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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a much broader role in oxygen homeostasis than simply regulation of erythropoietin. They control the expression of a large number of oxygen-regulated genes with functions in diverse processes including angiogenesis, metabolism, and apoptosis.[1,2] The identification of multiple hypoxically regulated HIFα isoforms raises obvious questions about their functional roles and the selective pressures that have led to their evolution. Recent studies on HIF-1α and HIF-2α, including the paper by Bernhardt *et al*. in this issue,[3] are beginning to illuminate this debate.

A further renal link arises from the discovery that mutations in the von Hippel-Lindau (VHL) gene (which occur in the majority of sporadic renal-cell carcinomas and underlie VHL disease) are associated with HIF activation.[4] This led to the elucidation of the mechanism of oxygen sensing that controls the regulation of HIF in all cells. Regulation of HIF complexes is mediated by two distinct but similar post-translational mechanisms. HIFα chain abundance is decreased in the presence of oxygen by enzymatic hydroxylation of two critical prolyl residues within the HIFα proteins, which is accomplished by a family of three HIF prolyl hydroxylases (PHD1, PHD2, and PHD3) and leads to VHL E3 ligase-dependent ubiquitylation and subsequent proteasomal degradation. Similarly, when oxygen is available, coactivator recruitment is blocked by factor inhibiting HIF (FIH)-mediated hydroxylation of an asparaginyl residue of HIFα. All four of these HIF hydroxylase enzymes are oxygen- and 2-oxoglutarate-dependent dioxygenases; when oxygen availability falls, their activity is limited, their inhibitory effects are removed, HIF complexes are activated, and gene expression increases. Again, the presence of multiple HIF hydroxylases raises questions about their possible redundancy and diverse roles. Indeed, these oxygen-sensing enzymes show differential patterns of organ expression, intracellular localization, inducibility by exogenous stimuli, and substrate selectivity.[2]

The mechanisms regulating the abundance and activity of HIF-1α and HIF-2α isoforms are largely concordant, although not identical. This is reflected in striking sequence similarity in critical functional regions, with minor sequence differences presumably explaining features that, although subtle, may be very significant biologically, such as precise targeting of individual prolyl residues by particular prolyl hydroxylase enzymes. There is also high homology between the HIF-1α and HIF-2α isoforms at the domains involved in DNA binding and dimerization. However, relatively large parts of these protein molecules show low levels of sequence homology/identity, suggesting that these regions of the different isoforms contribute differing functional capabilities, presumably by interacting with other proteins in a cell type- or stimulus-dependent manner.

Evidence for differential roles of HIF-1α and HIF-2α comes from their differing patterns of organ expression, and the different cell types within organs that express each α isoform. HIF-2α was originally reported to have a very restricted pattern of expression in the mouse endothelium, contrasting with a wide distribution of HIF-1α expression, and resulting in its original naming as endothelial PAS protein 1. Recently, distinct expression patterns have been demonstrated by immunohistochemistry in cell lines and adult tissues, and it has become clear that HIF-2α is more widely expressed than originally reported. In the hypoxic kidney, this work has shown HIF-2α expression in the erythropoietin-producing interstitial fibroblasts, whereas HIF-1α is the dominant form expressed in tubular epithelial cells.[5]

Further evidence of distinct functional roles has arisen from studies of knockout mice, in which the phenotypes of HIF-1α and HIF-2α knockouts are different. HIF-1α knockout mice suffer embryonic lethality with enhanced mesenchymal-cell death and defects in angiogenesis that involve both the yolk sac and embryonic tissues. By contrast, HIF-2α knockout mice have a variable phenotype, with some homozygotes surviving to the perinatal period. Different reports have indicated problems with vascular remodeling; catecholamine production; lung maturation and surfactant production; and fatty acid oxidation.[6] Defects in kidney development in these mice have not been investigated in depth, as it is difficult to disentangle primary effects from the secondary consequences of vascular abnormalities. However, important new work by Bernhardt *et al*[3] goes some way toward redressing this, reporting differing patterns of expression of HIF-1α and HIF-2α proteins in the normoxic fetal kidney. This extends the understanding of the developmental roles of the HIFα proteins, suggesting differential functions in tubular and vascular genesis and perhaps a synergistic role in formation of glomeruli.

To understand these differing roles it will be important to define the patterns of gene expression elicited by each HIF isoform. Additional evidence of functional disparity comes from studies of those genes that are selectively activated by individual HIFα isoforms. Specific examples of this include the discordant expression of carbonic anhydrase IX, dominantly driven by HIF-1α, and erythropoietin, dominantly driven by HIF-2α.[7,8] Microarray technology is allowing more complete descriptions of gene expression, and this has increased the number of known HIF-2α-responsive genes, although genes that respond solely to HIF-2α appear to be substantially rarer than those that respond only to HIF-1α. The report by Bernhardt *et al*.[3] of colocalization of HIF-2α with the hypoxically regulated endoglin gene product (the gene mutated in hereditary hemorrhagic telangiectasia (Rendu-Osler-Weber syndrome)) provokes the speculations that endoglin may be another HIF-2α-responsive gene and that endoglin might have a role in renal cancer pathogenesis. The mechanisms responsible for selective HIFα gene regulation and the tissue specificity of VHL-associated malignancies are subjects of active study.

In addition to the different physiological functions of the HIFα isoforms, there appear to be differences in the action of HIF-1 and HIF-2 in the pathogenesis of renal cancer. In the kidneys of VHL patients, where HIF is activated because of genetic mutation rather than environmental factors, the earliest lesions in both proximal and distal tubules express HIF-1α protein but not HIF-2α protein, whereas HIF-2α protein is more abundant in later tumor nests, which tend to have a distal tubular phenotype/location and to be more aggressive.[9] In keeping with this, many VHL-negative renal cancer cell lines,

including those derived from metastases, show preferential expression of functional HIF-2α over HIF-1α. Additionally, a further increase in HIF-2α levels enhances growth of renal cancer xenografts, whereas overexpression of HIF-1α inhibits it.[10,11]

Differences in HIF isoform expression and function are also manifest in other tumor types, presumably reflecting the net selective advantage of the particular spectrum of downstream genes activated in a particular cellular background by environmental factors. In tumors derived from ES cells, variable effects of loss of HIF-1α expression on solid tumor growth have been reported, probably depending on the balance of effects on microenvironmental hypoxia, apoptosis, and stress-induced proliferation.[12,13] More recently, studies on teratomas derived from ES cells in which an HIF-2α allele has been knocked in at the HIF-1α locus indicate that HIF-2α promotes tumor growth more effectively than HIF-1α.[14] In keeping with this, a recent study of melanoma patients has reported that, in multivariate analysis, HIF-2α staining is an independent indicator of poor prognosis whereas HIF-1α staining is not.[15]

Tumor development often involves reversion to a fetal or embryonic cellular phenotype, with earlier markers generally indicating a more aggressive phenotype. In the fetal kidney, tubular HIF-1α expression is seen in normoxia, but this disappears once nephrogenesis is complete. Whether HIF-2α contributes at an earlier stage of renal development than that studied by Bernhardt *et al*.[3] is unknown. However, the role of HIF-2 in renal vasculogenesis that they describe is of particular interest given the highly vascular nature of VHL-associated tumors.

Understanding the functional differences between the HIFα isoforms and their roles in tumor formation is a prerequisite for attempts to manipulate this important homeostatic mechanism to therapeutic advantage, be that to enhance endogenous erythropoietin production, affect tumor growth, facilitate therapeutic angiogenesis in ischemic tissues, or help preserve tissues for transplantation. The work reported here by Bernhardt *et al*.[3] represents another important contribution in dissecting the developmental, physiological, and pathological roles of HIF.

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Acute renal failure outcomes in children and adults

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Acute renal failure (ARF) in hospitalized patients remains associated with significant morbidity and mortality. Most reports have detailed the in-hospital outcomes in ARF patients. This commentary focuses on the outcomes (including quality of life) of ARF patients, both adults and children, after discharge from the hospital.

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With rapid advances in medical technology, the epidemiology of acute renal failure (ARF) has dramatically changed. Over the past few decades, obstetrical and traumatic causes of ARF have continued to decline, with a corresponding increase in the number of elderly patients with complicated medical and surgical conditions.[1] Thus, the typical ARF patient at this time possesses a large burden of comorbid illnesses and extensive extrarenal complications.[2] Despite major advances in dialysis and continuous renal replacement therapies, the mortality rate from ARF has not changed. The adult mortality rates average approximately 50% and have remained stable over the past 50 years.[3] The outcome is

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