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Receptor-mediated actions of renin and prorenin

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Renin can induce renal disease by generating angiotensin II and, thereby, increasing fibrosis. Huang *et al* describe a new mechanism of action whereby the renin–angiotensin system can also exert this effect. Direct activation of the renin/prorenin receptor in mesangial cells induced synthesis of TGF-β and profibrotic proteins. Hence, like other proteases such as thrombin, renin and prorenin are capable of receptor-mediated cellular signaling.

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The renin–angiotensin system is a central regulator of extracellular fluid volume and blood pressure. Fifty years after the discovery of renin, the landmark experiments of Goldblat established that the system could also be responsible for disease (renovascular hypertension). Though tantalizing but controversial clinical observations by Laragh's group suggested that the system, independently of renovascular hypertension and of hypertension itself, could have a widespread pathogenic role in the cardiovascular system,[1] the importance of the system in directly causing disease was first firmly established in the kidney. Brenner and coworkers,[2] and others, showed that inhibition of angiotensin II (Ang II) action ameliorated the usually inexorable progression of glomerular sclerosis that characterizes many renal diseases. The recognition by Border and Noble[3] that Ang II stimulation of extra-cellular matrix (ECM) protein synthesis in mesangial cells was, at least in part, mediated by TGF-β provided an explanation for the sclerosing action of angiotensin and further highlighted the importance of TGF-β in the pathogenesis of glomerulosclerosis.

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Elucidation of the multiple roles of the renin–angiotensin system in normal homeostasis as well as its involvement in disease has been greatly facilitated by the availability of a variety of compounds that block its actions at different sites. Researchers can make use of inhibitors of renin enzymatic action, inhibitors of angiotensin-converting enzyme, and competitive blockers for the receptors of Ang II, the last two of which are also available to the clinician. Use of these probes, in laboratory and clinical settings, has established beyond doubt that inhibition of Ang II ameliorates a variety of renal and cardiovascular conditions characterized by progressive expansion of ECM and fibrosis.

Despite that use of inhibitors of the renin–angiotensin system is firmly established in clinical practice, several aspects of the system still remain poorly understood. Among the most significant issues is the exact function of the renin precursor circulating in plasma. This protein, termed 'prorenin,' contains a 43-amino acid peptide in the N-terminal of renin and, under normal conditions, lacks renin enzymatic activity (that is, it cannot generate angiotensin I from angiotensinogen), probably because the propeptide covers the enzymatic site and prevents access to angiotensinogen. Prorenin can be rendered enzymatically active by low pH (pH approximately 3.5; a reversible process) or by cleavage of the propeptide by a variety of proteases (kallikrein, trypsin, plasmin, and probably others, some of which are located in the kidney). Normally, the concentration of plasma prorenin exceeds that of plasma renin,[4] and although under most steady-state conditions renin and prorenin in plasma change in parallel, when renin secretion is acutely altered — for example, stimulated by diuretic administration — the concentration of plasma active renin may suddenly exceed that of plasma prorenin, suggesting that prorenin is not stored in and secreted from renin-storage granules only. Further, in several conditions, such as pregnancy and diabetes mellitus with microvascular disease, prorenin in plasma is remarkably increased.[5] The significance of these observations remains to be determined, and little is known about factors that regulate prorenin secretion.

Another aspect of the renin–angiotensin system that needs further study is a group of renin- and prorenin-binding proteins,[5] among which the most intriguing is a 350-amino acid membrane-associated polypeptide recently cloned from a human kidney cDNA library by Nguyen and coworkers.[6,7] As both renin and prorenin are capable of binding to it at high affinity, the authors named it the renin/prorenin receptor. Remarkably, upon receptor binding, renin's enzymatic activity was increased, and when prorenin bound to the receptor, it showed enzymatic activity. This result suggested that the receptor, using either renin or prorenin, may be important in generating angiotensin in the surface of the cell, perhaps attaining concentrations of angiotensin much greater than those circulating in plasma (such a mechanism could explain why plasma concentrations of Ang II are in the picomolar range but binding affinities of this peptide to its receptors are in the nanomolar range)[8]. In addition, however, binding of renin/prorenin to the receptor in the presence of losartan led to intracellular signaling (by activating the pathway of the mitogen-activated protein kinases extracellular signal–regulated kinase-1 (ERK1) and ERK2), which suggests

that renin and prorenin may have novel mechanisms of action that are independent of angiotensin generation and are receptor mediated. The renin/prorenin receptor localized, as shown by immunomicroscopy, to mesangial and vascular smooth muscle cells, and northern blotting showed the receptor's mRNA to be widely distributed.

In this issue, Huang *et al.*[9] provide additional evidence that renin and prorenin have direct, receptor-mediated actions that appear to be independent of angiotensin generation. In the tradition of the authors' laboratory, they carried out an impressive series of carefully designed experiments that yielded very interesting results. Their data can be summarized as follows. Using human renin and rat prorenin, they found that these proteins increased the synthesis of TGF-β in mesangial cells, cells previously found to contain renin/prorenin receptors.[6] This effect was not altered by pharmacological inhibitors of renin enzymatic action, inhibitors of the converting enzyme, or blockers of angiotensin receptor-I, which strongly suggests that the TGF-β-stimulating effect of renin and prorenin is independent of the generation of Ang II. Renin also enhanced synthesis of fibronectin, collagen I, and plasminogen activator inhibitor-1, and, where tested, these effects were partially blocked by an inhibiting antibody against TGF-β. The increase in TGF-β mRNA that was induced by renin could not be inhibited by losartan or enalaprilat (the active form of enalapril). To demonstrate that the effect of renin on TGF-β was receptor mediated, they transfected the cells with a small interfering RNA that decreased the mRNA of the renin/prorenin receptor; under these conditions renin was unable to increase the abundance of the mRNA for TGF-β. Because mesangial cells have been reported to contain all components of the renin–angiotensin system, Huang *et al.* considered the possibility that the observed effects of renin and prorenin were due to Ang II generation. They provide several lines of evidence suggesting that this is very unlikely. Perhaps the most convincing results are that mRNA for angiotensinogen was not found in the mesangial cells and that Ang II was undetectable in the cell lysates. In sum, Huang *et al* found that receptor binding of renin and prorenin increased synthesis of TGF-β in mesangial cells and, at least in part by the action of this cytokine, increased the synthesis of fibronectin and collagen I, components of fibrotic ECM. Because blockade of Ang II is only partially effective in slowing the progression of glomerulosclerosis, these important results should stimulate additional research directly relevant to clinical practice. Such work may profit from previous research with other proteases such as thrombin, where it has long been recognized that receptor-mediated cellular signaling is an important mechanism regulating cardiovascular function and disease.[10]

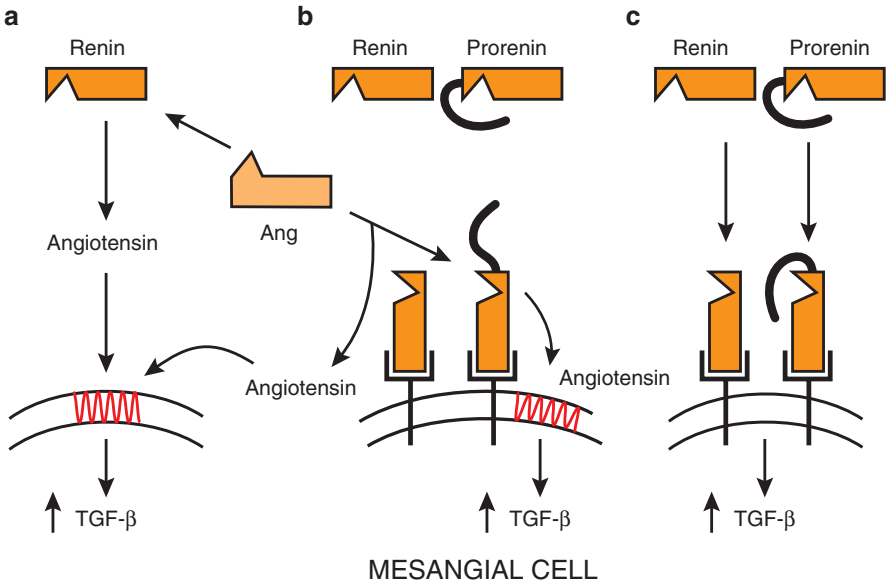

Figure 1 | Potential pathogenic mechanisms of the renin–angiotensin system in mesangial cells. (**a**) Generation of angiotensin I by renin circulating in plasma (and subsequent generation of angiotensin II by converting enzyme) leads to angiotensin II receptor activation and synthesis of TGF-β by the cell. (**b**) Binding of renin and of prorenin to the renin/prorenin receptor leads to activation of the enzymatic action of prorenin and enhancement of renin's catalytic activity. This may result in elevated concentrations of angiotensin (depicted as 'Angiotensin' and meant to convey both angiotensin I and II) next to the cell surface. (**c**) As shown in the article by Huang *et al.*, direct activation of the renin/prorenin receptor can also lead to TGF-β generation. Ang, angiotensinogen.

That activation of the renin/prorenin receptor may be an important mechanism of disease is supported by other observations. Transgenic rats expressing prorenin only in the liver had markedly increased plasma prorenin and minimally elevated plasma renin activity.[11] Interestingly, these animals did not have increased blood pressure but developed glomerulosclerosis, arterial wall thickening, and myocardial hypertrophy and fibrosis, observations consistent with a direct pathogenic effect of prorenin. Recently, Ichihara *et al.*[12] used a decapeptide derived from the 43-amino acid sequence of prorenin, which is attached to the N-terminal of renin. The peptide blocked the enzymatic activation of prorenin upon binding to the renin/prorenin receptor. Remarkably, when administered to diabetic rats, this peptide prevented the development of diabetic nephropathy without affecting the hyperglycemia. These provocative results urgently require confirmation and differ from the findings of Huang *et al.* in that the effect of prorenin appears to be dependent, at least in part, on its potential enzymatic activity, which is expressed when it is bound to the renin/prorenin receptor. It thus appears that prorenin, like renin, has angiotensin-dependent (that is, enzymatic) and direct, receptor-mediated actions. Regardless of the relative contributions of these two mechanisms of action, it is clear that the roles of prorenin and of the renin/prorenin

receptor in normal physiology and disease require in-depth study. For example, generation of mice with genetic deletion of the renin/prorenin receptor gene is needed. Also, analysis of the actions of renin and of prorenin in mesangial cells from mice with genetic deletions of different components of the renin–angiotensin system may facilitate elucidation of the receptor-mediated (that is, angiotensin-independent) effects of renin and prorenin. Animals lacking angiotensin-I receptors, converting enzyme, or angiotensinogen are available, and their mesangial cells could be used.

In sum, a significant body of evidence now indicates that activation of the renin–angiotensin system (Figure 1)can contribute to the induction and/or progression of renal and cardiovascular disease by at least three direct mechanisms (that is, independently of the hypertensive effect of the system): (1) by direct activation of angiotensin receptors by Ang II; (2) by activation of the enzymatic action of prorenin and enhancement of renin's catalytic activity upon binding of these proteins to the renin/prorenin receptor; and (3) as shown by Huang *et al*, by direct activation of the renin/prorenin receptor and generation of TGF-β. Needless to say, all these pathogenic mechanisms raise the intriguing question of why normal physiological conditions in which the renin–angiotensin system is markedly and chronically activated (for example, with a low-sodium diet), and in which plasma renin and prorenin as well as Ang II are markedly elevated, are not associated with disease. Answering this important question would clarify the mechanisms by which these hormones contribute to disease.

Although pharmacological blockers of the renin–angiotensin system are clearly effective in many renal and cardiovascular diseases, their effectiveness is limited, and exciting novel therapeutic avenues are likely to open through research on receptor-mediated renin and prorenin actions. In addition, because inhibitors of the converting enzyme are currently among the most frequently prescribed antihypertensive medications, in view of the results of Huang *et al.*, an analysis of the potential harmful effects of the markedly elevated concentrations of plasma renin caused by these drugs is urgently needed.

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Hypoxia-inducible factors: where, when and why?

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Hypoxia-inducible factor (HIF) is a family of transcription factors that regulate the homeostatic response to oxygen deprivation during development, physiological adaptation, and pathological processes such as ischemia and neoplasia. Our understanding of the function of different HIF isoforms is being advanced by understanding the processes that regulate their activity, learning where and when they are expressed and what genes they regulate.

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Hypoxia-inducible factor-1 (HIF-1) is an oxygen-regulated transcriptional complex, originally identified by its role in the control of erythropoietin gene expression in response to changes in oxygen tension. The original HIF components were identified after affinity purification of proteins from hypoxic cells capable of binding the oxygen-regulated erythropoietin 3′ enhancer DNA sequence. The genes encoding these proteins were identified as a novel α chain known as HIF-1α and a β chain that was found to be identical to the previously described aryl hydrocarbon receptor nuclear translocator, which is constitutively expressed.[1,2] Subsequent work has defined two further HIFα chains, HIF-2α and the much less well studied HIF-3α, one splice variant of which was originally identified as inhibitory PAS protein 1.

Since the identification of HIF-1, it has become clear that the HIF complexes have

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