Unraveling the glomerular RAS: one peptidase at a time

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Progressive glomerular diseases are linked to activation of the intraglomerular renin-angiotensin system (RAS). Angiotensin converting enzyme (ACE) inhibitors (ACEI) and angiotensin type 1 (AT\(_1\)) receptor antagonists, principal therapeutic approaches blocking the RAS, influence both circulating and renal levels of various angiotensin (ANG) peptide fragments; therefore, it is important to understand their renal actions. In an issue of the *American Journal of Physiology-Renal Physiology*, Velez et al. (12) establish the biotransformation pathways of ANG peptides in primary human glomerular endothelial (hGEnCs) and human mesangial cells (hMesCs), as well as conditionally immortalized human podocytes (hPODs). The authors report that the predominant metabolites of ANG I-(1–10) are ANG II-(1–8) and ANG-(1–7) in hGEnCs and hMesCs, whereas hPODs primarily convert ANG I into ANG-(2–10) and ANG-(1–7). The hGEnCs possess prominent ACE activity, as well as prolylendopeptidase (PEP), ACE2, prolylcarboxypeptidase, aminopeptidase (AP) N, and aspartyl AP involved in ANG peptide cleavage. Surprisingly, the hGEnCs possess modest ANG II-metabolizing activity, while hPODs exhibit significantly less ANG II formation. These studies extend previous reports by the authors on RAS enzyme expression in cultured mouse podocytes (11) and isolated rat glomeruli (13). The sophisticated methodological approach allowed for the quantification of specific ANG peptides produced by enzymatic cleavage combined with pharmacological inhibitors to identify the relevant carboxypeptidases, endopeptidases, APs, and serine proteases in human glomerular cells in culture. Peptide quantification was established by absolute quantification of isofoms for ANG II, ANG-(1–7), ANG III-(2–8), and ANG IV-(3–8), which are 6 Da larger than the native peptide as a result of \[^{13}\text{C}_{15}\text{N}\]valine incorporation into the amino acid sequence and are easily visible on the matrix-assisted laser/desorption/ionization time-of-flight mass spectrum. This technically superb study readily demonstrates the complex cell-specific processing mechanisms contributing to the formation of ANG peptides (12).

In hGEnCs, coincubation of ANG I with an ACEI led to a substantial reduction in ANG II; however, a chymase-serine protease inhibitor had no effect. Renal microvascular responses to ANG I in the presence of an ACEI were significantly suppressed in kidneys of control, but not in diabetic, mice (9). In this case, the vascular responses to ANG I were attenuated in the presence of a serine protease inhibitor in diabetic kidneys only (9). Thus non-ACE-dependent generation of ANG II may play a central role in the regulation of renal hemodynamics during the progression of diabetic nephropathy (8). Despite the lack of nepriylin expression, essentially equivalent formation of ANG II and ANG-(1–7) was evident in the hGEnCs. Velez et al. (12) find that PEP contributed to 40% of ANG-(1–7) formation, but the remaining activity was not identified. Our laboratory reported that release of the endopeptidase thimet oligopeptidase (EC3.4.14.15) from the rat vasculature directly converted ANG I to ANG-(1–7) (2). Although primarily characterized as a soluble peptidase, membrane-associated or released forms of thimet oligopeptidase may constitute the additional ANG-(1–7)-forming enzyme in the endothelial cells. Distinct from endopeptidase-dependent processing of ANG I to ANG-(1–7), hGEnCs PEP (and ACE2) also exhibits carboxypeptidase activity that converts ANG II to ANG-(1–7), while these cells express APs to form ANG III and ANG IV. Thus the hGEnCs exhibit quite efficient proteolytic mechanisms to clear the extracellular compartment of ANG II, as well as generate peptides with functions distinct from the ANG II-AT\(_1\) receptor pathway. In addition to the ANG-(1–7)-Mas receptor axis, ANG III may be the endogenous ligand for the renal AT\(_2\) receptor, and ANG IV may function through the insulin-regulated AP (see Fig. 1). We await further studies to establish the role of these novel processing products in glomerular function.

A major finding in the present study is the predominant processing of ANG I to ANG-(1–7) through non-ANG II pathways in hPODs. Increasing evidence suggests that the ANG II-AT\(_1\) recepto...
tor axis may induce deleterious actions within the podocytes, leading to oxidative stress, cytokine production, and cellular apoptosis (6). Importantly, podocytes constitute a critical component in the filtration barrier of the glomerulus, and early injury to these cells may begin a complex series of events leading to albuminuria, proteinuria, and more extensive renal damage. The novel aspect here reflects the concept that alternative generation of ANG-(1–7) is not simply a degradatory pathway, but shifts the functional balance or expression of the RAS (1). The authors demonstrate direct formation of ANG-(1–7) from ANG I in podocytes, although the identity of the endopeptidase(s) that cleave the Pro7-Phe8 bond of ANG I was not elaborated. Previously, this group demonstrated the endopeptidase nephrilysin accounted for the majority of ANG-(1–7)-converting activity from ANG I in mouse podocytes (11). Moreover, an ACEI increased the expression of ANG-(1–7), further emphasizing the independence from ANG II in these cells (11). Chronic administration of ANG-(1–7) reduces proteinuria in diabetic hypertension (3), stroke-prone spontaneously hypertensive rats (5), and anti-Thy-1-induced glomerulosclerosis (14), although not all models of renal injury are responsive to the peptide, which may reflect differences in the sensitivity or metabolism of ANG-(1–7) (4, 10). It is not clear whether podocytes maintain a significant degree of “ANG-(1–7) tone” that promotes a beneficial phenotype in lieu of an intrinsic RAS expressed by these cells and the selective pattern of peptidase expression that favors ANG-(1–7) formation.

We note that these experiments were conducted in intact cells, such that the peptidase activities represent ectocellular enzymes, either anchored to the plasma membrane, or secreted into the media. In fact, blocking AT1 receptors or interrupting trapping by endocytosis did not influence the profile of ANG II fragmentation, suggesting that intracellular enzymatic activity had little impact on extracellular ANG processing. An emerging concept of the RAS, particularly within the kidney, is the expression of intracellular components of the system, including AT1, AT2, and AT7 receptors on cellular organelles (7). In this regard, the robust biochemical approach utilizing the highly specialized matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and isotope-labeled peptide quantification by Velez and colleagues is equally appropriate to elucidate the intracellular peptidase domains within the renal cells (12).

The present results indicate that hGEnCs possess prominent ANG II-forming capabilities, whereas podocytes possess major ANG II-metabolizing activity. Does this imply that podocytes degrade ANG II synthesized by the endothelial cells? This leads one to propose that the glomerular endothelial and mesangial cells are predominantly equipped to form ANG II, while the glomerular podocytes are the predominant ANG II degrading cells in the glomerulus. Therefore, one might imagine glomerular pathological conditions in which there is an imbalance in the ANG II synthetic and degradation pathways as a result of selective podocyte damage. Under these circumstances, an augmented role of ANG II in glomerular disease progression may predominate, reflecting, in part, an attenuated response in the complement of podocyte peptidases.

**REFERENCES**


