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Megalin as a protein of major impact in the renal proximal tubular epithelium

The proximal tubule of the kidney reabsorbs low-molecular-weight proteins (<70 kDa) that are filtered by the glomerulus, up to 600 mg protein/day in a healthy adult. Glomerular damage leads to markedly increased glomerular filtration of serum proteins. While protein reabsorption by the proximal tubule can increase dramatically, spillage of proteins into the urine can nevertheless occur, producing the nephrotic syndrome. Unfortunately, the increased tubular reabsorption of protein is itself nephrotoxic, as the endocytic capacity of the proximal tubule becomes saturated.

Apical endocytosis in the renal proximal tubule epithelium is largely dependent on the scavenger receptor megalin, a member of the low-density lipoprotein receptor gene family. A mechanism for linking receptor-mediated endocytosis with intracellular signalling events, regulated intramembrane proteolysis (RIP), has been shown recently to act on the low-density lipoprotein receptor-related protein. RIP is an evolutionarily conserved mechanism in which sequential, regulated proteolysis of membrane proteins leads to release of the intracellular domain of the protein from the membrane. The released intracellular domain can translocate to the nucleus to act as a transcription regulator, or may activate cytoplasmic signaling pathways. For RIP to occur, the ectodomain of the receptor protein must be shed. This occurs as multiple steps, mediated either by members of the metalloprotease-disintegrin family of proteases (designated ADAMs; a disintegrin and metalloprotease domain), or by matrix metalloproteases (MMPs). The membrane-associated carboxyl-terminal fragments (MCTF) are in turn substrates for γ -secretase. This enzyme acts at a site within the transmembrane domain, enabling release of receptor peptide fragments into the cytoplasm. Of note, aberrations in the RIP processing have been shown to be relevant to the accumulation of the A β peptide fragments in plaques of Alzheimer's disease. The general relevance of RIP in disease processes is thus considerable.

Zou *et al*¹ have now demonstrated that megalin participates as well in RIP. High levels of γ -secretase activity were found in renal brush border membrane vesicles, and incubation of opossum kidney proximal tubule cells (OKP) with a γ -secretase inhibitor led to accumulation of megalin-derived MCTFs. Appearance of MCTF was increased by incubation

with the protein kinase C activator phorbol 12-myristate 13-acetate, a process which was blocked by metalloprotease inhibitors. These data indicate that megalin is a substrate for γ -secretase and metalloprotease-mediated ectodomain shedding; production of the resultant MCTF from megalin is induced by protein kinase C. The authors have thus provided evidence that enzymatic processing of megalin represents part of a novel ligand-dependent signaling pathway in the proximal tubule that links receptor-mediated endocytosis with cell signaling. The next investigative step will be to identify the intracellular signaling events activated by RIP processing of megalin. In this issue, Akihito *et al*² have demonstrated megalin-mediated proximal tubular uptake of L-FABP, and the likelihood that such uptake leads to direct nephrotoxicity due to the accompanying ligands endocytosed with L-FABP. Together, these two studies underscore the important role of megalin in the function of proximal tubular cells of the kidney, and the unwitting role megalin may play in the progression of nephropathy in the nephrotic syndrome.

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References

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Genetic signatures to predict lymphatic metastasis

Genetic signatures of malignant cells in primary tumors have been documented in a growing body of literature, with special interest given to their correlation with aggressive behavior and risk of metastasis. The fundamental premise is that the differences in gene expression of primary tumors without metastases, vs those with concurrent or subsequent metastases, might be of prognostic value.

In a recently published article, O'Donnell *et al*¹ investigated whether differences in gene expression between lymph-node-positive and lymph-node-negative primary tumors could be used to predict lymph node metastasis, and whether such a discriminatory gene signature would be similar to signatures predicting hematogenous metastasis.

Oral squamous cell carcinomas were chosen for this study, as they typically cause lymphatic metastasis and not hematogenous metastasis. Total RNA was isolated from tumor samples for microarray analysis. Genes with differential expression were identified using a permutation technique and were further verified by quantitative RT-PCR and immunohistochemistry. The authors identified a signature set of 116 genes using permutation-based method, which in hierarchical clustering showed separation between the primary tumors with nodal metastatic and the nonmetastatic primary tumors. This signature gene set also correctly predicted metastases in an independent set of patients. None of the genes in the signature set had been previously associated with lymph node metastasis in head-and-neck squamous cell carcinoma, although some of them had been previously associated with an aggressive phenotype in other types of cancers. Unexpectedly, CXCR4, which had been previously correlated with lymph node metastasis in oral squamous cell carcinoma, did not emerge as a differentially expressed gene. The absence of CXCR4 and the lack of overlap with other gene signatures defined for hematogenous metastasis indicated that metastases through lym-

phatic and hematogenous routes employed different pathways. A recent article by Uchida *et al.*,² however, pointed to the role of CXCR4 as critical for oral squamous cell carcinomas to metastasize to cervical lymph nodes.

These studies demonstrate measurable differences in gene expression between lymph-node-positive and lymph-node-negative primary tumors, and demonstrate that these differences are sufficient to predict the lymph node status of these patients. Identification of specific gene signatures in primary tumors at the time of biopsy thus may have value in prompting clinical testing for metastatic potential.

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