fibroblast cultures. TWEAK also downregulates ECM proteins in cultured renal fibroblasts and this may contribute to the delayed, rather than early, decreased ECM deposition in vivo. Furthermore, we also report for the first time the involvement of Ras GTPases in TWEAK signaling.

The demonstration that chronic TWEAK overexpression promotes renal fibrosis in mice without underlying kidney injury and that TWEAK deficiency decreases renal fibrosis in the UUO model supports a key role for TWEAK in kidney fibrosis. In this regard, in the UUO model the obstruction is

not released. Thus, protection from fibrosis in TWEAK deficient mice was observed despite the continuous presence of the stimulus for renal fibrosis. This assessment of both the chronic effects of TWEAK on kidney fibrosis as well as of the effects of TWEAK deficiency on a chronic persistent kidney injury model is a key point of the study. These results may be relevant in many human kidney diseases where the kidney insult is persistent.

Dissociation between protection from tubular injury/inflammation and protection from fibrosis has been observed in the UUO model when targeting pro-inflammatory molecules, illustrating the complex relationship between inflammation and fibrosis. Toll-like receptor-2 (TLR2) deficiency decreases tubular apoptosis, inflammation and the activated fibroblast population following UUO [52]. However, TLR2 absence did not modify ECM deposition in obstructed kidneys. Tumor necrosis factor-alpha (TNFα) targeting decreased early apoptosis, inflammation and interstitial volume, but there was no significant difference in the extent of renal fibrosis after UUO [53-56]. TNFα repression of collagen type I gene transcription, as observed in human dermal fibroblasts [57], may prevent further reduction of fibrosis when TNFα is targeted. By contrast, despite a direct suppressive effect on ECM production in cultured fibroblasts, TWEAK promoted fibroblast proliferation and accumulation and the overall effect of TWEAK absence in vivo in UUO was to decrease renal fibrosis.

Our study in the UUO model also provides novel perspective on the contribution of upregulated TWEAK, as well as Fn14, to chronic pathology. In prior studies of AKI, Fn14 gene expression is increased out of proportion compared to TWEAK expression, raising the potential for Fn14 self-oligomerization and activation. Nonetheless, TWEAK targeting protected from AKI induced by folic acid [19, 21] and Fn14 targeting protected from acute injury induced by transient kidney ischemia-reperfusion [19]. Not unexpectedly, given the transient nature of the kidney insult, protection by Fn14 targeting at the time of the transient ischemia-reperfusion period was followed, when perfusion was restored, by decreased residual renal fibrosis. However, these studies did not address whether TWEAK overexpression may promote renal fibrosis, whether TWEAK deficiency might protect from renal fibrosis, whether protection from renal fibrosis might be observed in the presence of a persistent kidney insult or whether TWEAK has direct actions on renal fibroblasts. Our study provides novel information on all these four aspects. Of particular interest, this is the first time that the kidney effects of endogenous TWEAK overexpression have been studied. TWEAK overexpression was enough by itself to trigger kidney fibrosis in mice without underlying kidney disease. Current thinking is that upregulation of Fn14 expression during

kidney injury is a key factor in sensitizing the kidneys to the deleterious effects of constitutively expressed TWEAK. However, these experiments show that a primary increase in TWEAK expression may also result in kidney injury and fibrosis. This finding suggests the pathological relevance of increased levels of kidney TWEAK found in experimental models and leukocyte, kidney and urinary TWEAK found in clinical kidney injury [21, 27, 28]. Specifically, increased urinary TWEAK is correlated with the renal disease activity of lupus nephritis, a condition known to evolve to kidney fibrosis if untreated [28].

Renal fibrosis is characterized by an increased myofibroblast number, which leads to excessive ECM deposition [58]. Ras-dependent activation of ERK and the role of Ras in fibroblast proliferation are well established [4, 59, 60]. Indeed, myofibroblast activation and renal fibrosis following UUO are decreased when H-Ras is deleted [1]. Furthermore epigenetic modifications that silence the Ras inhibitor RASAL1 perpetuate fibroblast activation and fibroblast proliferation [4]. However, TWEAK activation of Ras or induction of renal fibroblast proliferation has not been previously described. In this regard, TWEAK induced renal and embryonic fibroblast proliferation through Ras/ERK signaling. In accordance with this *in vitro* observation, the absence of TWEAK was associated with a reduced kidney myofibroblast population following UUO. TGF-β1-induced MEF proliferation also requires N- and H-Ras isoforms and ERK pathway recruitment [59]. Since TGF-β1 can induce Fn14 expression in fibroblasts [61, 62], these pathways might cooperate to promote proliferation of fibroblasts.

The end-result of fibroblast activation and proliferation is ECM deposition and fibrosis. Since TWEAK deficiency decreased early tubular cell loss, and delayed interstitial inflammation and myofibroblast population after UUO, a decrease in ECM deposition was also expected at early time points. However no difference in Sirius red-stained ECM was observed at day 7. As renal fibrosis increased from 5-10% of the tubulointerstitial area at day 7 to about 25% at days 14-21 in WT mice, a reduced ECM deposition was eventually observed in TWEAK KO. Based on the cell culture data, we hypothesize that the effect of TWEAK deficiency on ECM deposition reflects the balance of TWEAK actions in promoting renal fibroblast expansion and limiting ECM expression. Since TWEAK promotes the proliferation and decreases ECM proteins in cultured renal fibroblasts, the reduction in fibrosis in TWEAK deficient mice may be delayed by loss of TWEAK direct effects on ECM. However, importantly, the net effect is reduced late-stage fibrosis. The effects of TWEAK on renal fibroblasts mirror its effects on other mesenchymal cell types. Thus, TWEAK promotes cell proliferation and inhibits differentiation [17].

Importantly, similar results were obtained with cultured renal fibroblasts and MEFs and in both cell types TWEAK effects were N- and H-Ras-dependent. N- and H-Ras are also negative intracellular regulators of ECM synthesis in response to TGFβ1 in MEFs [59]. Figure 10 shows a model reflecting the potential dual role of Ras in promoting fibroblast proliferation and inhibiting ECM production [59], integrated with our results demonstrating TWEAK-induced changes in fibroblast biology mediated by the Ras pathway that may impact on kidney fibrosis. In addition, TWEAK may have profibrotic actions on other renal cell types, such as tubular cells, not shown in the figure [16].

In addition to fibrosis TWEAK also regulated tubular injury and inflammation in the UUO model. Loss of tubular cells (tubular atrophy) often through tubular apoptosis is a key process in CKD progression [63]. TWEAK cooperates with other inflammatory cytokines to induce tubular cell apoptosis [19-22]. Tubular cell apoptosis is also an early feature of UUO [64, 65]. In this regard, TUNEL+ dead tubular cells were more common in WT type than in TWEAK KO UUO kidneys. TWEAK also regulates renal inflammation in toxic or ischemic AKI [19, 21, 22, 66]. Our results indicate that TWEAK also promotes renal interstitial inflammation after UUO in mice. Renal fibroblasts express chemokines [67]. TWEAK induced NF-κB activation and chemokine secretion in renal fibroblasts. TWEAK contribution to interstitial inflammation in UUO might thus be mediated through NF-κB signaling and chemokine secretion in renal tubular cells and fibroblasts. Indeed, NF-κB is a key mediator of renal inflammation [68] and NF-κB targeting decreased inflammatory cytokines and ECM proteins in murine UUO [69]. In addition, therapeutic targeting of CCR2, the MCP-1 receptor, reduced macrophage infiltration and interstitial fibrosis following UUO in mice [70].

5. Conclusions

TWEAK has Ras-dependent pro-proliferative and NFκB-dependent proinflammatory actions on kidney fibroblasts and TWEAK deficiency is associated with delayed accumulation of myofibroblasts and reduced late stage ECM deposition in an experimental model of persistent kidney injury. Furthermore, overexpression of TWEAK in the absence of underlying kidney disease resulted in kidney fibrosis. Potential anti-fibrotic actions of TWEAK targeting on human kidney disease where the kidney insult is persistent should be explored.

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