Angiotensin-Converting Enzyme-2 (ACE2)—A New Player in the Genesis of Glomerular Injury?


In the good old days, the world of the renin-angiotensin (ANG) system was clear and straightforward. In a first step, the protease renin cleaved the decapeptide ANGI (angiotensin 1-10) from the substrate angiotensinogen. In a second step, the dicarboxypeptidase angiotensin-converting enzyme (ACE) cleaved 2 amino acids from the carboxyterminus of ANGI to generate the active octapeptide ANGII (angiotensin 1-8). This scheme is not wrong, but as we know today matters are much more complex. By binding to a specific prorenin/rexin receptor in the plasma membrane (1,2), ANGI may be generated locally. Even further complexity was introduced by the discovery of a carboxypeptidase homologous to ACE, called ACE2, (3) sharing >60% sequence similarity with ACE. ACE2 was shown to convert the decapeptide ANGI into the nonapeptide angiotensin 1-9 (4), and as we know today converts with much greater catalytic efficiency the octapeptide ANGII into the heptapeptide angiotensin 1-7 (5,6). It was therefore argued that ACE2 antagonized ACE by shifting the balance of production away from ANGII toward ANG 1-7—acting functionally like a clearance mechanism for ANGII. ANGII was regarded to be the only physiologically active player, whereas ANG 1-9 and ANG 1-7 were initially considered to be relatively uninteresting breakdown products. This view has recently been changed completely (7) with the recognition that angiotensin 1-7 is a vasodilator that antagonizes the effect of ANGII (8), interacting with a specific G protein–coupled receptor, the mas oncogene (9), and for unknown reasons serving as the receptor for the coronavirus SARS (severe acute respiratory syndrome) (10,11). In a crucial study, Crackower et al. (12) produced targeted disruption of ACE2. The ACE2 knockout mice displayed a cardiac phenotype with a severe contractility defect; a cardiac phenotype was seen even in Drosophila with deletion of ACE2. Interestingly, simultaneous disruption of the gene coding for the classic ACE prevented the appearance of the cardiac phenotype. This observation led to the reasonable conclusion that the cardiac abnormalities were the direct or indirect consequence of an excess of ANGII, particularly because measurements yielded increased ANGII concentrations in the plasma and tissues, including the kidneys, of the knockout animals. The cautionary note “direct or indirect” is appropriate, as studies to date cannot distinguish whether the phenotype is the result of an excess of ANGII or of the lack of ANG 1-7 (7).

The expression of ACE2 is high not only in the heart, but also in other organs (13,14), including the kidney. In most studies such renal expression was diminished in adult hypertensive rodent models (12,15); yet matters are more complex, as the expression of ACE2 was increased in normotensive, neonatal, hypertension-prone spontaneously hypertensive rats (SHR) and persisted in the glomeruli of adult hypertensive SHR (16). The expression of ACE2 in the kidney was also diminished in animal models of diabetes (17). These observations lead to the question whether expression of ACE2 is altered, and might even play a causal role, in nondiabetic renal disease.

The ACE2 gene is coded for on the Y chromosome. Oudit et al. (18) examined male (ACE2+/y) and female (ACE2−/−) ACE2 mutant mice and followed them over 12 mo. They measured albuminuria, blood pressure, renal lipid peroxidation products, and activation of extracellular signal–regulated kinases (ERK) 1 and 2 in the glomeruli. In additional series, the effects of simultaneously knocking out the ACE gene (ACE−/−/ ACE2−/−) and of angiotensin receptor blockade were compared.

What were the salient findings? The kidneys of young, male, mutant mice (ACE2−/−) 4 mo old developed normally and showed no gross abnormalities by light microscopy. Even at that early stage, however, electron microscopy revealed mesangial injury with small foci of fibrilar
collagen deposition. At the age of 1 yr, however, light microscopy revealed diffuse glomerulosclerosis in the male, but not in the female, mice. The glomerular basement membranes were unchanged, but there was widespread segmental capillary loop hyalinosis and focal mesangial expansion with increased staining for collagen III and capillary microaneurysm formation. Mesangial cells showed changes resembling a smooth muscle phenotype. Widespread mesangial deposition of fibrillar collagen was also noted.

Plasma creatinine and urea values were normal, but albuminuria was found in the male ACE2−/− mice, but not in the ACE2+/− controls. The systemic BP was even lower in the 1 yr old male ACE2−/− mice compared with the ACE2+/− controls.

Of interest are the contrasting results of the double knockouts ACE−/−/−ACE2−/−/− compared with the angiotensin receptor blockade with irbesartan in the ACE2−/−/− mice. While severe malformation of the kidneys, interstitial fibrosis, and lymphocytic infiltrates, as well as small glomeruli with malformed capillaries, were seen the kidneys of the double knockouts ACE−/−/−ACE2−/−/−, in agreement with previous studies (19), the kidneys of the irbesartan-treated animals were normal and there was complete absence of albuminuria.

The authors assumed that the molecular mechanism involved in the genesis of the renal abnormalities of the male ACE2 knockout mice was the presence of oxidative stress provoked by excess ANGII. To prove this hypothesis, the authors measured the lipid peroxidation products hexanal, malondialdehyde, and hydroxynonenal. They were elevated compared with age-matched wild-type mice and compared with irbesartan-treated ACE2−/−/−. The number of nuclei staining for phospho-ERK[1/2] was unchanged in the tubuli, but significantly higher in the glomeruli of the male ACE2−/−/− mice. Presumably as a result of downregulation in response to increased ANGII, the expression of angiotensin receptor subtype 1 (AT1) receptors and ACE was downregulated, but this was normalized by irbesartan.

In summary, deletion of ACE2 caused a modest, but definite, renal lesion in male mice. The finding is compatible with the assumption that the lesions caused by the absence of ACE2 are due to the increased intrarenal concentrations of ANGII (or, as one must also consider, of decreased concentrations of ANG 1-7). Genetic deletion of other components of the renin-angiotensin system, e.g., angiotensinogen, renin, AT1a + AT1b, causes more or less severe renal malformation (20,21), arguing for a critical role of the renin-angiotensin system in renal organogenesis; this was not seen in mice with genetic deletion of the ACE2 gene.

The absence of glomerular lesions in female ACE2 knockout mice is in line with numerous studies that documented the protective effect of estrogen in renal damage models (22,23).

Why is this study of interest? It is not the severity of the lesion, which is of note. Rather, the study provides proof of the principle that ACE2 is not an innocent bystander in the kidney. When discussing the effects of the renin-angiotensin system or its blockade on the kidney, we have to consider a novel player. In the future ACE2 and its products may even become therapeutic targets. Certainly the presence of this homolog of ACE in the kidney will force us to reinterpret some data and to rewrite our textbooks.

References


The resolution of the sequence of the erythropoietin (EPO) gene and the availability of recombinant EPO for therapy have caused a true revolution in the management of the anemia of uremic patients. In addition, great progress has been made in the clarification of the unique mechanisms controlling the synthesis of EPO and of the molecular mechanisms of its action. Understanding the mechanisms of the regulation of EPO synthesis has contributed to the understanding of the broader issue of cellular adaptation to hypoxia (1).

Von Hippel-Lindau (VHL) disease, a rare genetic condition (2), and discovery of the mutated causal protein in this condition (3) have greatly helped clarify the mechanisms of regulation of EPO synthesis and issues beyond, not the least of which is renal carcinoma formation. The clarification of the molecular basis of the different forms of polycythemia (or erythrocytosis) (4–6) is another impressive confirmation of the concept of Sir Archibald Garrod: when analyzing alkaptonuria (7), he had stated with clairvoyance, much ahead of his time, that when one has understood the rare, genetically determined “inborn errors of metabolism,” one will also obtain insights into the molecular genesis of common polygenic diseases, as we would say today.

To understand the following it is useful to briefly recapitulate the molecular control of EPO synthesis (8). As part of the defense reactions against hypoxia, the transcription complex hypoxia-inducible factor-1 (HIF-1) controls EPO gene expression, mainly in the kidney, as well as ubiquitous hypoxia defense reactions in all mammalian cell types (1). The HIF-1 complex contains a constitutively expressed subunit and the HIF-α subunits HIF-1α or HIF-2α, the latter of which are regulated at the posttranslational level in an oxygen-dependent manner.

How does oxygen regulate the concentration of the HIF complex? The HIF system is primarily regulated not by modulating the synthesis, but mainly by regulating the breakdown via the proteasome pathway in an oxygen-dependent manner. In a two-step process, the HIF molecule is first given the Judas kiss, so to speak, by molecular oxygen reacting with a prolyl residue, which then permits in a second step interaction with and capture by the VHL factor. VHL in the HIF/VHL complex is the substrate recognition unit of a proteasomal E3 ubiquitin ligase complex, which destines the marked molecule to proteasomal destruction. Consequently, the HIF-1α protein is stable under hypoxic conditions, but is degraded with exquisite rapidity under normoxic conditions so that its half-life is one of the shortest of any protein.

The site-specific addition of oxygen to prolines of the α-subunits of HIF is catalyzed by proline hydroxylases, which are 2-oxoglutarate- and ascorbate-dependent and have bivalent Fe⁺⁺⁺ at the active center. Three proline hydroxylase isoforms have been identified, PHD1, PHD2, and PHD3, which are variably expressed in different tissues. Knockdown experiments with small interfering RNA showed that the three isoforms contribute in a nonredundant manner to the regulation of the HIF-1α and HIF-2α subunits, have different selectivities for the different hydroxylation sites within HIF-α subunits, and react differently to stimulatory signals (9). Experiments selectively silencing the PHD2 isoenzyme using short interfering RNA proved that this maneuver is sufficient to stabilize and activate HIF-1α under normoxic conditions in all human cells investigated, thus imitating the effect of hypoxia (10). In reticulocyte lysates, PHD2 is the most abundant isoform (9,11), which is also closely related to the ancestral proline hydroxylase in Caenorhabditis elegans (12). All these points are relevant for the selection of therapeutic targets discussed below.

The above paper by Percy et al. (6) concerns a family with polycythemia (erythrocytosis). It was recognized long ago that—apart from polycythemia vera, a malignancy characterized by
excessive proliferation of erythroid, myeloid, and megakaryocytic elements in the bone marrow—there are rare cases of familial polycythemia (or erythrocytosis), for which mutations in the EPO receptor have been made responsible, resulting in hyperresponsiveness to EPO and low EPO concentrations (4). A widely discussed case concerned the Finnish cross country skier Eero Mäntyranta, who won 4 gold medals, 1 silver medal, and 2 bronze medals in the 1960, 1964, and 1968 winter Olympics as well as several world championships. He had admitted to doping with amphetamine, but later it had also been shown that he had a familial form of autosomal dominant erythrocytosis characterized by increased sensitivity to EPO (13), thought to result from a gain of function mutation of the EPO receptor (4). He had hemoglobin concentrations >20 g/dl since childhood (13). This case, as well as other genetic conditions impacting athletic performance (14), raised the hotly debated ethical issue of whether it is fair to let genetically unprivileged athletes compete with such genetically privileged competitors (15,16), as well as concerns about gene doping (17).

One interesting, endemic, autosomal recessive form of polycythemia, presumably resulting from a single founder in the distant past, had been observed in the Chuvash Autonomous Republic of the Russian Federation in the mid-Volga River region (5), and also, more recently, in southern Italy (18). Meanwhile, sporadic cases have been observed as well (19,20). Customarily one distinguishes primary polycythemias (i.e., inherited or acquired mutations of erythroid progenitor cells rendering them hypersensitive to EPO and causing downregulation of EPO concentrations in the circulation) from secondary polycythemias (i.e., caused by extrinsic factors such as elevated EPO concentrations with normal EPO responsiveness of progenitor cells). Interestingly, the Chuvash polycythemia is characterized by features of both primary and secondary polycythemia, i.e., hypersensitivity of erythroid precursors to EPO and elevated EPO concentrations. A recent paper identified homozygosity with respect to a missense mutation in the VHL protein due to an Arg200Trp substitution as the molecular basis of both the Chuvash polycythemia (21) and the sporadic form of the disease (19), although conceivably compound heterozygotes might have an analogous presentation (20). In the VHL syndrome, mutations of VHL are characterized by hemangioblastomas, pheochromocytomas, and renal cell carcinomas. Although the mutation in the Chuvash polycythemia concerns the VHL protein, such features are not seen, suggesting that either VHL has additional functions unrelated to the HIF system or alternatively that the Chuvash mutation causes a milder dysfunction of the VHL protein, which causes only decreased interaction between HIF-1α and VHL, reduced degradation of HIF-1α, and elevated HIF-1α concentrations under normoxic conditions. As a result, despite the absence of hypoxia, the transcription of HIF-1α-dependent genes is increased, particularly EPO, causing polycythemia, but also—without clinical sequelae affecting the phenotype—other HIF-1α-dependent genes such as glucose transporters, transferrin, transferrin receptor, and vascular endothelial growth factor.

It had long been recognized, however, that forms of familial polycythemia that fail to have mutations of the VHL gene exist (4), raising the issue whether genetic abnormalities of other proteins in the above cascade can produce polycythemia as well.

The above observation of Percy et al. (6) now adds a new twist to this issue by identifying a form of genetically determined autosomal dominant polycythemia that is caused by the mutation of another player in the hypoxia response cascade, upstream of the VHL step, i.e., a mutation the PHD2 isoform of prolyl hydroxylase. The authors investigated a family with polycythemia of father, daughter, and son. They all had somewhat variable mildly elevated hemoglobin values with inappropriate failure of EPO concentrations to be suppressed. There were no features of VHL phenotype. Sequencing revealed in all three family members a heterozygous C to G change at base 950 of the coding sequence of PHD2, resulting in a proline to arginine exchange at codon 317. It had been suspected that this proline is crucial for the coordination of the Fe²⁺ ion at the active site.

Of obvious interest are the functional consequences of this mutation. Without going into details, mutant PHD2 bound more weakly to HIF-1α than wild-type PHD2. Does less binding translate into less hydroxylation of HIF-1α and HIF-2α? In a proline hydroxylase assay in the
presence of the necessary cofactors (2-oxoglutarate, ascorbic acid), the HIF hydroxylase activity of the mutant PHD2 was significantly diminished and it was also less able to suppress the HIF-1α or hypoxia-induced activation of a reporter gene.

This rare and, clinically speaking, not extremely important form of familial polycythemia is of great interest also to nephrologists and is informative in several respects. First, as outlined above, PHD2 is the key PHD isoform that regulates HIF under physiologic conditions. Of interest with respect to erythropoiesis, it is also the isoform that is most abundant in reticulocyte lysates (9,11). PHD2 also appears to be the weakest link in the chain of the hypoxia response cascade, as apparently near-haploinsufficiency for PHD2 in the EPO-producing cells of these patients was sufficient to cause polycythemia, suggesting that in these cells there is not much PHD2 reserve capacity, making this an obvious target for intervention. This raises the issue whether pharmacologic inhibition of this weak spot would make sense. Of the three major factors necessary for proline hydroxylation, \textit{i.e.}, ascorbate, Fe\textsuperscript{3+}, and 2-oxoglutarate, 2-oxoglutarate appears to be an obvious candidate. Indeed, pharmacologic inhibition by what presumably is an analog of this cofactor (22) and can be administered orally has been tried successfully in a small number of patients with renal failure who responded with a significant increase of Hb concentration (23).

Apart from pharmacokinetics and potential toxicity, the issue now is long-term safety, as stabilization and upregulation of HIF cause a wide spectrum of reactions (1), of which increased angiogenesis (24) and tumor growth (22) are theoretically of greatest concern. It is here that both the observations in Chuvash polycythemia (21) and particularly the polycythemia of PHD2 malfunction (6) is somewhat reassuring, as no major clinical problems, particularly no malignancies, were seen with the exception of esophageal carcinoma in the propositus of the paper by Percy \textit{et al.} (6), which in a smoker might have another explanation. It should not give the nephrologist a headache whether low molecular weight inhibitors, presumably difficult to detect in urine samples that are not strictly timed, might further increase athletic performance in future Tours de France.

References


