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Hepatocyte growth factor: New arsenal in the fights against renal fibrosis?

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Hepatocyte growth factor (HGF) has emerged as a potent, endogenous antifibrotic factor that shows an impressive efficacy in ameliorating tissue fibrosis in a wide variety of animal models. Herrero-Fresneda *et al.* provide new evidence demonstrating that intramuscular injection of HGF gene reduces mortality, inflammation, and renal fibrosis in chronic allograft nephropathy.

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After injury, tissues in multicellular organisms often have a reparative and regenerative response in an attempt to repair the damaged structure and to restore the lost function. An intensive search for the substance responsible for triggering liver regeneration after partial hepatectomy led to the discovery of hepatocyte growth factor (HGF) about two decades ago.[1] In retrospect, the name of HGF merely tells a small proportion of its function, because HGF is widely expressed in many different organs, including the kidney, and possesses multiple biological activities. Besides its well-described regenerative property, studies from numerous laboratories have indicated that HGF is an endogenous, antifibrotic factor that is capable of ameliorating fibrotic lesions and preserving organ functions in a wide variety of experimental animal models.[2]

Herrero-Fresneda and colleagues[3] (this issue) provide new evidence for a therapeutic effect of exogenous HGF in rat chronic allograft nephropathy, a model characterized by chronic inflammation and progressive tissue scarring. Delivery of human HGF, via intramuscular injection of its gene, reduces animal mortality, inhibits inflammatory infiltration, and ameliorates renal fibrosis. In the context of numerous previous reports,[2,4,5] this comprehensive study offers a new affirmation of a potential utility of HGF as a therapeutic agent in combating renal fibrotic diseases.

The rationale and mechanisms behind the beneficial effect of HGF in renal fibrosis appear to be quite compelling (Figure 1). HGF expression is increased initially after various insults, perhaps in an effort to repair the damage. However, chronic exposure to injurious stimuli eventually leads to the downregulation of HGF, along with a progressively increased transforming growth factor-β1 (TGFβ1). As a result, the ratio of HGF to TGFβ1 in a chronically injured tissue is dramatically shifted in favor of a fibrotic milieu. Both HGF and TGFβ1 can induce their own expression via a positive autoinduction mechanism *in vivo* (Figure 1), and they reciprocally inhibit each other. In this regard, supplementation of exogenous HGF would lead to a restoration of the balance between HGF and TGFβ1 in the diseased kidney, thereby eradicating the fibrogenic actions of TGFβ1. We have recently carried out a series of experiments that offers significant insights into the mechanisms by which HGF antagonizes TGFβ1 action. Those studies unravel that HGF effectively intercepts TGFβ1–Smad signaling by diverse mechanisms in different types of kidney cells.[2] HGF inhibits myofibroblastic activation from quiescent interstitial fibroblasts by blocking the activated Smad from undergoing nuclear translocation. In glomerular mesangial cells, HGF induces expression of the Smad corepressor TGIF by stabilizing its protein against degradation.[6] HGF has also been shown to block tubular epithelial-to-mesenchymal transition by inducing gene expression of the Smad corepressor SnoN.[7] Collectively, HGF is able to antagonize TGFβ–Smad signaling in diverse types of kidney cells and virtually eliminates the activation of fibrogenic cells in the injured kidney.

The beneficial action of HGF in chronic kidney diseases may go beyond the inhibition of TGFβ signaling. The results of Herrero-Fresneda *et al.*[3] as well as those from previous reports reveal that HGF is able to attenuate renal infiltration of inflammatory cells in different models of chronic kidney diseases.[3,8] As inflammation is a major pathologic feature that often occurs before any significant fibrotic lesions, the anti-inflammatory activity of HGF probably represents another mechanism leading to amelioration of renal pathologic lesions. HGF inhibits the expression of numerous proinflammatory cytokines, such as tumor necrosis factor-α, interferon-γ, monocyte chemoattractant protein-1, and interleukin-12b, and blocks infiltration of monocytes/macrophages and T cells into the kidney.[3,8] Emerging evidence suggests that HGF may target multiple cells during the different stages of inflammation. For instance, HGF is known to potently suppress dendritic-cell function and downregulate the antigen-induced immune response both *in vivo* and *in vitro*.[9] HGF also targets vascular endothelial cells by suppressing E-selectin expression, thereby attenuating the E-selectin-mediated monocytic adhesion to endothelial monolayers.[10] Finally, HGF inhibits chemokine expression in kidney parenchymal cells and thus decreases chemokine gradient that is critical in attracting inflammatory cells.

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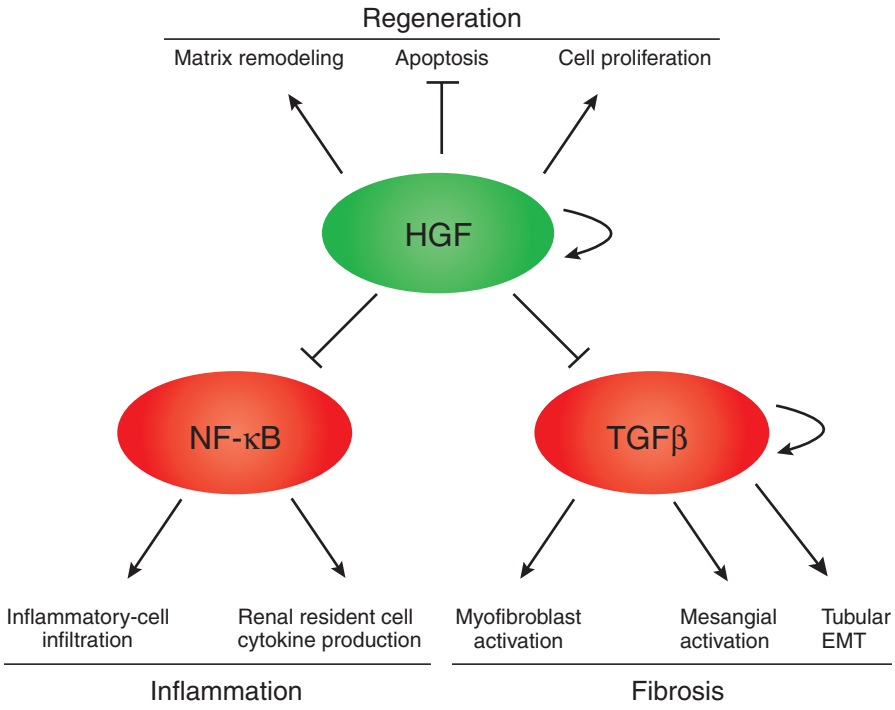

Figure 1 | Anti-inflammatory, antifibrotic, and pro-regenerative actions of hepatocyte growth factor in chronic kidney disease. Hepatocyte growth factor (HGF) elicits its antifibrotic and anti-inflammatory effects by blocking TGFβ–Smad and nuclear factor-κB (NF-κB) signaling. By intercepting TGFβ–Smad signaling through diverse mechanisms, HGF inhibits myofibroblastic activation from renal interstitial fibroblasts and glomerular mesangial cells and blocks tubular epithelial-to-mesenchymal transition (EMT). Both HGF and TGFβ autoinduce themselves *in vivo*, whereas they reciprocally inhibit each other. HGF, via inhibition of NF-κB signaling, also exerts an anti-inflammatory activity by blocking inflammatory-cell infiltration and inhibiting cytokine expression. In addition, HGF promotes kidney regeneration by accelerating matrix remodeling and cell proliferation and inhibiting apoptosis.

The anti-inflammatory action of HGF is believed to be mediated by its inhibition of nuclear factor-κB signaling (Figure 1), although the molecular details remain to be worked out.

As a regenerative factor, HGF possesses a unique ability to promote renal regeneration, which could lead to the recovery of kidney structure and function after chronic injury (Figure 1). This implies that HGF not only hampers the progression of renal disease but may potentially induce a regression of the established renal fibrosis as well. Along this line, it is of interest that HGF is capable of modulating matrix remodeling by influencing the matrix metalloproteinases and their inhibitors. In addition, HGF is a survival factor for tubular epithelial cells by inhibiting apoptosis and preferentially promotes proliferation of tubular epithelial cells. The integration of such diverse effects would render HGF well suited to reconstitute renal parenchyma via regeneration after injury.

Given its antifibrotic, anti-inflammatory, and pro-regenerative properties (Figure 1), HGF may hold promise as a new arsenal in our fights against renal fibrotic diseases. There are, however, several obstacles in translating the experimental results into benefit for patients in the clinical setting. One issue comes from the rapid clearance of HGF from the circulation, which makes it difficult to sustain a substantial level of its protein *in vivo*. To circumvent this problem, one might want to use a gene therapy approach to continuously produce HGF protein *in vivo*. Indeed, delivery of human HGF gene using a hydrodynamics-based strategy results in a significant, therapeutically effective amount of HGF protein in the circulation and in the kidney.[11] Nonetheless, such a gene delivery approach in the present form, albeit simple and efficient, cannot be applicable to humans. It is in this context that the study by Herrero-Fresneda *et al.*[3] is specifically interesting. The authors used a simple, clinically applicable approach, in which delivery of HGF gene is achieved by intramuscular injection of plasmid vector, followed by electroporation. The expression of exogenous human HGF is detectable, though low, in the circulation; and, importantly, exogenous HGF is obviously functional and therapeutically effective. As judged by the simplicity of the methodology, it appears that this gene delivery procedure might be readily adaptable to clinical situations.

The story of HGF in chronic kidney diseases illustrates how a naturally occurring, endogenous regenerative factor helps to boost the chronically injured tissue to repair and recover. If one considers renal fibrosis as a failed wound-healing process, supplementation of extra HGF to re-energize the injured tissue to repair for the sake of therapy makes sense. However, the role of HGF in renal fibrogenesis is not without controversy. At least one report indicates that administration of exogenous HGF protein in mice worsens renal dysfunction in diabetic nephropathy,[12] although three subsequent, independent studies confirm the beneficial effects of HGF on kidney structure and function in diabetic animals.[5,11,13] The reason for this discrepancy remains unknown, but it suggests that one should be cautious when taking HGF to clinical trials.

Our current options for the therapy of chronic renal fibrosis are limited, despite the introduction of angiotensin-converting enzyme inhibitors and angiotensin receptor blockers into standard medical practice in the past decade. HGF appears to be able to tackle multiple facets of the pathology associated with renal fibrosis. In addition, combination of HGF and anti-angiotensin II therapy results in a synergistic effect that leads to dramatic attenuation of renal fibrotic lesions.[14] So, is HGF administration, alone or in combination with anti-angiotensin II, a plausible approach to the future treatment of the patient with chronic kidney insufficiency? A definite answer to this question, of course, has to await well-designed clinical trials.

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The need for reliable serum parathyroid hormone measurements

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Serum parathyroid hormone (PTH) is a recognized marker of bone remodeling in patients with renal osteodystrophy. However, identification of N-terminal truncated PTH fragments and a new form of PTH that interfere with second-generation PTH assays may be responsible for the great variability of PTH values and the difficulties of implementing the recommendations of the National Kidney Foundation/Kidney Disease Outcomes Quality Initiative.

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Parathyroid hormone (PTH) is a polypeptide molecule of 84 amino acids with a molecular weight of 9500 daltons. It is synthesized by and secreted from the chief epithelial cells of parathyroid glands.

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Developmentally, there are four orthotopic parathyroid glands derived from the pharyngeal pouch; however, supernumerary, scanty, and/or ectopic parathyroid glands can be seen in a small percentage of subjects.[1] The formation of these glands is under the control of a key regulatory gene coding for the transcription factor *Gcm-2* (glial cell missing), which is almost exclusively expressed in these cells.[2] PTH is synthesized as a 115-amino acid precursor molecule called pre-pro-PTH, which is encoded by a single gene located on the short arm of chromosome 11 (11p15). This gene has three exons separated by two introns; the first exon contains most of the 5′ non-coding region of the gene, and the second exon codes for most of the pre-pro-PTH sequence. The third exon codes for three regions: the cleavage site Lys-Arg, the mature 1–84 PTH sequence, and the untranslated 3′ region.[3] The mature PTH is stocked in secretory granules that fuse with the cellular membrane and release PTH in response to reduction in the extracellular ionized-calcium concentration. Most of this PTH is secreted in its intact form, 1–84; however, it can also be secreted as N-terminal truncated fragments or C-terminal fragments after intracellular degradation, as in case of hypercalcemia.[4] No PTH fragment has been found to be the result of another gene or alternative splicing of the PTH gene.

Normally, on the basis of the percentage of the total immunoreactivity detected by different PTH assays, sometimes validated by the evaluation of PTH molecular-form concentrations derived from high-performance liquid chromatography profiles,[4] approximately 10%–20% of the total circulating PTH molecules are in its intact and bioactive form (1–84) (Table 1). Indeed, once the secreted 1–84 PTH reaches the circulation, it is rapidly taken up by the liver and the kidney and metabolized by several endoproteases in two main fragments: N-terminal and C-terminal fragments (the half-life of the entire molecule varies between 2 and 5 minutes). In the liver, all intact and N-terminal fragments are metabolized, and no N-terminal fragment is released. This degradation gives rise to several C-terminal fragments, starting from residues at positions 34, 37, 41, and 43 and ending probably at position 84 or shorter, which are often released back into the circulation.[5,6] Quantitatively, less than 20% of the intact PTH is converted into C-terminal fragments by the liver. However, because of their longer half-life (five to ten times longer than that of the intact form) and because they are normally cleared by the kidneys, those C-terminal PTH fragments represent 80% of the total circulating PTH molecules in normal individuals