Discarding the haystack to examine the needles: the potential role of urinary exosome analysis

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EXOSOMES ARE TINY VESICLES (usually in the order of tens of nanometers), released by several cell types after fusion of multivesicular bodies with the plasma membrane. For many years, exosomes, described for the first time over 30 years ago (8), were thought to represent but an alternative to hydrolysis and proteosome degradation for the disposition of obsolete proteins and intracellular organelle debris (2). Recent studies, however, have provided evidence that exosomes may play an important role in the communication between cells, given the ability of these nanovesicles to convey at the same time proteins, mRNA and miRNA, which can be inoculated into target cells, thus influencing their physiology, gene expression, and protein synthesis (2). The implications of these discoveries are wide. Exosomes can transmit information from macrophages to dendritic cells and from these to T lymphocytes, thus participating actively in specific immune responses (7). Cancer cells can exchange information or influence vascular cells and the tumor microenvironment through the release of exosomes (4). In the kidney, exosomes released by podocytes can be detected in urine (5), whereas those derived from the proximal nephron may interfere with more distal tubular segments (2), thus allowing the nephron to operate in an integrated manner.

In view of their ubiquity, it is hardly surprising that exosomes can be found in many biological fluids such as blood, ascitic fluid, and cerebrospinal fluid, where they can serve as diagnostic tools and as a potential source of information about the pathophysiology of a number of diseases. In the particular case of urine, exosomes can provide valuable information about functional and or structural changes taking place in podocytes and tubular cells (5, 10). Because exosomes can be isolated from urine (and from other fluids) by ultracentrifugation and disrupted, the minute amounts of proteins and nucleotides they carry can be quantified in a much more favorable condition than if they were directly assessed from urine. However, the assays usually employed are labor-intensive, hard to develop in large scale, and lack standardization, precluding the widespread utilization of exosome analysis at the present moment.

In an issue of the American Journal of Physiology-Renal Physiology, Isobe and associates (3) utilized urinary exosome analysis to gain insight into the distal convoluted tubule (DCT), a nephron segment that, although responsible for no more than 5 to 10% of the filtered load of sodium, has been recently shown to play a key role in sodium and potassium homeostasis (1). Isobe and co-workers developed a highly sensitive ELISA sandwich technique that allows the measurement in human urine of fentomol amounts of exosome-linked sodium-potassium cotransporter (NCC), unique to the DCT, and of its active, phosphorylated form (pNCC). They initially showed that in patients with Gitelman’s syndrome the excretion of exosome-linked NCC was absent or very low, while in Gordon’s syndrome (pseudohypoaldosteronism Type II, or PHAII), markedly increased amounts of pNCC were detected. In additional assays, they were able to show decreased urinary pNCC in patients with chronic kidney disease, likely reflecting an adaptation to volume expansion, thus opening the possibility that the technique be useful for the clinical assessment of other changes of effective arterial volume, such as congestive heart failure and chronic liver disease, and even to predict the natriuretic response to thiazides.

The possible applications of urinary exosome analysis, especially if facilitated by techniques such as the one developed by Isobe et al., clearly transcend the DCT. Considering previous results obtained with more complex techniques, other molecules present at other nephron segments and likely dragged into the urine, such as aquaporins and components of the renin-angiotensin system (9), can be assessed in this manner, allowing a more comprehensive analysis of the renal response to a number of pathological situations, and even the association of distinctive profiles to specific diseases or clinical situations.

Urinary exosome analysis can be extended further to include molecules not directly involved in transport, such as cell constituents, enzymes, and adhesion molecules. It can contribute to the early detection of podocyte injury in glomerulosclerosis and other glomerulopathies, and help to understand the pathogenesis of these diseases. In addition, it can be helpful in the current quest for a sensitive biomarker suited to predict acute kidney injury, allowing early diagnosis of this condition. Other conceivable applications of this technique include early detection of acute rejection in transplanted kidneys, and of neoplasms from the prostate or urinary tract.

The ELISA technique described by Isobe and co-workers, although simplified compared with other methods, still requires ultracentrifugation to sort out exosomes from the molecular maze present in the urine, thus restricting its potential use to large medical centers. However, recent developments may allow exosome isolation by more accessible means in the not so distant future (6). When such methods are standardized, along with techniques such as the one described by Isobe and co-workers, a powerful clinical tool may become available, allowing practical and noninvasive diagnosis of a myriad of conditions, as well as a better comprehension of renal physiologic and pathophysiologic phenomena.

DISCLOSURES
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