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*Review*

# Kidney Fibrosis and Matrix Metalloproteinases (MMPs)

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**Abstract**

Chronic kidney disease (CKD) is a disorder that causes changes in both the structure and function of the kidneys, causing complications such as hypertension, edema, and oliguria. Renal fibrosis is also a common pathological feature of CKD. Matrix metalloproteinases (MMPs) are endopeptidases that degrade extracellular matrix (ECM) proteins. The proteinase domain consists of a zinc ion in the active site, which contributes to its stabilization with another zinc and three calcium structural ions. Many cellular processes are controlled by MMPs, such as cell–cell interactions and various signaling pathways, while they are also involved in degrading substrates on cell surfaces. Tissue inhibitors of metalloproteinases (TIMPs) are key regulators of metalloproteinases, and both are involved in regulating cell turnover, the regulation, and the progression of fibrosis and apoptosis in the tissue. MMPs play a role in renal fibrosis, such as the tubular cell epithelial–mesenchymal transition (TEM), activation of resident fibroblasts, endothelial–mesenchymal transition (EndoMT), and pericyte–myofibroblast transdifferentiation. This review aims to show the mechanisms through which MMPs contribute to renal fibrosis, paying particular attention to MMP-9 and the epithelial–mesenchymal transition.

**Keywords:** cell signaling; chronic kidney disease; renal fibrosis; apoptosis; TIMPs; renal tubular epithelial cells

## 1. Introduction

Chronic kidney disease (CKD) is a pathological condition that occurs when the structure and function of the kidney changes, which in turn can lead to complications, such as hypertension, edema, and oliguria, as well as high levels of serum creatinine or blood urea nitrogen [1]. Kidney fibrosis (KF), the most prevalent histological hallmark of CKD, is a condition marked by an increased deposition of extracellular matrix (ECM) in the interstitium. KF disrupts kidney architecture and exacerbates kidney dysfunction [2]. Nowadays, it is widely accepted that KF involves an epithelial–mesenchymal transition (EMT) of renal tubular epithelial cells prompted by abnormal activation of myofibroblasts and subsequent ECM deposition. Transforming growth factor $\beta$ (TGF-$\beta$) regulates the most important processes of this complex mechanism. The first phase of EMT is characterized by both the loss of stability of the various cell–cell contacts and the disruption of apical–basal polarity. This destabilization is caused by stimulating tubular epithelial cells through cytokines, such as the epidermal growth factor (EGF), basic fibroblast growth factor (FGF-2), and TGF-$\beta$1. These cytokines are secreted mainly by infiltrating mononuclear cells, partially reducing epithelial membrane junctions and tight junction proteins, such as E-cadherin and zonula occludens. In the intermediate phase, there is a shift towards the expression of new mesenchymal proteins, including $\alpha$-smooth muscle actin ($\alpha$-SMA) and fibroblast-specific protein 1 (FSP-1) synthesis. In this stage, there is a protein reorganization of the cytoskeleton and an upregulation of both matrix metalloproteinases (MMP-2 and MMP-9) and enlarged spindle-shaped myofibroblasts, which show increased capacity for cell migration and invasion into the tubular interstitium. The observation that epithelial markers are expressed in interstitial cells in biopsies from End Stage Renal Disease (ESRD) patients suggests that EMT is involved in KF. The detection of FSP-1 in the tubular epithelium is another sign of EMT [3]. In human biopsy samples, there is a correlation between tubular cells presenting EMT markers, the appearance of interstitial fibrosis, and functional involvement [4]. However, there is a lack of support from *in vivo* cell-fate tracking experiments in showing the direct involvement of EMT in contributing to the myofibroblast population [5]. Recent studies have shown that a partial EMT (pEMT—wherein tubular epithelial cells acquire one or two pheno-

typic markers without leaving the local microenvironment) was induced by Snail1 or Twist-related protein 1 (TWIST1) in tubular epithelial cells, leading to the onset of fibrosis. Consistent with this, pEMT has been observed in clinical samples and was found to correlate with the progression of fibrosis in renal allografts [5]. Inflammatory processes, beginning with the infiltration and activation of macrophages, are considered to be major factors leading to fibrotic diseases [6]. The recruitment of macrophages in the glomerular or tubular interstitium is necessary to promote innate immune responses and important defensive and disruptive reactions in renal disease. The subsequent macrophage infiltration leads to kidney structure disruption and tissue fibrosis development. Glomerular and interstitial macrophages, although believed to be associated with the development of interstitial fibrosis, play an important role in stromal remodeling during tissue repair [7,8]. In addition to releasing inflammatory cytokines, activated macrophages also secrete matrix metalloproteinases (MMPs), including high levels of MMP-1, -3, -7, -9, -10, -12, -14, and -25, along with lower levels of MMP-2, -3, -8, -10, -11, and -12 [9]. An inflammatory injury in the kidney is also the result of these MMPs contributing to the degradation of the extracellular matrix [10]. The profibrotic changes in tubular epithelial cells [11] and the proteolytic activation of osteopontin in kidney fibrosis led to macrophage recruitment, indicating that MMP-9 causes fibrosis [12].

## 2. Matrix Metalloproteinases

Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidase enzymes. They function to remodel the ECM and are fundamental to tissue development and homeostasis. Previously, MMPs have been considered to have a negative impact on human health; today, their role in physiology and general health is being progressively clarified [11–13]. In fact, MMPs regulate many physiological processes. These include angiogenesis, embryogenesis, morphogenesis, and wound healing, although they may also induce various pathological conditions, similar to myocardial infarction, fibrotic diseases, osteoarthritis, and cancer.

Some studies demonstrated that specific increases in MMP could play a role in arterial remodeling, aneurysm conformation, venous dilatation, and pulmonary embolism diseases [14,15]. Many MMPs are secreted as pro-MMPs, requiring activation through prodomain cleavage, typically by plasmin or other MMPs. Indeed, 24 *MMP* genes have been identified in the human genome. Notably, *MMP-23A* is a duplicated version of *MMP-23B* and is likely a pseudogene [16,17]. Each MMP contains the following components: (1) an N-terminal signal peptide that directs the enzyme to the secretory pathway and (2) a prodomain with a conserved PRCGXPD sequence responsible for the latency of the enzyme bound to a conserved HEXGHXXGXXH motif [18]. Four twisted $\beta$-sheets are present in the hemopexin-like domain's 'propeller', allowing substrate recognition [14] Together, the hinge region and the hemopexin-like domain break down substrate proteins for further degradation. The C-terminal domain interacts with Tissue inhibitors of metalloproteinases (TIMPs) and participates in substrate recognition [19]. MMPs are also able to cleave a range of non-ECM substrates, such as cell adhesion molecules (cadherins and integrins), as well as growth factors and their receptors (TGF-$\beta$), fibroblast growth factor receptor 1 (FGF-R1) [20]. Tissue inhibitors of metalloproteinases or TIMPs, which have been identified in vertebrates, control the activities of MMPs. There are four TIMP members, TIMP-1, -2, -3, and -4, that inhibit all MMPs, except for TIMP-1, which does not inhibit MMP-14, -16, or -24 [21]. TIMPs can induce intracellular signaling such as proliferation, angiogenesis, and apoptosis [22]. Based on substrate and sequence homology, MMPs are classified into six distinct groups: collagenases (MMP-1, -8, -13, -18), gelatinases (MMP-2, -9), stromelysins (MMP-3, -10, -11), matrilysins (MMP-7, -26), membrane-bound matrix MMPs (MMP-14, -15, -16, -17, -24, -25), and the "other MMPs" (MMP-12, -19, -21, -23a, -23b, -28) [21]. TIMPs are also classified numerically from TIMP-1 to TIMP-4 [23]. Gelatinases are responsible for cleaving and further degrading triple-helical fibrillar collagens. They also degrade basement membrane constituents; for example, MMP-2 (but not MMP-9) can degrade laminin and fibronectin [24]. Stromelysins degrade extracellular matrix proteins but not triple-helical fibrillar collagens. Membrane-bound MMPs activate many proteases and growth factors. All TIMP molecules have 190 amino acids, which are folded into two distinct domains. These domains comprise a larger N-terminal domain (approximately 125 amino acid residues) and a smaller C-terminal domain. Three conserved disulfide bonds stabilize each domain. The N-terminal domain alone can fold individually and is fully functional for MMP inhibition. While the function of the C-terminal domain is not fully understood, it has been shown to tightly bind to the hemopexin domain of latent MMPs [20]. Regulation of MMP activity may occur through several mechanisms: (1) gene expression, by both transcriptional and posttranscriptional modifications; (2) compartmentalization that occurs by incorporating cell-specific and tissue-specific release of extracellular MMPs; or (3) through the zymogen activation of the pro-enzyme forms of MMPs achieved by the removal of the 80–84 residue terminal domain; (4) inhibition by TIMPs. While TIMPs 1–4 all share some sequence similarity, TIMPs 2, 3, and 4 are more identical than TIMP-1. Furthermore, TIMP-2 is unique in that it can both inhibit and activate MMPs [25]. All TIMPs inhibit the pro- and active forms of the MMPs, although with relatively low selectivity, and with which they form tight non-covalent complexes. TIMP-1 has shown low inhibitory activity against MMP-19 and membrane-bound MMP-14, -

16, and -24 while displaying greater potency against MMP-3 and MMP-7 than TIMP-2 and TIMP-3 [26]. TIMP-2 has an effective inhibitory activity against all MMPs. Similarly, TIMP-3 inhibits all MMP members while extending its inhibitory profile to include members of the ADAMs (a disintegrin and metalloprotease) family of metalloproteinases. MMP-1, -2, -3, -9, -11, -13, -14, -24, -25, -27, -28 and TIMP-1, -2, -3, -4 are expressed in the kidney [27]. The distribution of MMPs and TIMPs in human kidney tissue and their localization at the subcellular level and immunohistochemical study are available at https://www.proteinatlas.org/ [28].

*Matrix Metalloproteinases in Kidney Fibrosis*

The accumulation of proteins in the extracellular matrix and an increased activity of myofibroblasts cause the development and progression of renal fibrosis. In this process, MMPs and TIMPs play a very important role, and this is demonstrated, for example, by the role that MMP-2 and MMP-9 play in particular. The latter causes the EMT of tubular cells. It favors the activation of resident fibroblasts, endothelial–mesenchymal transition (EndoMT), and pericyte–myofibroblast transdifferentiation [29]. Many cells, including mesangial, endothelial, epithelial, collecting duct tubular cells, macrophages, and fibroblasts, express MMP-2 and MMP-9, which could directly induce the entire course of renal tubular cell EMT, as demonstrated by *in vitro* studies [30,31]. MMPs are usually responsible for the degradation of mature ECM proteins, such as collagens and fibronectin. TIMPs decrease MMP activity and reduce ECM degradation rates. The accumulation of collagen types I, III, and IV in the glomeruli, interstitial tissue, and vessels causes progressive renal scarring. TGF-$\beta$1 induces increased MMP-9 expression and decreased TIMP-1 expression, altering ECM homeostasis and leading to kidney fibrosis. This process has been recognized as an essential component of EMT activation and permits the migration and invasion of newly transformed mesenchymal cells in the interstitial space, resulting in fibrosis progression through extracellular matrix deposition [32]. However, it should be noted that some investigators have reported an increased expression of TIMP-1 by TGF-$\beta$1. One study observed the induction of TIMP-1 by TGF-$\beta$1 via the proximal promoter activator protein 1 (AP1) site while having the opposite effect on MMP-1 expression [33]. Another study investigating animal models of intestinal fibrosis observed increased phosphorylation of Smad-2 and Smad-3 proteins upon treatment of myofibroblasts with TGF-$\beta$1 and increased TIMP-1 levels [34]. Akool *et al*. [35] also noted TIMP-1 expression induced by nitric oxide (NO) in mesangial cells—NO exerted this effect through the TGF-$\beta$1/Smad-2 signaling pathway. Yang and co-workers underlined the importance of MMPs in disrupting the tubular basement membrane and demonstrated that the tubular basement membrane integrity was preserved due to the indirect reduction of MMP-9 activity in mice with tissue-type plasminogen activator (t-PA) deficiency. This reduction caused a decrease in tubular cell EMT and kidney fibrosis in obstructive nephropathy. Furthermore, Fernandez-Patron and Leung [29] reported that MMP-2 could reduce inflammation because it antagonizes phospholipase A2 (PLA2). Additionally, MMP-2 may facilitate the activation of the MMP-1, MMP-9, and MMP-13 zymogens. MMP-2 performs many pathophysiological functions, creating a close association between fibrosis and inflammation. In fact, many studies report that it is produced in many nephron sites (such as the glomerular mesangial cells, renal tubular epithelial cells, and interstitial cells) and can play different roles during the progression of CKD. The rapid progression of CKD is also due to the ability of MMP-2 to degrade the basement membrane, alter the glomerular filtration membrane, activate TGF-$\beta$1, and promote the phenotypic transformation of tubular epithelial cells [36]. Deficient MMP-2 activity leads to renal tubular atrophy, fibrosis, and excessive ECM deposition in the basement membrane and interstitium, a sign of advanced chronic kidney disease. This not only contributes to ECM degradation and the development of chronic inflammation but also leads to insufficient degradation of endothelin-1, adrenomedullin, and calcitonin gene-related peptides by MMP-2, exacerbating the intrarenal arteriolar spasms and sclerosis [30]. MMP-7 may also contribute to renal fibrosis mainly through EMT transition, TGF-$\beta$ signaling, and ECM accumulation [31]. MMP-7 mediates EMT through three distinct pathways: Through the first mechanism, MMP-7 causes ECM destruction by cleaving collagen type IV and laminin. Secondly, MMP-7 mediates E-cadherin degradation, leading to a disruption of tubular epithelial integrity. The last process induces the expression of the Fas ligand (FasL) in interstitial fibroblasts, leading to apoptosis [31]. MMP-7 expression in the kidney is also upregulated by age, and its level correlates with renal healing [37]. The thickening of the basement membrane and mesangial expansion precede the onset of renal scarring and are favored by MMP-7. The latter also enzymatically cleaves numerous extracellular matrix proteins such as type IV collagen, laminin, fibronectin, proteoglycans, and entactin. Interestingly, in a rat model of age-associated renal interstitial fibrosis, MMP-7 expression is significantly elevated in fibrotic samples, whereas non-fibrotic samples do not show this expression profile. Furthermore, the expression of MMP-7 strongly correlates with the fibrosis degree [38].

Zhang *et al*. [39] investigated the role of TIMP-1 in fibrosis using a transgenic TIMP-1 mouse model and observed that intercellular adhesion molecule-1 (ICAM-1) expression was increased in these mice partly due to MMP-9 inhibition. They also noted that the increase in ICAM-1 level led to increased renal macrophage infiltration, kidney collagen levels, and renal fibrosis, which correlated with the age of the transgenic animal. Zhang *et al*. [39] sug-

gested that an imbalance between TIMPs/MMPs and inflammation could lead to fibrosis. A recent study utilizing TGF-$\beta$1 transgenic mice also demonstrated that renal fibrosis was correlated with TIMP-1 expression, and using an anti-TIMP1 neutralizing antibody ameliorated glomerulosclerosis and tubulointerstitial fibrosis [40]. They also observed increased TIMP1 levels in human kidneys with focal segmental glomerulosclerosis, suggesting a correlation between TIMP-1 and fibrosis. Other groups have also noted a correlation between TIMP-1 and renal fibrosis: Increased expression of TIMP-1 mRNAs in glomerular resident cells and tubular epithelial cells have been associated with interstitial injury [41]; a reduction in TIMP-1 mRNA levels was associated with improved symptoms of nephritis in a murine model of lupus nephritis [42]; decrease in TIMP-1 expression was also accompanied by a reduction in renal collagen expression and glomerulosclerosis in diabetic rats treated with the soluble guanylate cyclase activator cinaciguat [43].

## 3. MMP Gene Polymorphisms and Kidney Disease

Gene polymorphisms can influence both the expression and activity of MMP-9. Two functional polymorphisms affecting the transcriptional activity of the *MMP-9* gene were identified in the promoter region, namely the *C-1562T* (*rs3918242*) and microsatellite $(CA)_{n13-25}$ (*rs3222264*) polymorphisms [44]. In particular, the microsatellite (C*A*)*n* region located near the -90 positions functions as a binding site for a specific DNA regulatory protein and also promotes the unwinding of the DNA helix and the transcription process by allowing the DNA to adopt a Z structure. The longest repeat alleles of the microsatellite were correlated with the highest activity, as demonstrated *in vitro*. The single nucleotide polymorphism *C-1562T* prevents the nuclear repressor protein from binding to the *MMP*-9 gene promoter region, resulting in increased MMP-9 expression, as observed in some *in vitro* studies [45,46]. A large Japanese cohort study has highlighted that the *MMP-9-1562T/279R/668Q* haplotype reduced the risk of CKD compared with the *MMP-9-1562C/279R/668R*. Furthermore, the *MMP-9-1562TT/279RR/668QQ* genotype combination has been linked to a decreased CKD risk compared with the major combination of homozygous allele *MMP-9 -1562CC/279RR/668RR*. The association of these genotypes supports the idea that they play a protective role in kidney disease and could cause an increase in MMP-9 expression [47]. Earlier studies have also shown that the *CC* genotype for the *-1562 C/T* (*rs3918242*) polymorphism in the promoter region causes a significant increase in serum MMP-9 in hemodialysis (HD) patients compared with the *CT* genotype [48]. Conversely, in nephrolithiasis, the *-1562 TT* (*rs3918242*) genotype, but not the *Q279R* (*rs17576*) single nucleotide polymorphism (SNP) of the *MMP-9* gene, was associated with a higher risk of lithiasis [49]. It has been shown, in a 3-year study of HD patients, that the *2G/2G* homozygous genotype for MMP-1 was associated with increased mortality in contrast to the *6A/6A* homozygous genotype for MMP-3 in the same population [48]. These findings suggest that investigating the genetic polymorphisms in *MMP* genes might help identify high-risk populations that can benefit from a targeted treatment approach.

## 4. MMP-9 Signaling

Identifying the molecular components involved in the signal transduction pathways activated by MMPs may allow interventions to be developed by modifying their activity. However, there have only been a few studies investigating the signaling pathways involved in the cellular actions of MMP-9, with several molecules identified as playing a role in MMP-9 signaling, as discussed in the following subsections.

### *4.1 p38 Mitogen-Activated Protein Kinase and NF-kB*

In addition to its proteolytic action on the ECM, MMP-9 activates interleukin-1$\beta$ [50] and plays a role in cell signaling. Notably, a study by Al-Sadi *et al*. [51] reported that MMP-9, at clinically relevant concentrations, rapidly upregulated p38 mitogen-activated protein kinase (MAPK) levels in Caco-2 cells. This, in turn, led to an increase in myosin light-chain kinase gene and protein levels. However, no details on the mechanism through which MMP-9 caused activation of the MAPK were given. Furthermore, it was noted that MMP-9 did not activate the extracellular-signal-regulated protein kinases (ERK)1/2 and c-jun N-terminal kinase (JNK) members of the MAPK family [51]. In a subsequent report, the same authors observed that p38 activation led to the activation and subsequent nuclear translocation of the proinflammatory transcription factor nuclear factor kappa B (NF-$\kappa$B) [52]. Notably, the p38$\alpha$ isoform may regulate MMP-9 expression [53]. While these studies were conducted in non-renal cells, it is possible that MMP-9 could have similar effects in renal cells. In such a scenario, the p38/NF-$\kappa$B axis would lead to inflammation and increase MMP-9 levels, creating a positive feedback loop that could intensify the inflammatory milieu.

### *4.2 Collagen Degradation Products*

MMP-9 action on the ECM leads to the production of collagen fragments, such as the tripeptide N-acetyl Pro–Gly–Pro (Ac-PGP), a neutrophil chemoattractant [54]. AcPGP also binds to neutrophils to induce further production of MMP-9 via an ERK1/2–MAPK-dependent pathway [22], creating a positive feedback loop. AcPGP binding appears to be on the CXC motif chemokine receptor 1 and CXC motif chemokine receptor 2 (CXCR1 and CXCR2) receptors on neutrophils and may interact with these receptors wherever they are present in other cells, including renal cells [55].

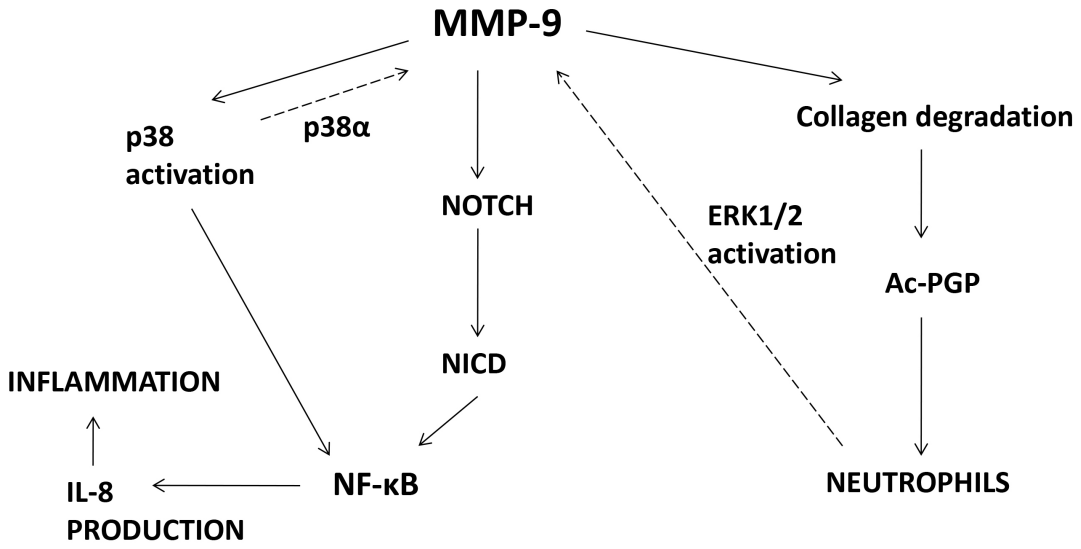


**Fig. 1. Signaling molecules affected by MMP-9.** MMP-9, matrix metalloprotease-9; Ac-PGP, N-acetyl–proline–glycine–proline; NICD, Notch intracellular domain; IL-8, interleukin-8; NF-$\kappa$B, nuclear factor-$\kappa$B; ERK1/2, extracellular signal-regulated protein kinase 1/2; p38, p38 mitogen-activated protein kinase.

*4.3 Notch Signaling*

Zhao *et al*. [56] reported the induction of Endo-MT in human kidney glomerular endothelial cells (HKGECs) by recombinant MMP-9 (rMMP-9). It was observed that rMMP-9 caused a decrease in Notch1 receptor levels while simultaneously increasing the levels of the Notch intracellular domain (NICD) [56]. The Notch signaling pathway consists of the Notch receptors together with several ligands, although in the canonical signaling pathway, Notch is cleaved to form the NCID, which is then transported to the nucleus to regulate transcription of its target genes [57]. Zhao *et al*. [58] also demonstrated that using a Notch pathway inhibitor, a $\gamma$-secretase inhibitor (GSI), inhibited the rMMP-9-induced morphological changes and decreased NICD levels in HKGECs. In a following study performed by the same group, Zhao *et al*. [58] used primary mouse renal peritubular endothelial cells (MRPECs) to observe Endo-MT following induction by MMP-9. Once again, rMMP-9 caused an increase in NICD, whose levels were decreased by GSI. Furthermore, in MRPECs isolated from MMP-9 knockout (KO) mice and subjected to TGF-$\beta$1, higher levels of Notch1 and lower levels of NICD were observed. Finally, Zhao *et al*. [58] performed unilateral ureteral obstruction (UUO) of the kidneys in MMP-9 KO and wild-type mice to induce fibrosis. Zhao *et al*. [58] noted that the MMP-9 deficient UUO kidneys had less fibrosis, and levels of hairy/enhancer-of-split related with YRPW motif 1 (Hey-1), a downstream target gene in the Notch signaling pathway, decreased, implicating Notch signaling in the MMP-9 mechanism of action. Interestingly, it is worth mentioning that a complex interaction between Notch and NF-$\kappa$B exists, which could activate the NF-$\kappa$B pathway (Fig. 1) [59].

## 5.MMP-9 as a Promising Therapeutic Target

Several studies have indirectly demonstrated the involvement of MMP-9 in renal fibrosis through the inhibition of t-PA (as t-PA is an inducer of MMP-9) and through knockout mice. To determine the role of MMP-9 *in vivo*, direct inhibition of MMP-9 activity is the recommended approach [60]. Indeed, a study [60] has shown that inhibiting the activity of the MMPs early, particularly of MMPs-2, 3, and 9, seems to protect against Alport's disease in mice deficient in the $\alpha$3(IV) chain of type IV collagen. The study showed that late-stage inhibition of MMP activity results in more rapid disease progression, which is associated with interstitial fibrosis and early mortality. Some macrophage phenotypes may contribute to fibrosis, and evidence suggests that neutralizing proinflammatory macrophages may prevent renal fibrosis [61]. *In vitro* studies have shown that MMP-9 cleaves osteopontin, inducing migration of macrophages, and that MMP-9 from both tubular epithelial cells (TECs) and macrophages induces EMT in tubular cells [12]. In the same study, a unilateral ureteral obstruction murine model of renal fibrosis was used to show that TECs were the main source of MMP-9 in the early stages of UUO, whereas TECs, macrophages, and myofibroblasts were the main sources in the later stages. Using a murine renal tubular epithelial cell line, a study investigated the effects of incubating

these cells in an activated macrophage-conditioned medium (AMCM) containing MMP-9 secreted by Lipopolysaccharide (LPS)-stimulated macrophages. They noted that the AMCM could induce EMT in tubular cells, and this effect was inhibited in the presence of MMP-9 inhibitors and by immunoprecipitation of MMP-9 from the AMCM [11]. A recent study also demonstrated how diosmin has a potential multicomponent molecular mechanism of action in treating renal fibrosis. The study indicates that caspase 3, MMP-9, annexin A5, and heat shock protein 90-alpha family class A member 1 could be important direct targets of diosmin and that the MAPK, Ras, phosphatidylinositol-3 kinase (PI3K) Protein kinase B (Akt), FoxO, and hypoxia inducible factor-1 (HIF) signaling pathways could play a major role in its mechanism of action. Further studies on diosmin in treating renal fibrosis should be performed to demonstrate its efficacy [62]. However, as evidence for the role of MMP-9 in renal fibrosis increases, understanding its secretion, regulation, and mechanism of action is necessary to develop therapeutics to regulate its action.

*Control of Secretion and Expression*

Bellosta *et al*. [63] reported that the hydroxyl methyl glutaryl coenzyme A (HMG-CoA) reductase inhibitor (statin), Fluvastatin, could reduce MMP-9 activity by up to 50% in human macrophages. Furthermore, they hypothesized that the statin interfered with the prenylation of small Ras-like guanine nucleotide-binding proteins (GTPases) that regulate membrane traffic involving endocytic and exocytic processes, leading to reduced MMP-9 secretion by macrophages. Other studies have also reported similar effects of various statins on MMP-9 secretion by different cell types and macrophages [64–66]. However, other workers have observed that under certain conditions, some statins may increase MMP-9 levels in macrophages [67]. Surprisingly, one report attributes the beneficial effects of a hydrophilic statin, rosuvastatin, on preventing an increase in MMP-2 and a decrease in MMP-9 in hypertensive stroke-prone rats [68]. Confocal microscopic analysis of kidney sections from Zucker diabetic rat kidneys showed increased MMP-9 in the glomeruli, particularly in the parietal epithelial cells (PECs) [69]. Albumin administration of cultured primary PECs resulted in a dose-dependent increase in MMP-9 in the culture medium, together with increased phosphorylation (activation) of extracellular-signal-regulated kinases (ERKs)1/2 of the mitogen-activated protein kinase (MAPK) family. Other kinases investigated included Akt and p38 MAPK, which showed no increase in their phosphorylation status. Furthermore, using the inhibitor U0126, which inhibits the upstream kinases, MAPK kinase1/2 (MEK1/2), of the ERK1/2 kinases, resulted in lower MMP-9 levels. Many factors positively regulate MMP-9 gene expression including transcription factors E-26 (Ets), NF-$\kappa$B, polyomavirus enhancer-binding protein 3 (PEA3), activator protein 1 (AP-1), specificity protein 1 (Sp-1) and serum amyloid A activating factor (SAF)-1 [70], all of which may be considered as viable targets for MMP-9 regulation, together with the signal transduction pathways that modulate their activity. However, more research must be undertaken to delineate these pathways in renal cells and determine the conditions under which they are activated.

## 6. Conclusions

Renal fibrosis is caused by excessive accumulation of the extracellular matrix (ECM), and its pathogenesis is a progressive process that leads to end-stage renal disease. The accumulation of ECM and uncontrolled matrix degradation play an important role, mainly involving MMPs and TIMPs, which are particularly important in the development and progression of renal glomerulosclerosis and tubular interstitial fibrosis. MMPs have traditionally been considered antifibrotic factors due to their proteolytic activity and extracellular matrix degradation. However, a decrease in MMP proteolytic activity or an increase in MMP tissue inhibitors (TIMPs) is thought to be responsible for extracellular matrix accumulation and fibrosis [27]. In particular, in this review, we have highlighted the important role that MMP-9 plays in up-regulating pathways involved in renal fibrosis [58]. Therefore, further studies are needed to demonstrate that inhibition of MMP-9 pathways could represent a therapeutic strategy for treating renal fibrosis in chronic kidney disease. In addition, it would be interesting to understand the intracellular signaling pathways involved in the action of MMPs, particularly the role of MMPs and TIMPs in the kidney, to elucidate the underlying mechanisms and develop biomarkers and therapeutic targets for renal fibrosis.

## Author Contributions

ALR, RS and MA designed the study. ALR, RS, TF, GCr, AB, GCo, DB, YB, NI, DC, AM and MA performed the search and analyzed the data. ALR, RS, AM and MA wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

## Ethics Approval and Consent to Participate

Not applicable.


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## Conflict of Interest

The authors declare no conflict of interest. Raffaele Serra is serving as one of the Editorial Board members of this journal. We declare that Raffaele Serra had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to Amancio Carnero Moya.

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