Activation of Canonical Wnt Signaling Meets with Podocytopathy

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WNT signaling regulates a variety of cell fate decisions and developmental processes in species ranging from flies and worms to humans. Altered function of the WNT/β-catenin pathway occurs in a diverse array of human diseases, highlighting a crucial role for canonical WNT signaling in the regulation of cellular homeostasis, organ function, and control of tissue patterning during vertebrate embryogenesis. WNTs encompass a family of 19 secreted glycoproteins, short-range signaling molecules activating canonical (β-catenin dependent) and noncanonical (β-catenin independent) intracellular signaling cascades through alternate binding to Frizzled and LDL receptor–related protein 5 and 6 transmembrane receptors. WNT signals control levels of cytosolic β-catenin by inhibiting glycogen synthase kinase 3β. Nuclear translocation of activated β-catenin results in interaction with a number of negatively charged ligands including the leukemia and the T cell factor transcription factors. In addition to its intracellular signaling function, β-catenin constitutes a central component of adherens junctions through its interaction with cadherins and other cell adhesion molecules.

A role for canonical Wnt signaling in renal development, specifically branching morphogenesis of the ureteric bud, is well established. Several Wnt components are implicated, including Wnt 4 and Wnt 9b. Deletion of β-catenin in renal epithelial progenitors results in failed condensation of metanephric mesenchyme with reduced nephron formation, whereas constitutive activation induces ectopic leukemia and the T cell factor–dependent transcripts. In the mature kidney, increasing evidence implicates Wnt-β-catenin signaling in the pathogenesis of renal interstitial fibrosis.

A connection between canonical Wnt signaling and glomerulopathy has, until now, been far more tenuous, although the β-catenin complex is expressed at the slit diaphragm. In addition, activation of integrin-linked kinase occurs in hereditary nephropathy, and unpublished observations in human podocytes identified nuclear translocation of β-catenin together with upregulation of glycogen synthase kinase 3β in association with specific NPHS1 mutations (A.K. and M. Saleem). Moreover, a functional consequence of integrin-linked kinase activation in puromycin/adriamycin-induced podocyte damage is nuclear translocation of β-catenin.

The study published in this issue of JASN by Dai et al. builds on these initial observations and provides innovative data that corroborate a role for aberrant activation of canonical Wnt signaling in the pathogenesis of glomerulosclerosis and proteinuria. Preliminary findings in adriamycin nephropathy established the pattern of Wnt activation. To test their hypothesis further, the authors focused subsequent functional experiments on the prototype of the group Wnt1, activated de novo by adriamycin injury. They demonstrated exacerbation of podocyte damage in adriamycin nephropathy by exogenous Wnt1, alongside rescue through blocking endogenous Wnt signaling using canonical pathway antagonist Dickkopf1. Subsequent experiments established a role for canonical Wnt signaling in glomerular protein leak by verifying that podocyte-specific ablation of β-catenin in mice protects against podocyte injury, whereas activation enhances proteinuria. A mechanistic explanation is partially provided by the observation that Wnt1-mediated induction of Snail, a known transcriptional repressor of nephrin, downregulates nephrin in cultured podocytes. Subsequent detection of Wnt-1/β-catenin activation in the podocytes of patients with FSGS and diabetic nephropathy suggests a link between experimental findings and human disease. Nevertheless, because Wnt-1/β-catenin activation was identified only in a circumscribed number of cases, it would be intriguing to examine sections from a wider range of primary glomerulopathy, including minimal-change and hereditary nephropathies, to help ascertain the spectrum and confirm whether activation might contribute to the pathogenesis of glomerular scarring analogous to processes seen within the renal interstitium.

Increased expression of mRNA encoding canonical WNTs and Fzd5 in podocytes occurs the day after adriamycin injection, whereas the increase in active β-catenin protein is observed 3 days after injection. Both time points precede onset of overt proteinuria, supporting activation of the canonical pathway during an early stage of injury rather than simply consequent to proteinuria. Interestingly, overexpression of exoge-

See related article, “Podocyte Glutamatergic Signaling Contributes to the Function of the Glomerular Filtration Barrier,” on pages 1929–1940.


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6. Increased expression of mRNA encoding canonical WNTs and Fzd5 in podocytes occurs the day after adriamycin injection, whereas the increase in active β-catenin protein is observed 3 days after injection. Both time points precede onset of overt proteinuria, supporting activation of the canonical pathway during an early stage of injury rather than simply consequent to proteinuria. Interestingly, overexpression of exoge-
nous Wnt1 exacerbates proteinuria and foot process effacement in response to adriamycin but does not produce a phenotype in normal podocytes. This protection may result from redundancy between WNT and interrelated pathways, with the functional consequences becoming unmasked only with additional triggers such as adriamycin. In human disease, chemical damage inducing glomerular proteinuria is unlikely, but it is tempting to speculate that the experimental findings in adriamycin nephropathy may represent situations in which WNT pathway activation becomes a key contributory factor. In contrast to the severe phenotype seen after inactivation of β-catenin targeted to ureteric epithelia, which results in renal dysplasia or aplasia, podocyte-specific deletion of β-catenin seemed dispensable to developing or mature podocytes because no glomerular phenotype is observed; however, β-catenin null mice challenged with adriamycin were protected against injury as compared with wild-type mice, again supporting a role for canonical signaling in podocyte injury. This protective effect is enhanced in outbred backgrounds, supporting the presence of genetic modifiers and/or epigenetic factors able to influence the phenotype.

The authors offer a mechanistic explanation for their data by showing that Wnt/β-catenin activation in podocytes induces Snail and represses nephrin, confirming a previous independent observation that Wnt2 activation induces Snail and transcriptional repression of nephrin. Conversely, recent findings in Xenopus demonstrating inhibition rather than activation of canonical Wnt signaling results in loss of expression of the Xenopus nephrin orthologue Xnphs1, which indicates an apparent lack of species conservation and may put into question the biologic significance of observations in adriamycin nephropathy. Conversely, these disparate observations may simply reflect the complexity of the Wnt signaling and potential differences in spatial/temporal inactivation in different models; however, whether canonical Wnt activation in diseased podocytes results from constitutive activation or occurs through reactivation of developmental signaling cascades by pathologic stimuli remains unclear.

Further studies of downstream targets of Wnt/β-catenin in podocytes including the influence of chromatin binding by β-catenin on the regulation of target gene activation, as well as the contribution of other Wnt ligands and pathway components, are now required to unravel how dysregulation of this ancient pathway challenges podocyte function. Canonical Wnt activation may, for example, be initiated through cross-talk with other pathways such as Notch and PAX2, which play a role of early podocyte differentiation. As the authors propose, delineating the exact role of Wnt activation in podocytopathy will undoubtedly aid an exciting quest for new directed anti-proteinuric therapy. Ultimately, there exists the potential for pharmaceutical modulators of the Wnt signaling pathway following small molecule strategies currently being developed for bone and central nervous system diseases as well as cancer, that in the future may also provide novel therapeutic opportunities to arrest podocyte damage and potentially enhance repair.

DISCLOSURES
None.

REFERENCES
Editorials


Measuring Patient Survival on Hemodialysis

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Considering the progression of ESRD—left untreated and in the context of maintenance hemodialysis—there can be little doubt that the delivered dose of hemodialysis impacts patient survival. What remains at issue is how best to operationally define hemodialysis dose, a necessary precondition to defining the dose that represents “enough” dialysis. Presently, hemodialysis dose is most often measured in terms of $Kt/V_{urea}^1$, a mathematical ratio that seeks to balance urea removal (rate of removal, $K$, times treatment time, $t$) with metabolic needs (represented loosely by the volume of distribution, $V$).$^2$ Curiously, data on the association between $Kt/V_{urea}$ and mortality are mixed, with some studies showing a potent and statistically significant relationship,$^3,4$ and others finding none.$^5,6$ In this issue of JASN, Argyropoulos et al.$^7$ examine data from a nationally representative cohort of incident hemodialysis patients to further examine the association between $Kt/V_{urea}$ and mortality and to explore incongruity between biologic mechanism and statistical modeling assumptions as one potential explanation for inconsistent findings among prior studies.

The authors analyzed data from 491 participants in the Choices for Health Outcomes in Caring for ESRD (CHOICE) study for whom complete covariate data were available, finding that each (unitless) increment in $Kt/V_{urea}$ is associated with a 42% (95% CI: 2–98%) increase in median survival time using adjusted accelerated failure time (AFT) analysis. The clinical significance of this finding should be judged by the magnitude of association. Smaller, more clinically relevant $Kt/V_{urea}$ increments, such as 0.2 and 0.4, correspond to more modest 7.5 and 15% increases in median survival time. Furthermore, given the observational nature of the study, results may have been influenced by residual confounding, particularly on the basis of dialysis access type, which was not considered as a covariate term despite known associations with both $Kt/V_{urea}$ and mortality.$^8,9$

Recently, nephrologists have challenged the validity of $Kt/V_{urea}$ as a metric of hemodialysis dose on both theoretical and empirical grounds. Some argue that given the known association between greater $V$ and improved survival, normalizing urea removal to $V$ (i.e., placing $V$ in the denominator of $Kt/V$) seems illogical and may lead to suboptimal dialysis delivery to patients with small body habitus.$^{10}$ Others argue, in an era where high-efficiency dialyzers enable rapid removal of low molecular weight compounds, use of any urea-based metric of hemodialysis dose may result in treatment durations that are too short to enable adequate removal of middle molecules and excess volume.$^4,11$ Assuming that residual confounding did not overly influence results, the authors’ demonstration that $Kt/V_{urea}$ is significantly associated with mortality offers a small modicum of support for the currently pervasive $Kt/V_{urea}$-centric paradigm of hemodialysis dose titration. However, without data on other urea- or non–urea-based measures of adequacy, these data cannot address whether other metrics may be superior to $Kt/V_{urea}$.

More than its clinical message, the methodological implications of the study of Argyropoulos et al. deserve notice. That $Kt/V_{urea}$ was significantly associated with mortality on AFT but not proportional hazards analysis may bear analytical relevance to future studies. Proportional hazards analysis is by far the most common analytical technique applied to time-to-event analyses in the medical literature. It makes the implicit assumption that the risk associated with exposure (e.g., $Kt/V_{urea}$) remains constant over time: for example, the hazard ratio in year 1 equals that in years 3, 5, etc.$^12$ This assumption is inconsistent both with the underlying biology of uremia—continued accumulation of uremic solutes in response to lower $Kt/V_{urea}$ causes risk to increase over time—and with the empirical data.$^7$ When data do not conform to the proportional hazards assumption, these models will generate biased and imprecise effect estimates.$^{13}$ Conversely, because AFT models do not require the data to conform to the proportional hazards assumption,$^{14}$ they render more accurate and precise effect estimates in instances where relative hazard varies over time. The authors show greater accuracy and precision of AFT (versus proportional hazards) models empirically in the form of greater agreement between predicted and observed (Kaplan-Meier) survival curves and narrower confidence intervals, respectively. Consideration of the proportional hazards assumption applies equally to observational and randomized studies; thus, both future epidemiologic studies and randomized trials of hemodialysis dose should consider using AFT models.

Successful application of AFT models requires appropriate specification of the baseline hazard (unlike proportional hazards models, which do not require specification of baseline hazard).$^{15}$ Correct specification leads to increased precision