Not Just an Inhibitor: A Role for p21 Beyond The Cell Cycle—“The Truth Is Rarely Pure and Never Simple”

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The global public health importance of the current accelerating epidemic of diabetes and its attendant complications cannot be overstated. The number of patients receiving renal replacement therapy in the United States is set to double over the next decade, with diabetic nephropathy (already the leading cause of end-stage renal failure) being the principle diagnosis in the majority. Nor will this increase be a transient phenomenon – it has recently been estimated that the life-time risk of developing diabetes for a child born in 2000 is of the order of 30% (1). The incidence of nephropathy in these individuals may approach 50%, further adding to the health, social, and economic burden of diabetes (2). While primary prevention has to be the cornerstone of management, a better understanding of the molecular mechanisms that lead to the changes occurring in the diabetic kidney is also critical in addressing this problem.

Elsewhere in this issue of JASN, Fan and Weiss (3) show that mesangial cell hypertrophy in response to high glucose can be reduced using antisense oligonucleotides directed at a cell cycle protein, p21. Why should we care about mesangial cell hypertrophy? Diabetic nephropathy is characterized by mesangial expansion, which is due to a combination of increased mesangial cell size (hypertrophy) and increased accumulation of extracellular matrix proteins. Although the consequent glomerular and kidney enlargement may be initially associated with hyperfiltration, glomerulosclerosis then develops with compromise of renal function. Studies have shown that reducing mesangial hypertrophy additionally reduces matrix deposition, indicating interdependence of these two pathologic processes, and has beneficial effects on renal function.

So what is p21, and what does it have to do with the development of hypertrophy? p21 is a p53-responsive gene and best known as a mediator of cell cycle arrest (4). The progression of the cell cycle from quiescence to mitosis depends on the sequential expression of cyclins, which then interact with and activate specific cyclin dependent kinases (CDK) (5). The activity of these cyclins is restrained by two classes of inhibitors: the INK4 proteins, which bind CDK4 and CDK6 and cause dissociation of the CDK-cyclin complex, and the Cip/Kip family, which includes p21 and p27. The Cip/Kip proteins interact with all CDK-cyclin complexes with varying avidity, without causing their dissociation, and may therefore exert their inhibitory function throughout the cell cycle. After initiation of the cell cycle in response to mitogens, cyclin D–CDK4/6 becomes active and protein synthesis, and hence cell size, increases in anticipation of mitosis. However, if subsequent cell cycle progression is blocked, for example by exposure to the anti-mitogen TGF-β, there is an increase in the levels of p21 and p27, cyclin E–CDK2 does not become active, and pRB remains hypophosphorylated and restrains the activity of the E2F transcription factors. The pro-proliferative E2F-responsive genes are therefore not transcribed, and the cell remains trapped in G1, where it enlarges, undergoing hypertrophy.

Several in vivo studies have indicated a role for the CDK inhibitors, both p21 and p27, in the glomerular hypertrophy associated with diabetic nephropathy. p21 was increased in the glomeruli of mice with experimental diabetes (6), and glomerular hypertrophy did not occur in diabetic p21−/− mice (7). Importantly, this abrogation of hypertrophy was accompanied by a decrease in immunostaining for specific matrix proteins, despite a comparable increase in TGFβ in both the p21+/+ and p21−/− mice, and the diabetic p21−/− mice did not develop proteinuria. Similarly, p27 was increased in the glomeruli of diabetic db/db mice (8), and there was no change in glomerular volume after the induction of diabetes in p27−/− mice (9). This study also reported a reduction in mesangial expansion despite an increase in TGFβ, and the p27−/− mice did not develop proteinuria. A plethora of in vitro data support these results for p27 (10–14), and the current paper by Fan and Weiss (3) in this issue of JASN uses human mesangial cells to further confirm the contribution of p21 to diabetic-induced cellular hypertrophy. Although an increase in p21 has been reported in mesangial cells derived from p27−/− mice (12), this does not appear to fully compensate for the lack of p27; indeed, the presence of both CDK inhibitors is required for the maximal hypertrophic effect of TGFβ in mouse mesangial cells (15).

Fan and Weiss (3) describe a reduction in human mesangial cell hypertrophy in response to high glucose and insulin-like growth factor-1 after the administration of phosphorothioated antisense oligodeoxynucleotides (ODN) to p21. Interestingly, when p21 is knocked down, the lack of hypertrophy is not a consequence of them completing the cell cycle, as proliferation rates (determined by 3H thymidine incorporation) in these cells were further reduced compared with control cells exposed to high glucose alone. The anti-proliferative effect of inhibiting p21 may reflect its positive cell cycle regulatory role as an assembly and nuclear import factor for cyclin D and CDK4/6 (16,17). Cytoplasmic localization of p21 has been reported to promote cell cycle progression (18,19). It may be speculated that the decrease in proliferation observed after inhibition of
p21 might be due to the subcellular localization of p21, with the p21 protein remaining after treatment with p21 ODN being confined to the nucleus.

The role of p21 in other cell processes beyond the cell cycle has recently attracted increasing attention (20). After injury, there are a number of stereotypical responses that may occur in different cell types, including proliferation, apoptosis, hypertrophy, and changes in cell shape. Involvement of p21 in these responses has been reported in several cell types, and its subcellular localization determined. Cytoplasmic localization of p21 appears to be critical in determining its ability to protect cells from apoptosis (21,22) and alter cell morphology (23,24).

Following the demonstration that the human genome encodes a mere 30,000 proteins, there has been increased appreciation of the vital importance of alternative splicing, post-translational modifications, and subcellular localization of transcribed proteins in augmenting the functional capacity of our thrifty DNA. The involvement of p21 in diverse cell processes exemplifies the potential contribution of an individual protein to several pleiotropic pathways. The development of specific antisense strategies and optimization of the techniques for their administration offers the opportunity to clarify the role of any protein in health and disease in vivo—where more surprises almost certainly await. For the patients swept up in the epidemic of diabetes with its consequent renal damage, this observation brings us one step closer to understanding the processes that cause diabetic nephropathy at a molecular level; with that, we are one step closer to successful therapeutic intervention.

References
