Targeting epithelial–mesenchymal transition

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The epithelial–mesenchymal transition (EMT) is a process in which epithelial cells dedifferentiate to become mesenchymal cells [1–3]. The process requires them to lose their cell polarity, to relinquish their cell–cell adhesion, and to gain migratory and invasive properties. The mesenchymal cells are multipotent stromal cells (mesenchymal stem cells) that can differentiate into various cell types. EMT is essential for the developing embryo to enable mesoderm and neural tube formation. EMT is also necessary for wound healing and other physiological processes. On the other hand, EMT can also be an initiator for metastasis, cancer progression, organ fibrosis, and other pathological conditions. These are disorderly processes.

The induction of EMT is complex (Fig. 1) [4]. A fundamental event appears to be the loss of E-cadherin. Transcription factors that can repress E-cadherin directly or indirectly are important to this process. SNAI1/Snail 1, SNAI2/Snail 2 (also known as Slug), ZEB1, ZEB2, E47, and Krüppel-like factor-8 (KLF8) can repress E-cadherin transcription by binding to its promoter. Indirect repressors, such as Twist, Goosecoid, TCF4 (sometimes referred to as immunoglobulin transcription factor 2), homeobox protein SIX1, and FOXC2 (fork-head box protein C2) are able to repress E-cadherin indirectly [5]. Other junction proteins, such as claudins and desmosomes, are also suppressed, facilitating EMT. Various transcription factors such as grainyhead-like protein 2 homologue (GRHL2) and ETS-related transcription factors ELF3 and ELF5 are downregulated during EMT [6, 7]. The induction

of EMT is dependent on numerous signaling pathways including TGF- β , FGF, EGF, HGF, Wnt/ β -catenin, Notch, and hypoxia-induced mechanisms. Ras-MAPK signaling activates Snail and Slug during EMT induction. Slug is involved in disrupting desmosomes, inducing cell spreading, and separating the cell–cell borders [8]. However, EMT involves a second phase that cannot be triggered by Slug. This phase includes the induction of cell motility, repression of the cytokeratin expression, and activation of vimentin expression [9]. Phosphatidylinositol 3' kinase (PI3K)/AKT-axis activation is emerging as pivotal to EMT. Similarly, hedgehog, nuclear factor- κ B (NF- κ B), and activating transcription factor-2 have been implicated [10]. Of course, the EMT processes are specific, so that while Wnt signaling pathways regulate EMT in embryonic development, cardiac valve formation, and cancer, other pathways are important to wound healing and fibrosis [11].

EMT is central to two recent J Mol Med contributions. Kimura et al. report on the attenuation of EMT in retinal pigment epithelial cells and subretinal fibrosis by a retinoic-acid receptor- γ (RAR- γ) agonist [12]. The retinal pigment epithelium belongs to the neural retina and is indispensable for vision. In humans, proliferation and transformation (EMT switching) of retinal pigment epithelial cells after injury causes retinal disorders and loss of vision [13]. On the other hand, EMT could develop into a therapeutic target [14]. Subretinal fibrosis contributes to the loss of vision associated with age-related macular degeneration (AMD), a devastating and common disease in older people.

The authors began with retinal epithelial cells in culture. When subjected to TGF- β 2, the cells expressed mesenchymal markers including α -smooth muscle actin and fibronectin. The cells also released interleukin-6. Paxillin was phosphorylated; MAPK constituents such as ERK, p38, and JNK were activated, as were Smad2 and AKT. A RAR- γ agonist, R667, attenuated the EMT responses. Furthermore, the cells treated

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Fig. 1 The effector molecules regulate the different stages epithelial-mesenchymal transition (EMT) and the reverse process, mesenchymal-epithelial transition (MET). The regulation of the tight junctions and the adherent junctions are crucial. Epithelial (E)-cadherin, extracellular

matrix (ECM), fibroblast-growth-factor receptor-2 (FGF-2), fibroblast-specific protein-1 (FSP-1), microfilaments (MF), and numerous other molecules are pivotal. Physiological transitions are orderly; nonphysiological transitions are not. Adapted from [4]

with R667 no longer released matrix metalloproteinases. The authors were successful in translating these cell-based findings to a mouse model of subretinal fibrosis. The expression of glial fibrillary acidic protein was reduced in response to R667 injected into the subretinal space, as was the fibrosis area. Interestingly, R667 is palovarotene, a highly selective RAR- γ agonist that is also under investigation as a potential treatment for emphysema, another chronic disease involving EMT [15].

Renal fibrosis is another condition in which EMT is implicated. Similar to the EMT occurring in AMD, the EMT in fibrosis as well as in cancer is an anarchic cellular process actually developing at the expense of the whole organism [16]. Kang et al. also first studied renal proximal tubular epithelial cells [17]. The cells were subjected to high glucose concentrations (30 mmol/L). E-cadherin expression was reduced, while vimentin, α -smooth muscle actin, fibroblast specific protein-1, matrix metalloproteinases, and collagen production were increased. This state-of-affairs were improved considerably when the cells were exposed to 5,7-dihydroxyflavone, better known as chrysin. This “health food” supplement has been used by body builders, rumored to raise testosterone levels, is claimed to be anti-inflammatory, and is claimed to be anxiolytic. The authors next treated db/db mice. The db/db mouse is a model of obesity, diabetes, and

dyslipidemia. Over time, the mice develop diabetic nephropathy. The leptin receptor activity is deficient in db/db mice because the mice are homozygous for a point mutation in the gene for the leptin receptor. Chrysin administration to the mice decreased collagen production in the kidneys. Furthermore, chrysin treatment reduced tubular-cell migration concurrent with decreased matrix metalloproteinase-2 activity. These effects indicated less epithelial cell derangement and tubular basement membrane disruption. The compound appeared to restore tight junction proteins and to inhibit EMT-mediated tubulointerstitial fibrosis.

Sounds quite good but how much does EMT really contribute to this model? The contribution of EMT to the formation of renal fibrosis has been debated in several lineage-tracing studies, with conflicting findings. Different disease models, the mouse strains, and type of genetic alteration used could confound results. To determine the contribution of these factors to EMT, Inoue et al. generated four kidney disease models in several mouse strains genetically modified to express enhanced green fluorescence protein (EGFP) in cortical tubular epithelial cells under the control of the γ -glutamyl transpeptidase promoter [18]. The approach allowed them to visualize EMT and quantify EMT-related effects based on a count of EGFP-positive interstitial cells in the fibrotic kidney sections of the four renal disease models that they could then

classify to be either EMT-prone or -resistant. The EMT-prone models were unilateral ureteral obstruction and ischemic nephropathy in SJL mice. The EMT-resistant models consisted of ureteral obstruction in C57B/6 and F1(C57B/6×SJL) mice, adriamycin nephrosis in 129 mice, and nephrotoxic serum nephritis in SJL mice. Analyses of these renal disease models suggested that EMT-derived fibroblasts arose in a disease-specific and strain-dependent manner. Thus, when considering molecular mechanisms and involvement of the EMT in renal fibrosis, the authors suggest that taking experimental conditions, particularly the mouse strain and type of disease model, is important. Before we can accept the idea that RXR- γ agonists or chrysin specifically act by inhibiting EMT, the role of EMT in these models should be more precisely defined.

Respectfully,

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