Are microRNAs the key to transforming renin progenitor cells in the afferent renal circulation?

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RENIN, PRODUCED IN JUXTAGLOMERULAR (JG) cells at the glomerular terminus of the afferent arteriole, is the critical regulatory enzyme in the pathway of angiotensin formation. When the kidney is chronically stressed with the need to produce more renin (as in renal ischemia, sodium restriction, or with converting enzyme inhibition), a number of vascular smooth muscle cells upstream in the afferent arteriole and interlobular arteries are transformed into a renin-producing phenotype by a process referred to as “recruitment” (7–9). While early on it was thought that there was some transformation of regular afferent vascular smooth muscle cells to a renin-containing phenotype, the laboratories of Dr. Ariel Gomez at the University of Virginia, with a number of collaborators, have been at the forefront of studying the signaling and transformation of a distinct population of renin progenitor cells. These cells are not the typical vascular smooth muscle cells, as they have been genetically programmed to be involved in the embryonic progression of renin expression associated with the developing kidney that is lost to an adult vascular smooth muscle phenotype (5, 7–9). These progenitor cells have the ability to transform back into their fetal heritage of a renin-containing cell under certain conditions in which renal homeostasis is challenged. This was determined (7) using a clever technique of incorporating expression within the renin progenitor cells in a dual-labeling technique, revealing that when they are not expressing renin (in the vascular smooth muscle phenotype) they are marked with a cyan fluorescent protein and when they are expressing renin they are marked with a yellow fluorescent protein. Thus the metamorphosis from the adult smooth muscle phenotype back into the embryonic renin-producing phenotype distinctly identifies these cells from other smooth muscle cells in the afferent circulation following the recruitment stimulus. The pattern of the progenitor cell distribution throughout the renal vasculature at different stages of embryonic development has been described in elegant studies using three-dimensional reconstructions (5). We now more fully understand the characteristics of these cells and how they are involved in the recruitment process, but the signaling and the control of this metamorphosis have remained largely a mystery.

cAMP is well established as the stimulatory second messenger which regulates renin secretion (1), and it has also been shown to be a critical factor in the stimulation and regulation of renin expression and synthesis via histone acetylation at the cAMP-response element (CRE) on the renin gene (7) under the control of the histone acetyl transferases CBP and p300 (2). These two transferases are essential for maintaining the integrity of the renin-containing phenotype. Interestingly, the production of cAMP linked to renin secretion is mediated selectively by the calcium-inhibitable isofrom(s) of adenyl cyclase (1, 3, 6), which colocalizes with the renin-containing granule (6), but it is not known whether the stimulation of cAMP-mediated renin gene transcription and expression are also controlled through this calcium-mediated pathway or by another adenyl cyclase isoform within the JG cell. Additionally, while cAMP mediates renin expression, it is not yet clear what its role in the signaling process of recruitment and metamorphosis of the progenitor cell into its renin-producing phenotype might be. So, the critical question is, what controls the “switch” in response to homeostatic challenge to transform these cells between their two phenotypes?

In a remarkable study, Medrano and the laboratories of Dr. Gomez (4) present the first data that really begin to resolve this question. They target the possible regulatory effect of microRNAs, which are a group of small (18–22 nucleotides) endogenous noncoding RNAs with cell specificity, suggesting they may be involved in characterizing individual cell phenotype and differentiation (10). Based on their recent findings that deletion of the microRNA processing enzyme “Dicer,” which is involved in producing mature microRNAs, confounded the expression of the JG cell phenotype (10), they set about trying to identify possible microRNAs that would express or decrease in association with stressing changes in the JG cell phenotype. To do this, the authors used microarrays to identify microRNA expression in renin-expressing vascular smooth muscle cells in culture, induced to transformation using forskolin and IBMX over 24 h, and kidney cortex from mice treated for 10 days with a low-sodium diet plus captopril to induce the transformation of the renin progenitor cells into the renin phenotype. Beginning with some 599 mature microRNAs, the authors focused on two in particular: miR-330, which was barely detectible under control conditions, and miR-125b-5p, which was found at high levels in a microarray. Both expressed exclusively in the renin progenitor cells and also changed in response to the stimuli for transformation. What they found was that miR-330 was upregulated after stimulation, but miR-125b-5p was downregulated by stimulation of transformation. Other microRNAs of interest did not produce changes in response to stimulation, suggesting that they were not candidates for the transformation process. Additionally, they found that miR-330 retarded while miR-125b-5p increased expression of the smooth muscle phenotype, inverse to and consistent with the transformation of these progenitor cells to the renin-containing phenotype.

There are several important milestones in their observations (4): first, that these microRNAs may be critical in mediating the transformation process, in either direction, and next that these two progenitor cell-specific microRNAs work in opposite direc-
tions; as one is upregulated, the other is downregulated, suggesting each is involved in a coordinated directing of the transformation toward one of the two particular phenotypes of this cell. From this they suggest that while “cAMP, via CRE and associated co-activators, initiates and maintains renin gene transcription” (4) and the renin phenotype, these microRNAs control or buffer the expression of the non-renin (smooth muscle) phenotype of the progenitor cells.

It is important to note that they did not depend only on a microarray but integrated a variety of novel and clever techniques including in silico analysis of smoothelin and other genes involved in the vascular smooth muscle phenotype, and cell transfections to manipulate the microRNAs. These all helped characterize which of the hundreds of renal microRNAs changed character (both directional changes) associated with the transforming challenges. From this the authors could determine which of these that changed were associated exclusively with the renin progenitor cells (only 2). Remarkable is the notion that if the authors had depended only on the typical microarray screening parameters, they would probably have never identified both miRNAs, especially miR-330, since under normal homeostatic conditions it is hardly expressed.

The study by Medrano et al. (4) presents us with the first real clue as to how these progenitor cells transform between phenotypes in the process of recruitment and will no doubt generate considerable interest in the role of microRNAs in regulating cell differentiation and expression in the field of renal physiology. While many questions remain about the regulation of this additional pool of renin from recruited renin-containing progenitor cells, we finally have a significant insight into what has been the mysterious process of phenotype transformation associated with renin recruitment.

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REFERENCES