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Epithelial protein lost in neoplasm modulates platelet-derived growth factor–mediated adhesion and motility of mesangial cells

Haruko Tsurumi[1], Yutaka Harita[1], Hidetake Kurihara[2], Hidetaka Kosako[3], Kenji Hayashi[4], Atsuko Matsunaga[1], Yuko Kajiho[1], Shoichiro Kanda[1], Kenichiro Miura[1], Takashi Sekine[1], Akira Oka[1], Kiyonobu Ishizuka[5], Shigeru Horita[5], Motoshi Hattori[5], Seisuke Hattori[6] and Takashi Igarashi[1,7]

[1]Department of Pediatrics, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan; [2]Department of Anatomy, Juntendo University School of Medicine, Tokyo, Japan; [3]Division of Cell Signaling, Fujii Memorial Institute of Medical Sciences, The University of Tokushima, Tokushima, Japan; [4]Department of Molecular Biology, Yokohama City University School of Medicine, Kanagawa, Japan; [5]Department of Pediatric Nephrology, Tokyo Women's Medical University, School of Medicine, Tokyo, Japan; [6]Department of Biochemistry, School of Pharmaceutical Sciences, Kitasato University, Tokyo, Japan and [7]National Center for Child Health and Development, Tokyo, Japan

Mesangial cell migration, regulated by several growth factors, is crucial after glomerulopathy and during glomerular development. Directional migration requires the establishment of a polarized cytoskeletal arrangement, a process regulated by coordinated actin dynamics and focal adhesion turnover at the peripheral ruffles in migrating cells. Here we found high expression of the actin cross-linking protein EPLIN (epithelial protein lost in neoplasm) in mesangial cells. EPLIN was localized in mesangial angles, which consist of actin-containing microfilaments extending underneath the capillary endothelium, where they attach to the glomerular basement membrane. In cultured mesangial cells, EPLIN was localized in peripheral actin bundles at focal adhesions and formed a protein complex with paxillin. The MEK–ERK (extracellular signal-regulated kinase) cascade regulated EPLIN–paxillin interaction and induced translocalization of EPLIN from focal adhesion sites to peripheral ruffles. Knockdown of EPLIN in mesangial cells enhanced platelet-derived growth factor–induced focal adhesion disassembly and cell migration. Furthermore, EPLIN expression was decreased in mesangial proliferative nephritis in rodents and humans *in vivo*. These results shed light on the coordinated actin remodeling in mesangial cells during restorative remodeling. Thus, changes in expression and localization of cytoskeletal regulators underlie phenotypic changes in mesangial cells in glomerulonephritis.

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Correspondence: *Yutaka Harita, Department of Pediatrics, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan. E-mail: haritay-ped@h.u-tokyo.ac.jp*

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Mesangial cell migration is essential in renal recovery after mesangial injury. In the Thy1 model, an experimental animal model of mesangial proliferative nephritis, mesangial injury causes mesangiolysis, as manifested by attenuation or dissolution of the mesangial matrix and degeneration of mesangial cells.[1] This process is followed by reconstitution of the mesangium, which is accomplished by coordinated migration and proliferation of mesangial cells[2] originating in the hilar and extraglomerular mesangium.

Glomerular reconstitution after mesangial injury is controlled by several growth factors.[3] Experiments involving infusion or systemic overexpression have characterized platelet-derived growth factor (PDGF) as the most potent stimulus of mesangial cell proliferation.[4] PDGF-mediated mesangial cell migration is also an essential process in glomerular development. Deletion of PDGF-B or PDGF receptor β, expressed in endothelial cells or mesangial cells in the glomeruli, respectively, causes defective migration of mesangial cells into the capillary tuft, resulting in a single ballooning capillary loop.[5–7] However, the mechanisms that regulate the morphology and migratory phenotype of mesangial cells remain unclear.

Epithelial protein lost in neoplasm (EPLIN) was originally identified as a genetic product that is downregulated or lost in a number of human epithelial tumor cells.[8] Two known isoforms of EPLIN, α and β, are generated from alternative promoter usage from a single gene and differ only at the 5′ ends.[9] Both isoforms contain a centrally located LIN11-ISL1-MEC3 domain that may allow homodimerization,[10] as well as N- and C-terminal actin-binding sites that flank the LIN11-ISL1-MEC3 domain. Through these sites, EPLIN cross-links and bundles actin filaments to form stable filament structures such as stress fibers.[10] Extracellular signal-regulated kinase (ERK)–mediated phosphorylation of EPLIN contributes to actin filament reorganization and enhances NIH 3T3 cell

motility.[11] Decreased EPLIN expression in some cancer cells results in enhanced motility and transformation due to instability of the actin filament structures and rapid filament turnover. Although the relevance of EPLIN in cancer cells has been documented,[12,13] few studies have examined the roles of EPLIN in normal tissues or in the pathophysiology of noncancerous diseases.

EPLIN transcript levels are particularly high in the kidney.[8] In this study, we found a strong expression of the EPLIN protein in glomerular mesangial cells, particularly in the mesangial processes and mesangial angles, and a markedly decreased expression in mesangial proliferative nephritis. In addition, EPLIN is associated with stability of focal adhesion and regulates PDGF-induced mesangial cell migration.

RESULTS

EPLIN expression in adult and developing glomeruli

As a previous study using northern blot analysis demonstrated a strong expression of the EPLIN transcript in the kidney,[8] we examined whether either or both EPLIN isoforms was expressed. Using reverse transcription PCR amplification with isoform-specific primers, both isoforms of EPLIN mRNA were found to be expressed throughout the kidney in rats, including renal cortex, medulla, and glomeruli (Figure 1a). Immunohistochemical staining of adult rat kidney tissues revealed that EPLIN protein was expressed in glomeruli, tubular epithelial cells, and extraglomerular vascular endothelial cells (Figure 1b). Similar staining patterns were observed in human kidney sections (Figure 1c). To analyze the localization of EPLIN in glomeruli, cryosections of adult rat kidney were double-labeled with several glomerular cell markers. EPLIN colocalized with Thy1, a mesangial cell marker, but not with intercellular adhesion molecule-2, an endothelial cell marker, or zonula occludens-1, a podocyte marker; this indicates that EPLIN is highly expressed in mesangial cells in the glomeruli (Figure 2a). EPLIN was not completely colocalized with Thy1. Punctiform signals of EPLIN, distinct from Thy1 signals, were observed near the capillary endothelium and mesangial angles (Figure 2aJ, K and L). EPLIN signals corresponding to the mesangial angles were also detected in human glomerulus (Figure 2b). A more detailed localization of EPLIN was examined using immunoelectron microscopy (Figure 2c). Immunogold particles for EPLIN were mainly detected in the periphery of the cytoplasmic processes of mesangial cells at the mesangial angles (Figure 2cA and B) and in the mesangial cell–cell adhesion sites (Figure 2cC).

During glomerular development, mesangial cells populate the core of the glomerular tuft and connect the capillary loops via deposition of the mesangial matrix. In the late S-shaped body stage to early capillary loop stage, when the mesangial cells derived from the metanephric mesenchyme are recruited to the developing nephron, EPLIN was observed in the central mesangial areas, which is distinct from the outer layers formed by column-shaped podocytes (Figure 2d).

EPLIN expression is decreased in mesangial proliferative nephritis

As EPLIN is implicated in growth control or motility of invasive tumors, we hypothesized that glomerulonephritis may affect EPLIN expression *in vivo*; therefore, we investigated EPLIN expression in the rat anti-Thy1.1 model of glomerulonephritis in which the intraglomerular mesangium is destroyed by an antibody-mediated reaction followed by tissue repair mediated by migrating and proliferating mesangial cells originating from the extraglomerular or hilar mesangium.[14] Before anti-Thy1.1 injection (day 0), EPLIN protein was expressed in the mesangial area, whereas α-smooth muscle actin (α-SMA), a marker for activated mesangial cells, was hardly detected (Figure 3a). Three or five days after antibody injection, when α-SMA staining in the mesangial area was apparent, the immunofluorescence signals for EPLIN markedly decreased. Western blotting of glomerular lysates confirmed the decreased EPLIN expression during acute phase of the models (Figure 3b). On day 15, Thy1 expression recovered to normal levels (Supplementary Figure S1 online), whereas EPLIN expression was still significantly lower than that at day 0. These results indicated that *de novo* expression of α-SMA, a molecular marker for phenotypical changes in mesangial cells, inversely correlated with EPLIN expression. Furthermore, changes in EPLIN expression were not fully coincident with those in Thy1 expression.

To evaluate EPLIN expression in human mesangial proliferative nephritis, we performed immunohistochemical analysis using kidney biopsy samples from two patients with membranoproliferative glomerulonephritis (MPGN) and two patients with IgA nephropathy (Figure 3c and d). Both MPGN patients had presented with moderate hematuria and proteinuria at the time of renal biopsy. EPLIN expression at mesangial angles or in the mesangial cytoplasm contiguous with endothelial cells was segmentally decreased in both patients, especially in the area of mesangial proliferation in glomeruli (Figure 3c). In the glomeruli of two IgA nephropathy patients, EPLIN expression in the glomerular tufts with proliferating mesangial cells was segmentally decreased (Figure 3d). These results indicate that EPLIN expression is dynamically regulated during the mesangial proliferative process in rodents and humans *in vivo*.

EPLIN colocalizes with paxillin at focal adhesions in mesangial cells

In cultured human mesangial cells, intense signals for EPLIN were observed at the sites of focal adhesion, partially colocalizing with paxillin (Figure 4a), suggesting that a subfraction of the two proteins may form a complex in mesangial cells. EPLIN signals were also partially localized to peripheral actin bundles. As a result of their colocalization at focal adhesions, we performed co-immunoprecipitation experiment to investigate whether EPLIN interacts with paxillin. As shown in Figure 4b, we confirmed endogenous EPLIN–paxillin interaction in cultured human mesangial cells. To further verify this interaction, we performed *in situ*