



Epoxyeicosatrienoic acid activation moderates endothelial mesenchymal transition to reduce obstructive nephropathy

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Fibrosis is the hallmark of end-stage organ disease and accounts for 45% of deaths around the world. Fibrosis is a key component in the progression of chronic kidney disease and involves the activation and accumulation of fibroblasts or myofibroblasts and the deposition of an extracellular matrix. Although it was previously thought that all collagen-producing fibroblasts originated solely from resident fibroblasts, other important sources of fibroblasts have been proposed, such as pericytes, circulating fibrocytes of bone marrow origin, and fibroblasts that originate from epithelial mesenchymal transition (EMT) and endothelial mesenchymal transition (EndMT) [1]. However, the contribution of these different sources to myofibroblast formation is under debate, and no consensus has been reached, particularly with respect to EMT and EndMT [1].

EndMT is a process by which endothelial cells acquire mesenchymal and stem cell-like phenotypic characteristics. Earlier reports indicated that 30% to 50% of renal interstitial myofibroblasts originated from the endothelium, although recent investigations demonstrate a lower contribution ranging from 10% to 23%. The presence of EndMT has also been confirmed in human patients with

primary nephropathy (e.g., immunoglobulin A nephropathy) and secondary nephropathy (e.g., diabetic or lupus nephritis) due to double labeling of CD31 and α -SMA expression in the endothelium [2]. Several molecules, including activation of transforming growth factor (TGF) β and Notch and chronic activation of the Wnt- β -catenin pathway, are implicated in EndMT [3].

Inhibition of EndMT in kidney disease has been reported to ameliorate renal fibrosis. The use of the anti-diabetic drug DPP4 inhibitor ameliorated kidney fibrosis in diabetic mice by inhibiting EndMT and restoring the microRNA mir-29 both *in vivo* and *in vitro* [4]. Yang et al [5] investigated the effects of inhibition of soluble epoxide hydrolase (sEH) on EndMT and renal fibrosis in the unilateral ureteral obstruction (UUO) model of chronic kidney disease.

Epoxyeicosatrienoic acids (EETs) are arachidonic acid metabolites with biological effects, including vasodilation, angiogenesis, anti-inflammatory, anti-fibrotic, and anti-apoptotic functions. sEH-mediated hydrolysis of EETs to their corresponding and less-potent dihydroxyeicosatrienoic acids attenuates these effects. A role for EETs and sEH has been well established in renal fibrosis. The genetic or pharmacologic inhibition of sEH protects mice from renal interstitial fibrogenesis and inflammation after UUO. The mechanism by which sEH inhibition prevents injury has been proposed to be via EET activation, which may act through peroxisome proliferator-activated receptor gamma to suppress TGF- β 1 and TGF- β -independent mechanisms, including activation of nuclear factor- κ B [6,7]. sEH inhibition was also shown to be renoprotective in an experimental model of adriamycin-

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cin-induced nephropathy [8]. However, these studies did not address whether sEH inhibition has a role in EMT or EndMT in these models. In a recent study, the chronic administration of 14,15-EET or its synthetic analog EET-A, in which the carboxyl group at carbon 1 of the EET pharmacophore was replaced by aspartic acid, reduced kidney fibrosis induced by UUO in mice by decreasing the renal expression of EMT inducers Snail1 and ZEB1. These results suggest that EET-A is a novel anti-fibrotic agent that reduces renal fibrosis by decreasing renal EMT [9].

A study by Yang et al [5] demonstrated that sEH inhibition using the sEH inhibitor 12-(3-adamantan-1-ylureido)-dodecanoic acid (AUDA) can decrease myofibroblast formation by attenuating EndMT in a mouse UUO model. EndMT was histologically detected in mice kidneys using double labeling of fibroblast markers (FSP-1 or α -SMA) and endothelial marker CD31. UUO increased the level of fibroblast markers, while AUDA treatment considerably reduced the number of FSP-1/CD31 or α -SMA/CD31 double-positive cells. The reduced EndMT was further confirmed by flow-cytometric analyses using CD31/FSP1 double-positive cells.

In an *in vitro* model of EndMT where the human umbilical vein endothelial cells (HUVEC) were treated with TGF- β 2, sEH inhibition decreased the activity of EndMT as indicated by reduced fibroblast-like morphology and levels of FSP-1. Interestingly, in an *in vitro* EMT model, where tubular epithelial cells (TEC) were treated with TGF- β 2, the differentiation to fibroblast-like cells was not prevented by AUDA treatment. However, when the cells were co-cultured with HUVEC cells, AUDA treatment reduced EMT of TECs. It is proposed that the EETs produced in the HUVECs may be required to prevent EMT. The mechanism of action of sEH inhibition was blood pressure-independent, as previously shown by other reports [7].

As in any study, several concerns can be raised about the current experiment. sEH inhibition is demonstrated to attenuate TEC injury and the release of a variety of factors that could prevent fibrogenesis. It is unclear from the data if TEC injury and the release of these factors may drive EndMT. The use of the non-specific marker FSP-1 for fibroblasts and the omission of VE-cadherin as an endothelial cell marker in histological sections are two weaknesses of this study. *In vitro* studies would have

benefited if the study used a kidney-derived endothelial cell line. The role of EMT in renal fibrosis has been questioned in the literature, and the use of EMT to assess the role of endothelial-derived EETs in myofibroblast formation is not justified. The co-culture system would have benefited if endothelial cells were co-cultured with fibroblasts.

Despite these minor weaknesses, the study by Yang et al [5] demonstrated for the first time that EETs could reduce EndMT and further reiterated the potential clinical use of EETs, their agonists or sEH inhibitors in the treatment of kidney fibrotic diseases. However, future studies determining the renal benefits of sEH inhibitors and EET analogs should be approached with caution; one of the caveats of using sEH inhibitors or high concentrations of EETs is their potential to induce tumors [10]. Further, our understanding of the mechanisms by which EETs may offer renoprotection is still ongoing; a complete investigation of the various signaling pathways activated by EETs is warranted. Defining these signaling mechanisms will provide novel opportunities to develop strategies to augment EET signaling by modulating their downstream signaling pathways instead of the EETs themselves. New agents with improved pharmacokinetic properties are expected and, in particular, EET agonists and some compounds displaying both EET analog and sEH inhibitor dual activity could be of increased therapeutic value.

Conflicts of interest

The author has no conflicts of interest to declare.

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