Using Transgenic Mice to Analyze the Mechanisms of Progression of Chronic Renal Failure

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Abstract. An understanding of the mechanisms underlying the formation of renal lesions is necessary for the development of strategies aiming to delay the progression of chronic renal failure. The generation of transgenic mice in the past 20 years has contributed significantly to the study of this phenomenon. Overexpression and/or inactivation of single factors in renal tissue demonstrated that molecules such as growth factors, proto-oncogenes, and renin-angiotensin system elements play major roles in renal deterioration. Several mouse models of renal injury have been developed in the past 10 yr. Transgenic mice that exhibit a normal phenotype under physiologic conditions allow analysis of the roles of single factors in the progression of chronic renal failure when renal injury models are used. Using this strategy, it was demonstrated that vascular adaptation, which is a process that involves the endothelin/nitric oxide balance, is essential for the survival of mice after nephron reduction and that the epidermal growth factor/activator protein-1/Bcl-2 pathway is involved in the development of renal lesions after renal injury, possibly via adjustment of the proliferation/apoptosis balance. Moreover, it was demonstrated that selective inhibition of epidermal growth factor signaling in the kidney successfully prevents the progression of chronic renal failure. These results indicate the power of transgenesis for elucidation of the pathogenesis of renal disease.

The relentless progression of human renal disease to end-stage renal failure is a phenomenon that remains largely unexplained. Recently, two approaches have given new impetus to the study of progression. The first is the development of several mouse models of renal injury that mimic human renal disease. The second is the generation of several lines of transgenic mice that lack or overexpress factors thought to be involved in renal deterioration. We combined these two approaches to investigate the different steps in the cascade of events leading to renal destruction after renal injury.

Role of the Genetic Background in Experimental Models of Chronic Renal Injury in Mice

In the past century, several models of chronic renal injury have been developed in rats for analysis of the consequences of chronic renal failure, such as metabolic disorders, hypertension, and renal deterioration. The reduction of renal mass is the most widely used model to investigate the molecular mechanisms underlying the progression of chronic renal failure (1). Indeed, numerous studies have demonstrated that severe nephron loss induces renal deterioration independent of the initial process (ischemic, toxic, or immunologic). Two models of nephron reduction are currently used, i.e., unilateral nephrectomy and subtotal nephrectomy. Unilateral nephrectomy has been used mainly to study renal compensatory growth and to accelerate the progression of renal lesions in other models of renal injury, such as toxic agent-induced nephropathy, diabetest, or deoxycorticosterone acetate (DOCA)-salt hypertension (1). Unilateral nephrectomy alone induces very few lesions in animals (rats and mice), except in old ones. Subtotal nephrectomy is performed using two main procedures, i.e., ligation of the branches of the renal artery or ablation of the two poles of the left kidney, followed by contralateral nephrectomy. The first method causes severe and immediate hypertension because of renal ischemia; the second is less hypertensive and allows more accurate quantification of the degree of renal ablation. Whichever method is used, subtotal nephrectomy in rats induces (1) immediate and sustained vasodilation, which is paralleled by an increase in GFR in the remaining nephrons; (2) compensatory growth of the remnant kidney, which is observed between the first and second week after surgery; and (3) renal lesions (glomerulosclerosis, tubular atrophy and/or dilation, and interstitial fibrosis), which develop approximately 1 mo after surgery and lead to complete destruction of the remnant kidney. In rats, the evolution toward end-stage renal failure depends mainly on the severity of the initial renal ablation and may be modified by environmental factors, such as diet or drugs (1). To date, very few data on the natural history of renal mass reduction in mice are available. It is clear that, in mice as well, hemodynamic adaptation occurs and plays a major role in maintaining renal function, as discussed below. The kinetics and magnitude of renal compensatory growth are apparently similar in mice and rats. We observed that, 14 d after 3/4 nephrectomy, the weight of the remnant kidney doubled in rats and mice and became close to that of control animals (2) (unpublished observations). However, the
development of renal lesions is not constant in mice and depends on the genetic background. Indeed, in C57BL6×DAB2 mice, 75% reduction of the total renal mass induced only moderate tubular lesions even 8 mo after surgery. In contrast, in FVB mice, similar nephron reduction resulted in severe glomerulosclerosis and tubular cystic dilations as early as 2 mo after surgery (unpublished observations). Along the same line, unilateral nephrectomy induced glomerulosclerosis in ragged oligosyndactyly pintail mice but not C57BL6 mice (3), a strain that is resistant to the development of renal lesions up to 44 wk after subtotal nephrectomy (4). In contrast, subtotal nephrectomy induced severe glomerulosclerosis in 129/sv mice 4 mo after surgery (5). Taken together, these results emphasize the key role played by genetic factors in the response to nephron reduction and suggest a role of “modifier” genes in the evolution of renal diseases.

To use lines of C57BL6×DAB2 transgenic mice, we developed three other experimental models of chronic renal injury, i.e., (1) prolonged renal ischemia (50-min clamping of the renal vascular pedicle), (2) folic acid-induced nephropathy (a single intraperitoneal injection of folic acid), and (3) cisplatin-induced nephropathy (a single subcutaneous injection of cisplatin). In C57BL6×DAB2 mice, all three procedures induced intense tubular cell proliferation, followed by the development of numerous areas of atrophic tubules, marked interstitial fibrosis, and mild multifocal mononuclear cell infiltrates. These lesions were less severe in folic acid-treated animals. It is noteworthy that the same procedures did not result in chronic renal lesions in rats, again demonstrating the difficulty of adapting the well known rat models of renal injury to mice. However, mice, like rats, are susceptible to the development of moderate glomerular lesions after anti-Thy antibody injection and severe renal atrophy after ureteral obstruction.

Two Different Strategies to Analyze Renal Deterioration Using Transgenic Mice

Several hypotheses have been proposed to explain the development of renal lesions after nephron reduction, i.e., glomerular hyperfiltration, glomerular hypertension, tubular hypermetabolism, and renal hypertrophy (6). All hypotheses rely primarily on differences observed between protective and nonprotective conditions, particularly the changes induced by protein or sodium diets and by antihypertensive drugs. The complexity of the pathways modified by these treatments made it difficult to reach conclusions regarding the roles of single factors. Indeed, an ideal model to study the direct effect of a candidate factor on the development of renal lesions requires that the kidney be continuously and selectively exposed to or deprived of that single factor. This goal could be achieved by inactivation of the gene encoding a specific factor or introduction of a gene into renal tissue, via germline gene manipulation or in vivo gene transfer (7,8). Selective renal overexpression can be obtained by using promoters that target transgene expression to different segments of the nephron (7). Similarly, the use of these promoters in the Cre/lox system could allow target gene inactivation in the kidney. To date, hundreds of different transgenic mouse lines have been generated, and a number spontaneously exhibit a renal phenotype (9). Several mouse lines have been produced to investigate the roles of growth factors (9,10), the fibrinolytic system (11), and the angiotensin II pathway (12) in renal deterioration. These models provided clear evidence that several of these molecules are involved in the development of renal lesions. However, their role in the study of chronic renal failure is limited by the fact that these mice spontaneously developed renal lesions, in the absence of an exogenous injury. This led to increased interest in the use of transgenic mice, which reproduce without an obvious phenotype under physiologic conditions, to analyze the effects of renal injury. In our laboratory, we chose this strategy to investigate the role of vascular adaptations and renal growth in the development of renal lesions.

Study of Hemodynamic Adaptation to Nephron Reduction Using Vimentin-Null Mice

Reduction of renal mass is known to induce immediate and sustained vasodilation, which is paralleled by increases in GFR in remaining glomeruli. Several studies suggested that these hemodynamic adaptations participate in the development of renal lesions by stretching mesangial cells, changing the capillary permeability, and damaging the surrounding endothelial and epithelial cells (6).

Vimentin is a class III intermediate filament protein that is mainly expressed in mesenchyme-derived cells, including endothelial and vascular smooth muscle cells (13). In normal adult kidney, vimentin is strongly expressed in both vessels and glomeruli. The observation that the vimentin network is modified by mechanical stress in vitro (14) suggests that this protein is involved in the regulation of vascular tone, including vascular adaptation to nephron reduction. A line of mice bearing a null mutation of vimentin gene has been generated (15). Interestingly, these animals develop and reproduce without an obvious phenotype. Moreover, they exhibit normal renal morphologic features and functions under physiologic conditions (16). Therefore, we used homozygous vimentin-null mice (Vim−/−) and wild-type littermates (Vim+/+) to investigate the role of vascular adaptation to nephron loss (17). Ablation of 75% of the total renal mass was lethal, within 3 d, for 100% of Vim−/− mice, whereas no lethality was observed among their Vim+/− littermates. Death in Vim−/− mice resulted from end-stage renal failure. In fact, plasma creatinine concentrations were higher in Vim−/− animals than in Vim+/+ littermates and were comparable to values observed for wild-type binephrectomized mice. Because the morphologic features of Vim−/− remnant kidneys were normal, we hypothesized that renal failure in Vim−/− mice resulted from increased arterial vasoconstriction and/or reduced arterial vasodilation. To test this hypothesis, we analyzed the ability of renal resistance arteries from mutant and wild-type mice to respond to chemical and mechanical stimuli in vitro. In renal arteries from Vim−/− mice, the contractile response to endothelin (ET) was selectively increased, whereas sensitivity to acetylcholine, a nitric oxide (NO)-dependent relaxing agent, was reduced. With re-
spect to the responses to mechanical stimuli, the myogenic tone (stretch stress) was increased in Vim\(^{-/-}\) renal arteries, whereas flow-induced dilation (shear stress), which depends on endothelial NO synthesis, was decreased. Similarly, remnant kidneys from Vim\(^{-/-}\) mice synthesized more ET but less NO, compared with kidneys from wild-type animals. Finally, infusion of bosentan (an ET-1 receptor antagonist) prevented death in 100% of Vim\(^{-/-}\) nephrectomized mice and restored renal function to a range similar to that observed for wild-type littermates. Taken together, these results illustrate the extent to which the ET/NO ratio is crucial in the maintenance of renal function after nephron reduction.

The molecular mechanisms responsible for vascular dysfunction in mice lacking vimentin remain unclear. Cytoskeletal proteins are thought to participate in the mechanotransduction of shear stress by modulating the transcription of several molecules, including ET and NO synthase (18). It was recently suggested that nephron reduction induced flow-induced dilation of renal arteries. We propose that, in mutant mice, the lack of a vimentin network impaired nephrectomy-induced shear-stress transmission, giving rise to the ET/NO imbalance.

The fact that Vim\(^{-/-}\) mice die within 72 h after surgery precludes conclusions regarding the role of hemodynamic adaptation in the renal deterioration process. However, our results provide the first evidence that these processes are necessary for survival after renal mass reduction, and they support the careful use of drugs aiming to reduce vascular modifications, at least in the early period after nephron loss.

**Role of the Epidermal Growth Factor Pathway in Renal Growth and Renal Deterioration**

Reduction of renal mass triggers molecular and cellular events that promote compensatory growth of the remaining nephrons, which precedes glomerular and tubular lesions and ultimately leads to end-stage renal failure (6). The mechanisms that link compensatory renal growth to renal deterioration remain poorly understood. The occurrence of both glomerular enlargement and cystic tubular dilation, as well as expansion of the interstitial space, suggests that excessive cell proliferation and/or defective cell death plays a key role. Both proliferation and apoptosis of renal cells could be triggered by a number of growth factors and/or proto-oncogenes that are synthesized within the kidney and act through specific membrane-bound receptors, possibly through autocrine or paracrine pathways (10). The expression of these molecules in the kidney is increased in experimental and human nephropathies (10). Among these paracrine modulators, epidermal growth factor (EGF) is of special interest because (1) its expression is increased in several renal pathologic conditions characterized by intense cell proliferation, such as nephron reduction (19), polycystic kidney disease (20), and adenocarcinoma (21); (2) transgenic mice that overexpress different molecules of the EGF pathway develop renal lesions (22–24); and (3) experimental approaches aiming to block EGF receptor (EGFR) signaling halt the evolution of renal disease in polycystic kidneys (25) and adenocarcinomas (26). Moreover, we demonstrated that the entire EGFR/EGF/activator protein-1 (AP-1) pathway is overexpressed after nephron reduction in rats (10) and this upregulation is blunted by moderate restriction of dietary sodium, which reduces the progression of renal lesions (27,28). These results suggest that EGFR activation could be a major determinant in the development of renal lesions, possibly via adjustment of the proliferation/apoptosis balance. To test this hypothesis, we used three different lines of transgenic mice, i.e., dominant-negative EGFR mice, Bel-2 mice, and junD-null mice.

**Study of the EGF Pathway in Renal Deterioration Using Dominant-Negative EGFR Mice**

Genetic approaches aiming to block EGFR signaling face two major difficulties, namely that EGFR-null mice are not postnatally viable and that inactivation of one of the numerous EGFR ligands might not be informative because of ligand redundancy. Therefore, we used a dominant-negative strategy to generate transgenic mice expressing a carboxyl-terminally truncated EGFR under the control of the kidney-specific type I \(\gamma\)-glutamyl transpeptidase promoter (29). As expected, the transgene was expressed exclusively in basolateral membranes of proximal tubular cells. Under physiologic conditions, mice exhibited normal renal morphologic features and functions. The mutant receptor behaved as a dominant-negative effector because it prevented EGF-signaled EGFR autophosphorylation after EGF infusion. Transgene expression did not affect kidney development, probably because of the weak expression of the \(\gamma\)-glutamyl transpeptidase promoter during development. In addition, phosphorylation of the endogenous receptor was not completely prevented by this dominant-negative strategy. It is noteworthy that kidneys also developed normally in waved-2 mutant mice, in which the mutated EGFR is only partially impaired (30).

We next investigated the effect of inhibition of the EGF pathway on the development of renal lesions in two models of renal injury, i.e., subtotal nephrectomy and prolonged renal ischemia. Eight months after 75% ablation of the total renal mass, tubular lesions were less severe in transgenic mice than in wild-type littermates. This effect was associated with a reduction of tubular cell proliferation, suggesting that activation of EGFR is one of the first events triggering cell proliferation after nephron reduction. A similar mechanism was recently implicated in the development of tubular dilation in several models of polycystic kidney disease (31). Twenty-eight days after prolonged ischemia (50-min clamping of the left renal vascular pedicle), tubular atrophy and interstitial fibrosis were reduced in transgenic mice, compared with wild-type littermates. The beneficial effects of the transgene included reductions in tubular cell proliferation, interstitial collagen accumulation, and mononuclear cell infiltration. Involvement of the EGF pathway in fibrogenic processes was previously observed in lung, using a transgenic approach. Indeed, mice that selectively overexpressed transforming growth factor-\(\alpha\), a
ligand of EGFR, in lung developed several areas of fibrotic lesions (32). These lesions were reversed in double-transgenic mice that expressed both transforming growth factor-α and a dominant-negative mutated EGFR (33). The mechanism by which tubular EGFR induces interstitial fibrosis in kidney remains to be elucidated. From in vitro results, we speculate that, in proximal tubules, EGF could stimulate (1) the conversion from tubular cells to fibroblasts, via increases in fibroblast-specific protein-1 expression (34), (2) the synthesis of matrix collagens (35), and (3) the production of tubular cytokines, which mediate the cross-talk between tubular and interstitial cells (36).

Study of Proliferation/Apoptosis in Renal Deterioration Using Bcl-2 and junD-Null Mice

Apoptosis (programmed cell death) is now recognized to play an important role in the regulation of the number of renal cells in both acute and chronic renal failure (37). Bcl-2, a potent inhibitor of cell death, is suspected to play a pivotal role in these processes (38). In proximal tubular cells, EGFR stimulates cell proliferation via AP-1 activation and inhibits apoptosis via Bcl-2. The respective roles of cell proliferation and cell death in renal deterioration were investigated by using Bcl-2- and junD-null mice (40). Both strains exhibit an apparently normal renal phenotype under physiologic conditions.

We demonstrated that, after prolonged renal ischemia, mice that overexpressed Bcl-2 in proximal tubular cells developed more severe tubular atrophy, interstitial fibrosis, and mononuclear cell infiltration than did wild-type littersmates (F. Terzi, A. Winckel, unpublished observations). This impairment was associated with a decrease in apoptosis but also with an increase in cell proliferation in proximal tubules. The stimulation of cell proliferation seems to be specific to renal tubular cells, because overexpression of Bcl-2 inhibits cell proliferation by blocking cell cycle entry in lymphocytes and thymocytes (41). Elucidation of the reason for this discrepancy requires further investigation.

AP-1 is a transcription factor that results from heterodimerization of one of the four Fos proteins (c-Fos, FosB, Fra1, and Fra2) with one of the three Jun partners (c-Jun, JunB, and JunD) (42). AP-1 activation mediates the mitogenic effects of polypeptide growth factors, including EGF. In previous works (2,28), we demonstrated that the expression of c-Fos and c-Jun proteins, the two major partners in the AP-1 complex, is increased after nephron reduction. Because JunD protein, in contrast to c-Jun, negatively regulates cell growth (43), we evaluated the effect of nephron reduction in mice lacking the junD gene (40). Two months after 75% nephron reduction, junD-null mice developed severe glomerulosclerosis and tubular dilations, whereas no lesions were observed in wild-type littersmates (F. Terzi, M. Burtin, unpublished observations). The more severe renal lesions were associated with increased cell proliferation, confirming the crucial role of AP-1 activation in these phenomena.

In conclusion, using several transgenic approaches, we demonstrated that the activation of the EGF/AP-1/Bcl-2 pathway is a major determinant in the development of renal lesions after renal injury, possibly via adjustment of the proliferation/apoptosis balance. Moreover, we demonstrated that selective inhibition of EGF signaling in the kidney successfully prevents the progression of chronic renal failure. A specific inhibitor of EGFR tyrosine kinase activity was recently produced. Its use halts the evolution of polycystic kidney disease in mice (44). Taken together, these findings strongly suggest that strategies aiming to block the EGFR pathway could be useful in delaying the progression of chronic renal failure in human patients.

References


