Megalin binds to NHERF1 and NHERF2 scaffold proteins

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Albumin endocytosis in the renal proximal tubule is regulated by the scavenger receptor megalin, as well as a number of transmembrane and accessory proteins. Previously we have demonstrated an essential role for the scaffold proteins NHERF1 and NHERF2 in albumin uptake. NHERF1 and NHERF2 are PDZ domain containing proteins that interact with specific sequences that form a PDZ binding domain (S/TXΦ) in the C-terminus of transmembrane proteins. Interestingly, megalin contains a functional PDZ binding domain (SDV), however an interaction between meaglin and the scaffold proteins NHERF1 and NHERF2 has not been investigated.

In this study we will investigate if there is an interaction between megalin and NHERF1 and NHERF2, and then characterize the specific domains required for this interaction. Initially, immunoprecipitation experiments were performed using anti-megalin, anti-NHERF1 and anti-NHERF2 antibodies that were incubated with rat kidney lysate. The immunoprecipitates were analysed by Western blot analysis using the anti-NHERF1 and anti-NHERF2 and anti-megalin antibodies, respectively. These studies clearly indicated that megalin bound to NHERF1 and NHERF2 *in vivo*. To determine which domains in NHERF1 and NHERF2 were required for this interaction, GST fusion proteins were generated as described previously (Hryciw *et al.,* 2006; Lee *et al.,* 2007). These fusion proteins included the full length NHERF proteins as well as their 2 PDZ domains (PDZ1 and PDZ2) and C-terminal ezrin binding domain. Incubation with rat kidney lysate and analysis by Western blot analysis indicated that megalin bound to PDZ2 of NHERF1 and PDZ2 and the C-terminal ezrin binding domain of NHERF2. Megalin binds to NHE3, an exchanger that that is also essential for albumin endocytosis. Interestingly, NHE3 also binds to in NHERF1 and NHERF2 using the same PDZ binding domains.

We also investigated the distribution of megalin and NHERF1 and NHERF2 in the opossum kidney (OK) proximal tubule cell line. OK cells are a well characterized model of albumin endocytosis that contains a large number of essential endogenous proteins including megalin, NHE3, NHERF1 and NHERF2. Confocal analysis of OK cells demonstrated that the distribution of megalin was predominantly apical with some cytosolic localization. Importantly, NHERF1 had a strong apical localization which overlapped with megalin. Further, as previously described (Hryciw *et al.,* 2006) NHERF2 was predominantly cytosolic, and this protein co-localized with megalin in this region. Therefore, we have described for the first time an interaction between megalin and the scaffold proteins NHERF1 and NHERF2. As the NHERF proteins have been shown to be required for the formation of macromolecular complexes in other cell systems, further investigation should determine if they play a role for the complex formation required for the regulation of megalin medicated albumin endocytosis in proximal tubule cells.

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