Hypoxia-Inducible Transcription Factors and Their Role in Renal Disease

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Summary: The 2 hypoxia inducible factors (HIF)-1α and HIF-2α are key mediators of cellular adaptation to hypoxia. They show a specific distribution pattern and possibly have complementary transcriptional targets in the kidney: HIF-1α is found mainly in tubular and HIF-2α in peritubular interstitial, endothelial, and glomerular cells. Both isoforms are regulated by oxygen-dependent hydroxylation of specific amino acid residues, which determines protein stability and transcriptional activity. Small molecule inhibitors of HIF hydroxylases act as pharmacologic inducers of HIF. HIF target genes are involved in cellular mechanisms that increase hypoxia tolerance or improve oxygen supply at the systemic or regional level, but also have been implicated in cellular apoptosis and profibrotic mechanisms. In experimental acute kidney injury the up-regulation of HIF either through endogenous hypoxia-sensing or after pharmacologic HIF stabilization confers tissue protection. Thus, HIF stabilization offers a promising novel and clinically feasible approach for nephroprotection. On the other hand, continuous activation of the HIF system occurs in kidney cancer and potentially promotes tumor growth. HIF therefore also is explored as a target for anticancer therapy.
Semin Nephrol 27:363-372
Keywords: *Hypoxia, ischemia, preconditioning, gene expression*

Hypoxia usually is not recognized as a major mechanism of renal injury, in contrast to the heart or the brain, for which ischemia is considered the most important pathomechanism. Nevertheless, because of steep oxygen gradients in the kidney, many parts of the renal parenchyma, in particular the medulla, but also certain tubular segments in the cortex, operate at the rim of an adequate oxygen supply. An imbalance between oxygen supply and consumption therefore can occur easily when the perfusion or architecture of renal vessels is disrupted. Acute tubular necrosis as a result of toxic and/or ischemic tubular damage is considered a hallmark of acute renal failure.

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0270-9295/07/$ - see front matter
 doi:10.1016/j.semnephrol.2007.02.007

On the other hand, reduced tissue oxygen tensions have long been considered largely irrelevant, as long as cellular energy production and thus cell viability is maintained. This view has entirely changed in recent years because it has been recognized that a reduction in oxygen tensions is an important determinant of gene expression. This transcriptional response is mediated largely by a family of hypoxia-inducible transcription factors (HIFs). HIF was first discovered by studying the oxygen-dependent regulation of erythropoietin (EPO),[1] but now has been shown to operate in most cells and tissues.[2-4] More than 100 HIF target genes probably exist, many of which regulate cell survival decisions, cell metabolism, or angiogenesis.[5] Although EPO production is confined to peritubular fibroblasts in the kidney cortex, HIF transcription factors are widely inducible in all parts of the kidney and their study reveals important insight into the development and consequences of hypoxia in different forms of renal injury.[6] Moreover, their oxygen-independent activation also plays a major role in most cases of

renal cancer. Understanding of the molecular control of HIF activation offers new insights into the progression of renal carcinoma and very promising opportunities for therapeutic intervention to protect the kidney from hypoxic injury.

OXYGENATION OF THE KIDNEY

Oxygenation of the kidney is characterized by an apparent paradox. Both kidneys receive approximately 25% of the total body oxygen supply and in relation to tissue weight renal oxygen supply is much higher than that of other organs. Nevertheless, oxygen tensions within the kidney are much lower than in most other tissues and usually are below those in the renal vein.[7] This paradox can be attributed to the unique architecture of the kidney, where arterial and venous vessels run parallel in close contact over long distances. The countercurrent arrangement allows oxygen to diffuse from arterial into venous branches before it has reached the peritubular capillary bed.[8] Oxygen tensions are particularly low in the renal medulla, but marked variability also has been measured in the renal cortex.[9] In fact, the comparatively low regional oxygen availability under normoxic conditions is considered a main reason for an extraordinary susceptibility of the kidney to acute hypoxic injury.[10] In addition, there is increasing evidence that regional hypoxia also plays an important role in the pathogenesis of chronic kidney disease.[11,12] Any disturbance in blood flow caused by renal atherosclerosis or distortion of the glomerular tuft inevitably will impair peritubular capillary perfusion. Moreover, chronic kidney disease appears to be associated with a marked reduction of peritubular capillaries,[11,12] which is likely to contribute to a reduction in tissue oxygen tensions. On the other hand, because renal oxygen consumption depends mainly on tubular sodium reabsorption, a reduction in glomerular filtration rate will reduce the oxygen need. Therefore, it is difficult to predict how tissue oxygen tensions change during different stages and in different types of chronic kidney disease. Actual measurements rarely have been performed and, rather surprisingly, in a model of 5/6 nephrectomy an increase in renal tissue pO_2 has been observed.[13]

MOLECULAR MECHANISMS OF HIF SIGNALING

HIF transcription factors are heterodimers, consisting of 2 Per-Arnt-Sim basic-helix-loop-helix proteins: a constitutive β-subunit, previously termed aryl hydrocarbon receptor nuclear translocator, and an oxygen-regulated α-subunit.[2,14,15] Two HIFα isoforms have been identified to play a major role in the transcriptional response to hypoxia: HIF-1α and HIF-2α. Both are regulated in a very similar fashion at the posttranslational level through oxygen-dependent destruction via the ubiquitin-proteasome pathway (Fig. 1). In the presence of oxygen, 2 defined prolyl residues in the oxygen degradation domain of HIFα are hydroxylated and this hydroxylation is a prerequisite for binding to a ubiquitin-ligase complex that targets HIF for proteasomal degradation. When oxygen is not available as a substrate for the hydroxylation reaction, HIFα does not enter the destructive pathway and forms a complex with HIFβ and transcriptional co-activators to induce target genes. Interaction with the co-activator p300/CBP also is regulated in an oxygen-dependent fashion by hydroxylation of a C-terminal asparagine residue. The enzyme that catalyzes this hydroxylation reaction initially was termed *Factor-inhibiting HIF-1* and behaves very similar to the prolyl hydroxylases.[2,14,15] Binding of the HIF transcriptional complex to hypoxia-response elements of HIF target genes then facilitates their transcriptional activity (Fig. 1).

The hydroxylation of HIF in the presence of oxygen is achieved by a group of specific HIF-prolyl-hydroxylases[16] (Fig. 1). Three functionally active isoforms of the HIF-prolyl-hydroxylases have been identified. The hydroxylation reaction is iron dependent and requires 2-oxoglutarate as a cosubstrate; thus, 2-oxo-glutarate analogues and iron chelators can be used to inhibit hydroxylation activity and to stabilize HIF under normoxic conditions. Only hydroxylated HIFα binds to the von Hippel-Lindau tumor suppressor protein (pVHL), which serves as the recognition component of the ubiquitin-ligase complex (Fig. 1). Structural alterations in pVHL that affect its ability to bind hydroxylated HIF result in the inability to degrade HIF in the

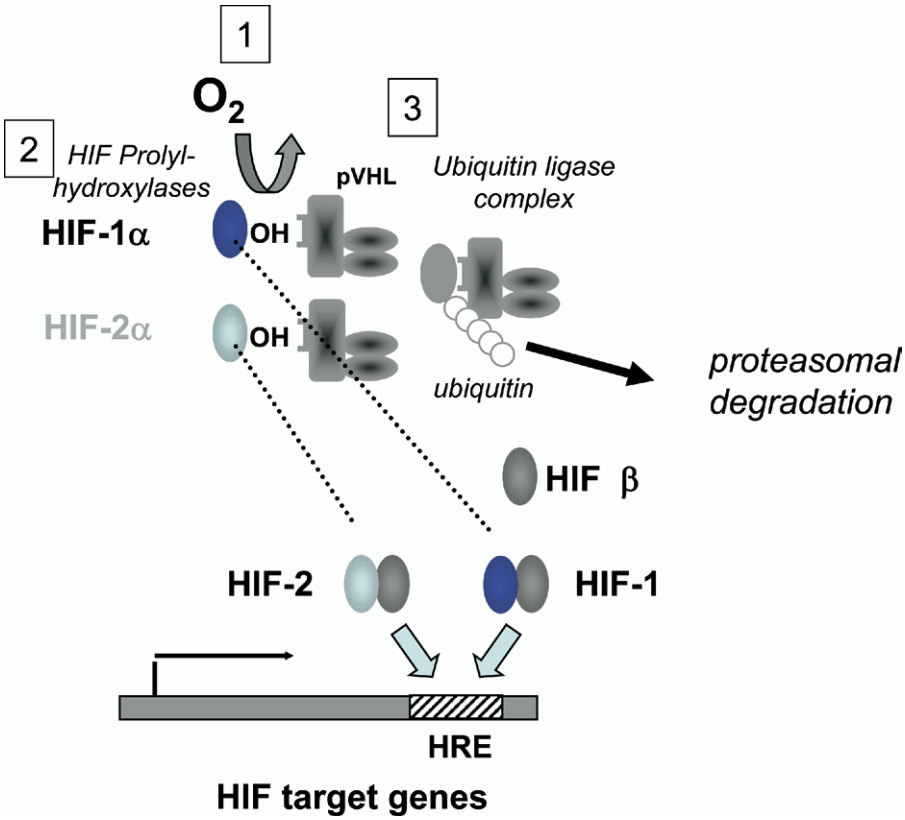

Figure 1. Simplified scheme of HIF regulation. The oxygen-dependent HIFα isoforms HIF-1α and HIF-2α are hydroxylated in the presence of oxygen at specific prolyl-residues. This hydroxylation is a prerequisite for HIF binding to a ubiquitin ligase complex that contains pVHL as a recognition component. The complex induces the attachment of ubiquitin molecules and subsequent proteasomal degradation of HIF. If the destruction of HIFα is inhibited it can bind HIFβ and activate hypoxia response elements of HIF target genes. There are 3 relevant ways by which HIF destruction can be inhibited: (1) lack of oxygen (hypoxia), (2) pharmacologic inhibition of HIF prolyl hydroxylases, and (3) inactivation of pVHL, which results in its inability to bind hydroxylated HIFα.

presence of oxygen and subsequent overexpression of HIF target genes.[17,18]

Apart from low oxygen concentrations or genetic activation, other factors have been shown to induce HIF, including, for example, nitric oxide, tumor necrosis factor α, angiotensin II, and reactive oxygen species.[19-22] However, their role in activating HIF in vivo is still unclear.

HIF EXPRESSION IN THE NORMAL KIDNEY

A limitation in oxygen availability is an important signal during embryonic development. Targeted disruption of HIF results in embryonic lethality and HIF-1α and HIF-2α expression has been shown in the developing human and rodent kidney.[23,24] The expression pattern of the HIFα subunits is stage and cell-type specific and correlates with the expression of important angiogenic factors.[23] HIF-1α occurs predominantly in collecting ducts, S-shaped bodies, and glomerular cells, whereas HIF-2α was found in podocytes, endothelial cells, and interstitial cells.[23,24] When renal morphogenesis has been completed, HIF proteins are usually not detectable in the normal kidney, despite its heterogeneity in oxygen tensions and the low oxygen concentrations that normally occur in the renal medulla.[23,25] In response to reduced renal oxygen availability caused by anemia, carbon monoxide poisoning, or a reduction in the arterial oxygen saturation, HIF is stabilized in different regions of the kidney in a pattern that resembles the embryonic expression and is determined by both local oxygen tensions and the endogenous properties of different renal cell

populations.[25] Corresponding to renal oxygen profiles, HIF induction under systemic hypoxia is most intense in the renal papilla. Marked nuclear accumulation of HIF also occurs in the outer medulla and the renal cortex, with a significant variability in different tubular segments. The connecting tubule and collecting ducts show higher HIF protein levels than the thick ascending limb of Henle and the distal convoluted tubule. Within the proximal tubule, HIF up-regulation is more prominent in the S1 and S2 segments than in the S3 segment. Furthermore, there is a distinctly separate expression pattern of HIF-1 and HIF-2. While tubular cells only express HIF-1, HIF-2 is found in glomerular cells, peritubular endothelial cells, and interstitial fibroblasts. Thus, despite a very similar mode of regulation, and despite the fact that many cell lines activate both transcription factors under hypoxia, HIF-1 and HIF-2 appear to have complementary rather than redundant roles in vivo. Different expression patterns of HIF-1 and HIF-2 also are found in other organs, but some cell types, such as cardiac myocytes, activate HIF-1 and HIF-2 simultaneously.[26,27] Of particular interest is the observation that only HIF-2α is found in the EPO-producing cells—the peritubular renal fibroblasts and hepatocytes—indicating that the oxygen-dependent control of red cell production is mediated by HIF-2. In cells that express HIF-1 and HIF-2, the 2 transcription factors do not overlap completely with regard to their ability to regulate specific genes.[28-30] Under such conditions, as yet undefined molecular mechanisms also confer a preferential or selective inducibility of the EPO gene by HIF-2.[29,31]

NEPHROPROTECTION THROUGH HIF ACTIVATION IN ACUTE RENAL INJURY

In a variety of experimental models HIF stabilization has been found in regions of the kidney that presumably are hypoxic as a consequence of different forms of acute injury. These settings include complete renal ischemia,[25,32] renal infarction,[33] contrast nephropathy[34] (Fig. 2), and hypoxic damage of isolated perfused kidneys.[35] In all these models HIF accumulation usually occurs in the vicinity of the most severely damaged tubules. This border-zone phenomenon is most obvious after renal infarction, during which a band of cells with nuclear accumulation of HIF can be observed between the infarct core and the surrounding normal tissue.[33]

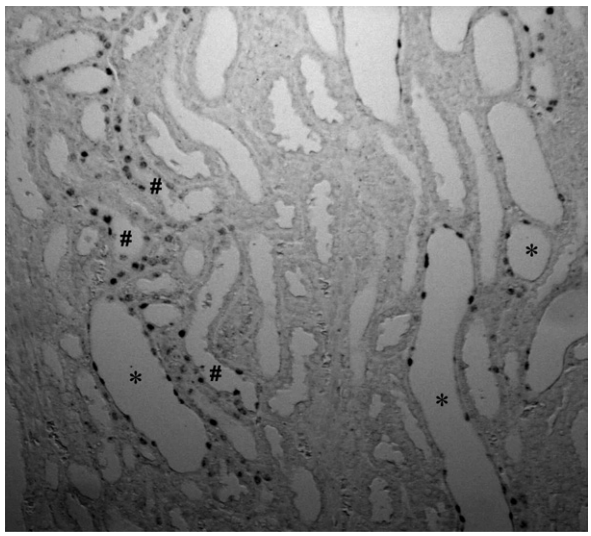

Figure 2. Expression of HIF-1α in a rat acute renal failure model (combination of indomethacin, iothalamate, and NG-nitro-L-arginine methyl ester (L-NAME). Nuclei in the #outer renal medulla thick ascending limbs of the loop of Henle (mTAL) and *medullary collecting ducts stain positive for HIF-1α protein.

Nevertheless, the accumulation of HIF is not uniform among different tubular segments and there is some evidence that the ability to induce HIF is related inversely to the severity of cell damage. For example, cells of the thick ascending limb in the outer medulla show comparatively little HIF accumulation in acute renal failure models. The application of inhibitors of active sodium reabsorption in this part of the tubule, such as furosemide or ouabain, are known to preserve cellular integrity and reduce damage.[36] Although inhibition of oxygen consumption through inhibition of sodium reabsorption increases tissue oxygen tensions,[36,37] more rather than less HIF activation occurs under these conditions.[34,35] It appears therefore that the ability to induce HIF signaling requires a specific low range of oxygen tensions that may vary between different cell types, and the system cannot be activated when oxygen tensions are either above or below this range. Inhibition of sodium reabsorption may bring cells operating below the lower border into a window of opportunity for HIF signaling. It is

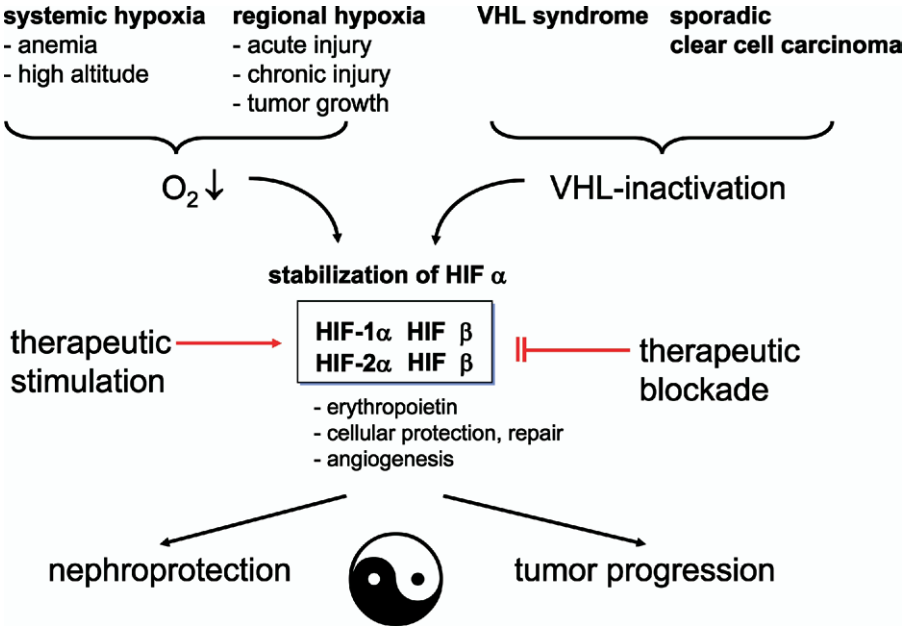

Figure 3. Conditions of HIF activation in the kidney. Both systemic and regional hypoxia stabilize HIF through inhibition of HIF prolyl hydroxylases. In addition, inactivation of pVHL in renal tubular cells in patients with the VHL syndrome and sporadic clear-cell renal cancer stabilizes HIF independently of oxygen availability. The activation of HIF target genes, including erythropoietin, several other genes that improve hypoxia tolerance, and genes that promote angiogenesis, may lead to nephroprotection. In renal tumors the same mechanisms may promote survival and growth of cancer cells. Thus, both activation of the HIF system to promote nephroprotection and its inhibition in patients with kidney cancer are attractive therapeutic approaches.

tempting to speculate that activation of the HIF system through the induction of protective genes plays a role in the functional and structural preservation observed in kidney injury models under these conditions (Fig. 3).

Indeed, evidence has been generated by several groups showing that HIF accumulation protects against a subsequent ischemic insult. The methods used to achieve activation of the HIF pathway include systemic hypoxia,[38] cobalt chloride,[32] and 2-oxo-glutarate analogues that inhibit HIF-prolyl-hydroxylases[38] (Fig. 4). The latter group of pharmacologic inducers of HIF is of significant interest for clinical applications.[6,39] Such compounds can be administered orally and parenterally and clinical trials already are ongoing to test their ability to treat anemia by stimulating endogenous EPO production.[40,41]

In principal, the advantage of targeting HIF rather than trying to use single gene products for tissue protection not only may reside in the ease of pharmacologic intervention with small molecules, but also in the broad spectrum of target genes induced through the master switch of oxygen-dependent gene regulation. However, some of the proteins coded by these genes have been found to be sufficient to induce nephroprotection. The probably best studied is EPO, which, when administered at high doses, confers protection against acute renal injury in rodent models.[42-44] It is not unlikely therefore that increased EPO gene expression in the kidney may contribute to the protective effects of experimental HIF induction. However, the relative importance of EPO gene induction in the context of the whole spectrum of HIF target genes is difficult to decipher and remains unclear so far.

It is also not entirely clear yet whether activation of the HIF pathway can protect against the exposure to toxic compounds, which damage tubular cells through mechanisms other than hypoxia. In models of cisplatin toxicity hypoxia was proposed to reduce the rates of tubular apoptosis, but divergent results have been reported regarding the role of HIF under these conditions.[45,46]

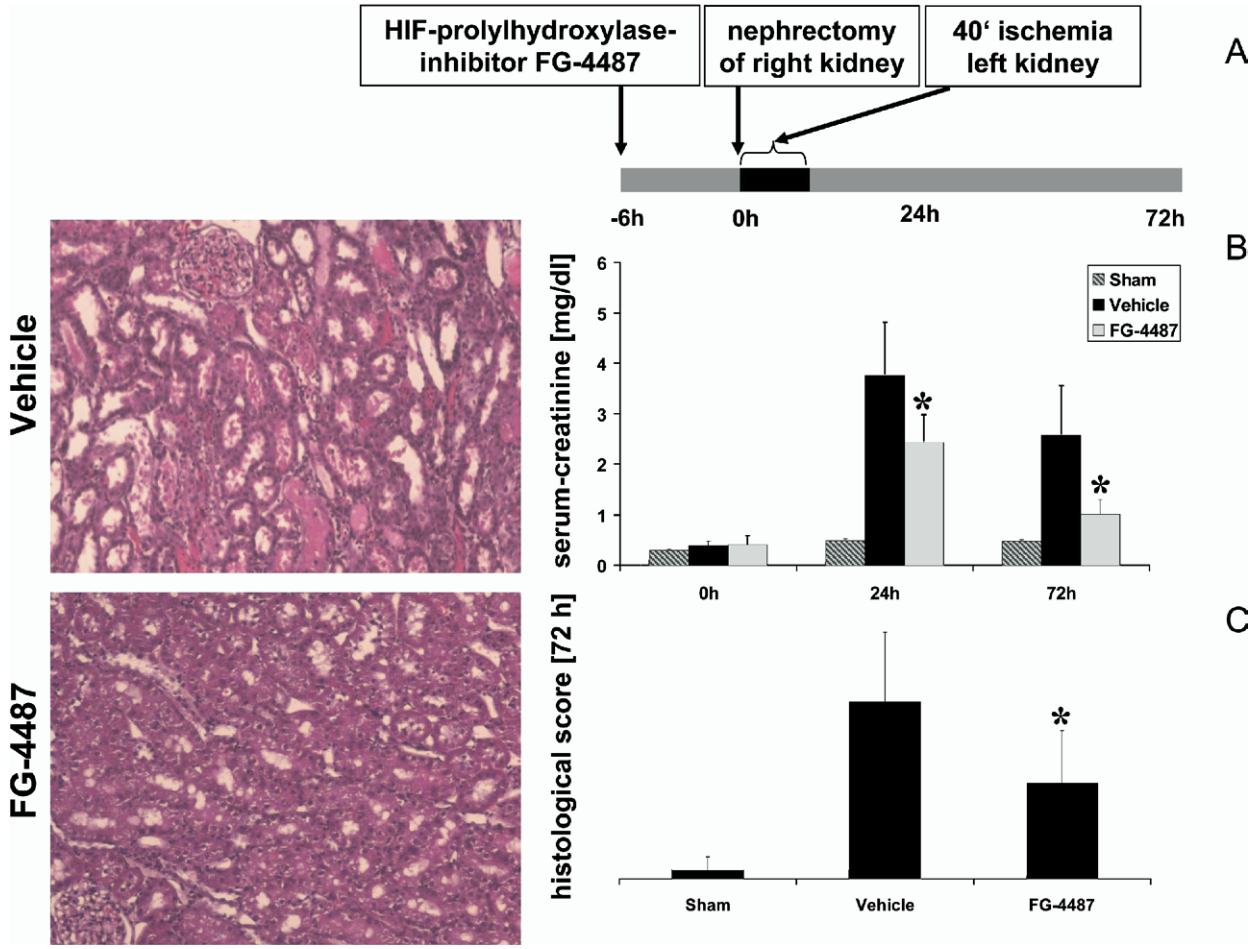

Figure 4. Preconditional activation of HIF by inhibition of the HIF-prolyl-hydroxylases protects the kidney from ischemía/reperfusion injury. (A) Six hours before right-sided nephrectomy in combination with a 40-minute clamping of the left renal artery, the PHD-inhibitor FG-4487 was injected into rats. (B) In comparison with vehicle-treated animals, FG-4487 treatment led to a significant amelioration of renal function, as indicated by a less marked increase in the serum creatinine level. (C) Histologic scoring also revealed a clear reduction of the renal damage at 72 hours after ischemia/reperfusion. (D) Representative hematoxylin-eosin stains of vehicle and FG-4487–pretreated animals, showing reduced tubular necrosis, reduced tubular cast formation, and less interstitial bleeding in animals with preconditional HIF activation (*$P < .05$). Data from Bernhardt et al.[38]

Future experiments also will have to determine if and to what extent HIF activation can alter and improve the course of acute renal injury when stabilization of HIF occurs after renal damage already has been initiated.

POTENTIAL ROLE OF HIF IN CHRONIC RENAL DISEASE

The role of HIF in chronic renal disease has been less well studied. Because tissue hypoxia probably occurs in different types of renal disease, it is not unlikely that HIF is activated as well. Indeed, renal hypoperfusion was found to precede tubulointerstitial inury.[47] Transgenic rats expressing a reporter gene under the control of a hypoxia-response element were found to activate the reporter in the remnant kidney and the puromycin model, suggesting that HIF signaling occurs under these conditions.[48] The functional consequences, however, are less clear. Some of the downstream effects of HIF, such as the formation of novel capillaries, may retard disease progression.[49] In line with this hypothesis, chronic administration of cobaltous chloride, which can stabilize HIF, was reported to ameliorate tubulointerstitial injury.[50,51] On the other hand, HIF signaling also has been suggested to promote the progression of renal

disease through a variety of mechanisms, including profibrotic effects, a possible role in epithelial to mesenchymal transition, and an impact on inflammatory processes.[52] Several genes that are implicated in renal fibrogenesis are direct HIF-1 targets (eg, tissue inhibitor of metalloproteinases-1,[53] plasminogen-activator-inhibitor-1,[54] and connective tissue growth factor[55]). Furthermore, hypoxia may promote the thrombospondin-dependent release of transforming growth factor-β[56] and synergistic effects between hypoxia and transforming growth factor-β1 on collagen synthesis have been shown.[57] The transition of epithelial to mesenchymal cells, which is considered to play an important role in the development of tubulointerstitial fibrosis, was found to be increased under hypoxia,[58] and HIF-1 has been suggested to play a key role in this process.[39,52]

The impact of HIF signaling on inflammatory processes has only just started to be recognized. HIF-1 can promote leukocyte adhesion during hypoxia,[59] it modulates lymphocyte function and T-cell receptor signaling,[60-62] and it appears to be essential for myeloid-cell–mediated inflammation.[63] The potential implications of these effects of HIF in renal disease models have not yet been defined.

Recent evidence has indicated that the HIF system also may be involved in the induction and/or progression of glomerular lesions. Podocyte-specific inactivation of pVHL was found to induce foot process effacement, proteinuria, and crescent formation.[64]

Another very interesting aspect of renal pathology in which the HIF system may play an important role is cyst formation. Specific inactivation of VHL in the proximal tubule of mice led to the development of multiple renal cysts.[65] In line with these findings, renal cysts frequently occur in patients with VHL syndrome.[66] The development of cystic lesions in these patients also may precede the formation of renal cell carcinomas (see later). Activation of HIF and up-regulation of its target genes was found to be an early event in this process.[67] Very recently, it was reported that cells lining these cystic lesions lose the cilium, which normally protrudes into the tubular lumen and is believed to serve as a mechanosensor of tubular flow.[68] Alterations in the signal transduction through this sensor probably play a key role in the pathogenesis of polycystic kidney disease.[69] Although inactivation of pVHL has several potentially relevant consequences that are independent of HIF, the loss of cilia in tubular cells with inactive pVHL was suggested to be mediated through up-regulation of HIF-1.[68] In addition, we recently found that kidneys from patients with polycystic kidney disease, and in a genetic rodent model of cystic kidney disease, show significant up-regulation of HIF in both the epithelium lining the cysts (HIF-1) and the stroma of the cyst walls (HIF-2).[70] This corroborates earlier findings, indicating that cystic kidneys can produce significant amounts of EPO, resulting in a less severe anemia compared with other types of kidney disease,[71] and that active angiogenesis plays an important role for cyst growth.[72,73] It is possible therefore that cyst expansion results in tissue hypoxia, hypoxia-induced stabilization of HIF, and induction of HIF target genes, which promote further cyst growth.

GENETIC ACTIVATION OF HIF IN RENAL CANCER

A genetic, oxygen-independent stabilization of HIF through inactivation of pVHL occurs in patients with the autosomal-dominant VHL disease and in the majority of sporadic clear-cell renal cell carcinomas, which represent the most frequent type of renal cancer. Patients with the VHL syndrome carry a germ-line mutation of the VHL gene and the second allele is believed to be inactivated through a second hit (Knudson's hypothesis).[74,75] In sporadic clear-cell renal carcinoma both alleles subsequently are inactivated.[76] Under both conditions the inactivation of pVHL leads to a loss of its ability to bind hydroxylated HIFα and HIF is stabilized independently of the oxygen concentration.[77,78] There is interesting evidence that this process also is associated with a shift from the expression of HIF-1 in tubular cells to HIF-2 and that the latter is of particular relevance for tumorigenesis.[79,80] Activation of HIF target genes can explain many of the characteristic findings in this type of renal cancer, including its high

degree of vascularization and occasional erythrocytosis.[78,81]

Because HIF activation is considered a key component in the adaptation of tumors to local hypoxia, the inhibition of HIF appears as an attractive approach to retard cancer progression.[82] Given the very prominent expression of HIF in clear-cell renal cancer, caused by VHL inactivation rather than regional hypoxia, clear-cell kidney cancer appears as a particularly promising target for such strategies (Fig. 3) and may allow proof of principle of their efficacy.

CONCLUSIONS

The discovery of the HIF system in the context of studies addressing the regulation of erythropoietin production has provided new insights into a fundamental biologic principle by which cells and organisms adapt to variations in oxygen supply. Although our knowledge on the regulation of this system in the kidney still is fragmentary, available evidence indicates that HIF is involved in the pathophysiology of many acute and chronic kidney diseases. Important insights from experimental pharmacologic modulation and genetic manipulation of the HIF pathway are likely to follow within the next years. Although it is remarkable that translation of this knowledge into therapeutic application already has started, increasing knowledge of the various aspects of HIF biology will be required to fully exploit the therapeutic potential of manipulating the system inside and outside of the kidney. The master switch function of HIF may convey important advantages when complex adaptive responses, such as tissue protection are aimed for. On the other hand, limiting the effects of HIF activation or blockade to certain tissues, cells, or sets of target genes is a challenge for approaches that aim to specifically induce certain downstream effects, such as red cell production. In this respect the complexity of HIF regulation with at least 2 oxygen-dependent isoforms, each containing more than 1 hydroxylation site, the prolyl-hydroxylase family of enzymes important for their ongoing destruction, the dependence of these enzymes on cofactors, and the variability in the sensitivity of different target genes to HIF activation, may offer important opportunities.

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