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Commentary

Size does matter: Will knockout of $p21^{WAF1/CIP1}$ save the kidney by limiting compensatory renal growth?

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When the work load presented to an organ increases, its functional response is proportionately augmented, a finding that forms one of the self-evident truths of homeostasis. For large and chronic increases in work load, homeostasis is often maintained by an increase in the number of functioning units. For example, in breast tissue, new acini develop during lactation, and similarly, chronic hypoxia induces an increase in the number of erythrocytes. However, in many organs, there is a predetermined limit to the total number of functioning units. For instance, in the kidney and lung, the number of nephrons and alveoli is set early in life, and no matter how high the demand, that number of functional units does not increase. Similarly, in response to increased work loads, there are no increases in the number of fibers in cardiac and skeletal muscle or in the number of absorptive villi in the intestine. The inability to reinitiate the developmental processes responsible for functional unit generation "forces" the organ to hypertrophy, i.e., to increase the physical size of the unit to increase work capacity. The signaling mechanism or mechanisms that initiate the hypertrophic growth process remain elusive. However, it is known that, after removal of one kidney, compensatory hypertrophy is mediated by more than just the increase in the filtration rate. More recently, it was discovered that a reduced number of functioning nephrons leads to an inexorable loss of the function of those remaining (1–3). Similarly, pressure-overload hypertrophy of the heart is associated with a progressive decline in its mechanical function (4, 5). Somewhat counterintuitively, limiting the compensatory growth response in these conditions is beneficial and has led to many of the recent advances in therapeutics.

The growth response to loss of renal mass depends on the amount of tissue loss. After the removal of one kidney, the resulting growth pattern is one of hypertrophy (increase in cell size) without hyperplasia (increase in cell number; ref. 6). This hypertrophy primarily involves the proximal tubules and correlates with an increase in kidney weight. In contrast, removal of more than one kidney, the so-called five-sixths nephrectomy remnant-kidney model that was used in the paper by Megyesi *et al.* (7), leads to a combination of hyperplasia and kidney enlargement; the kidney enlargement presumably reflects hypertrophy of the remaining nephrons. This observation suggests that the hyperplasia is induced by the renal injury, whereas the hypertrophy is associated with the increased demand for work capacity. A similar scenario exists for the glomerulus, in which hyperplasia is associated with glomerular injury but not with a reduction in glomerular number *per se*. Remarkably, cell-cycle proteins seem to be involved in both hypertrophy and hyperplasia (6). Hence, the availability of mice lacking one or another of these critical factors presents an opportunity, heretofore unavailable, to break into this complex pathway and examine its role in the genesis of progressive kidney failure induced by reduction in nephron mass. Megyesi *et al.* (7) have examined this progressive decline in kidney function in mice lacking $p21^{WAF1/CIP1}$ (p21), an inhibitor of the cyclin G_1 kinase. Removal of 80% of the kidney mass from wild-type mice results (as expected) in glomerular and tubular enlargement and eventual sclerosis and failure of the remaining nephrons. Remarkably, the mutant mice did not develop sclerosis or renal failure. They develop all of the characteristic changes associated with compensatory hypertrophy: their glomerular filtration rate increases initially, and their glomeruli enlarge, as does whole-kidney mass. Despite all of these well known (and well blamed) changes, the filtration rate of the remaining nephrons does not decline, and no sclerosis develops. How can removal of an inhibitor of the cell-cycle kinases protect the kidney from the devastation seen in this condition? Do these results suggest a new form of treatment of progressive renal failure?

Because of the extensive loss of renal function, the remnant-kidney model leads to a uremic state. Shortly after the reduction in kidney mass, the glomerular capillary pressure increases, leading to increases in filtration rate in the remaining nephrons and in the volume of remaining viable tissue (1). It has been difficult to arrive at a single pathway that explains the pathogenetic mechanism of this deterioration in kidney function. Progressive renal failure in this and other models can be prevented or reduced by a variety of procedures, including low-protein or low-phosphorus diets (reviewed in ref. 1). Recent evidence suggests that these disease processes increase the expression of transforming growth factor type β (TGFβ), which in turn induces the transcription of collagen I and III, thereby causing the fibrosis (8). The proximate signal for induction of TGFβ itself is unknown, but glomerular hypertension and angiotensin II have been implicated in some models. Prevention of TGFβ accumulation or interruption of the angiotensin II pathway leads to amelioration of the fibrosis and of the progression of renal failure (1, 9). Indeed, inhibition of angiotensin-converting enzyme reduces the level of angiotensin and is a widely used treatment.

The papers by Megyesi *et al.* (7) and Park *et al.* (10) show that some hyperplasia occurs in the remaining viable tissue. However, hyperplasia *per se* usually does not correlate with kidney enlargement, suggesting that there is also hypertrophy of the remaining functional nephrons. The cause of the progressive decline in renal function is still unknown. It could be caused by the toxic effects of the uremic state or by detrimental sequelae of the hyperplastic and/or hypertrophic growth processes. There had been a suggestion in the older literature that hypertrophy is maladaptive, whereas hyperplasia is protective (11). Can one actually implicate hypertrophy as opposed to hyperplasia as the cause of maladaptation?

Renal hyperplasia does not occur unless the cells progress successfully through at least one round of the cell cycle, which involves activation of the G_1 kinases and reduced expression or association of cyclin kinase inhibitors with these kinases.

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Compensatory renal hypertrophy and diabetes-induced renal hypertrophy are also associated with regulation of the G_1 kinases and G_1 kinase inhibitors (12). p21 is a member of the Cip/Kip family of cyclin kinase inhibitors that regulate G_1 kinase activity. Unlike other G_1 cyclin kinase inhibitors, p21 seems to function in a more complex manner. After DNA damage in cisplatin-induced acute renal failure, transient ischemic injury, or ureteral obstruction, p21 is induced and functions as a kinase inhibitor that blocks progression into S phase (12). Similarly, increases in p21 expression are associated with antiproliferative conditions, such as those that exist after induction of TGFβ expression. However, recent studies have suggested that it is the stoichiometric ratio of p21 to the kinase subunits that defines the role of p21. When the stoichiometric ratio is low, p21 facilitates kinase complex assembly and thus promotes kinase activation. At higher stoichiometric ratios, p21 serves as a kinase inhibitor and blocks cell-cycle progression (13). In contrast, association of p27, another member of the Cip/Kip family of cyclin kinase inhibitors, with G_1 kinases always results in reductions in kinase activity and in the rate of proliferation, regardless of its stoichiometric ratio.

Interestingly, proximal-tubule hyperplasia, as shown by increased proliferating cell nuclear antigen (PCNA) expression, is evident in the p21−/− but not p21+/+ mice (7). Recent studies by another group have shown that induction of a glomerular immunologic injury (by infusion of antiglomerular antibodies) results in an initial inflammation that is eventually followed by sclerosis. When the same disease is induced in mice lacking p21, there is excessive proliferation of visceral glomerular epithelial cells with consequent acceleration of renal failure (14). These two models of renal injury dissociate hyperplasia from renal failure. Clearly, increased proliferation of visceral glomerular epithelial cells after immune injury worsens renal function, whereas increased proliferation of proximal tubules in the remnant-kidney model is not associated with worsening renal function. What is clear from these studies is that p21 helps define the magnitude of proliferation in response to stress and injury. However, the role of hyperplasia in preventing renal failure is less clear.

We also noted that all kidney-growth parameters reported by Megyesi *et al.* (7) are lower in the p21−/− mice compared with the p21+/+ mice, although the differences are not large. Kidney growth can be estimated in a number of ways. One commonly used approach is to rely on the remarkably constant kidney-to-body weight ratio seen when a normal animal matures, a method that corrects for changes in body mass or altered metabolic states that might obscure changes in kidney growth. A comparison of the initial kidney-to-body weight ratio shows an average of 20% for p21−/− mice and 24% for p21 +/+ mice; these ratios suggest that mutant kidneys are smaller because of reduced growth rather than a decreased number of nephrons, because the number of glomeruli per kidney is similar. Furthermore, after a severe reduction in renal mass, the maximal kidney size seems to be limited in the p21−/− compared with p21+/+ mice. In the mutant mice, the kidneys grow to a maximum size of 65% greater than the initial weight by 6–8 weeks. This growth seems to be sufficient to normalize blood pressure but not to allow deterioration of glomerular filtration rate and glomerular sclerosis. On the other hand, in p21+/+ mice, there is a progressive increase in kidney weight, which seems to reach a maximum size that is 100% greater than the initial size by 14–16 weeks. This amount of growth seems to be detrimental: as hypertension persists, filtration rate declines, and glomerulosclerosis occurs. These studies show, in support of previous hypotheses, that some renal growth, presumably proximal-tubule hypertrophy, is beneficial after loss of renal mass but that too much growth is detrimental. Is there a regulatory mechanism that defines maximal nephron size? Why is compensatory renal growth self-limiting? What these studies show is that p21 may play a key role in determining how much renal growth occurs after the loss of renal mass.

It has been shown that, in compensatory renal hypertrophy, cells enter the G_1 phase of the cell cycle but that progression to S phase is prevented. This mechanism of hypertrophy is referred to as cell-cycle-dependent hypertrophy and is associated with activation of the early G_1 kinase, cdk4/cyclin D kinase, and with a failure to activate sufficiently the late G_1 kinase, cdk2/cyclin E kinase (6). This pattern of kinase regulation is associated with the proximal-tubule hypertrophy that occurs after removal of one kidney and in diabetes mellitus (15, 16). cdk2/cyclin E kinase, which is maximally activated in late G_1, hyperphosphorylates the retinoblastoma protein, rendering it inactive and permitting progression to S phase. It has been proposed that cdk4/cyclin D kinase regulates the physical cell growth that occurs in G_1 and that is part of both hyperplasia and hypertrophy, whereas the level of cdk2/cyclin E kinase activity defines the growth pattern as either hyperplasia or hypertrophy (6). Cells must complete all phases of the cell cycle for hyperplasia to occur, and cells must enter G_1 but not progress to S phase for cell-cycle-dependent hypertrophy to occur; hence, it seems that hyperplasia and hypertrophy cannot occur simultaneously. Indeed, the inability to have both hypertrophy and hyperplasia in the same cells at the same time has been observed in renal growth after induction of diabetes mellitus; 2 days after induction of diabetes, hyperplasia is seen with increased cyclin D and E kinase activities and BrdUrd incorporation. Later (days 5–10), the growth pattern is hypertrophic with an increased cyclin D kinase activity, decreased cyclin E kinase activity, baseline levels of BrdUrd incorporation, and proximal-tubule hypertrophy (15).

We suggest that the ability of p21 to have both a positive and negative effect on cell-cycle progression may explain the difference in growth patterns in the remnant-kidney model in p21+/+ and p21−/− mice. In the p21+/+ mice, the presence of p21, either at detectable levels or not, may be sufficient to prevent proximal-tubule proliferation via its role as a negative regulator of cell-cycle progression, thereby allowing more compensatory renal growth by arresting the cells in the G_1 phase. In the p21−/− mice, the opposite would be true. The lack of p21 eliminates the negative regulatory role, permitting proliferation and thereby limiting compensatory renal growth. Because the decline in renal function is associated with the greater amount of hypertrophic growth and not with hyperplasia, it is possible that the older literature is correct: hypertrophy is maladaptive (9).

This paper is the first to show that p21 might regulate nephron size during normal development and after reduction of renal mass (7). Many patients who donated or lost a kidney do not suffer long-term effects of renal function (17). Perhaps the answer lies in p21 expression; in human kidneys, p21 expression may be poised to limit compensatory renal growth, thereby preserving long-term renal function after removal of one kidney.

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