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Epithelial-Mesenchymal Transition and its Role in Renal Fibrogenesis

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HIGHLIGHTS

- Exploring the role of TGFβ and its associate genes in the pathogenetic mechanism of EMT in renal fibrosis.
- Possible immunohistochemical markers (Pancytokeratin, SMA and Vimentin) to identify the process of EMT.
- Availability of various EMT related anti-fibrotic therapy with emphasis on stem cell therapy.

Abstract: Renal fibrosis in chronic renal failure poses a major challenge with regard to providing specific therapeutic strategies.
The current hypothesis of the epithelial-mesenchymal transition (EMT) in renal fibrosis proposes that matrix producing fibroblasts and myofibroblasts generated in the diseased kidney causes fibrosis. This paper explores the pathogenetic mechanisms, the role of EMT in renal fibrogenesis in chronic kidney disease, biomarkers, and EMT related anti-fibrotic therapy.

Keywords: renal fibrosis; myofibroblast; epithelial-mesenchymal transition; chronic kidney disease; anti-fibrotic therapy.

INTRODUCTION

Epithelial-mesenchymal transition (EMT) was first described by Elizabeth Hay in 1995 as "epithelial-mesenchymal transformation", which was later modified to "transition" due to the phenomenon of phenotypic plasticity and distinction from neoplastic transformation [1]. It is a biological process wherein epithelial cells lose the epithelial markers and gain mesenchymal markers, resulting in a mesenchymal phenotype. These transformed cells have the properties of increased migratory capacity, the potential to invade tissues, marked resistance to apoptosis and enhanced production of extracellular matrix components[2]. Numerous well-coordinated molecular mechanisms are known regarding the initiation of EMT and its outcome, which includes transcription factor activation, the pronouncement of cell surface receptors, reorganization and expression of cytoskeletal proteins, production of extracellular matrix (ECM) degrading enzymes and alterations in specific microRNAs. In vivo, some of these factors are used as biomarkers to demonstrate the process of EMT [2]

Classification of epithelial-mesenchymal transition

The exact mechanisms as to how terminally differentiated cells are derived from the germ cell layers i.e. ectoderm, mesoderm and endoderm during embryogenesis and organ development are debatable. However, it is now known that cells within certain tissue compartments appear to be plastic and thus can transform from epithelial to mesenchymal states and vice-versa [2].

EMTs are divided into three distinct biological subtypes based on the different functional outcomes:

Type 1 EMT

This type of EMT plays a role in implantation and embryonic development where primitive epithelial cells (e.g., cells of paraxial mesoderm) give rise to mesenchymal cells-mesoderm, endoderm, and neural crest cells during organogenesis. An important feature of this type of transformation is its ability to undergo a reverse mesenchymal-epithelial transition (MET) to generate different types of epithelia[3].

Type 2 EMT

The second type of EMT is associated with wound healing. This process commences following trauma and inflammatory injury which leads to the production of fibroblasts as a response to inflammation. This results in functional cells being completely replaced by a fibrotic scar secondary to an unabated inflammatory response [2].

Type 3 EMT

The third type of EMT plays an important role in invasion and metastasis in cancer progression here, neoplastic cells develop a mesenchymal invasive phenotype that has the propensity to invade and metastasize to various organs [4].

Other cells capable of EMT include podocytes and endothelial cells in the kidney

Podocyte Epithelial-Mesenchymal Transition

Podocytes, one of the components of the filtration barrier in the kidney, are glomerular visceral epithelial cells that allow selective permeability owing to their unique structure and specialized function. Injury to the podocyte is an important cause of several glomerular diseases. One of the responses of podocytes to various injuries is EMT which results in depletion of these cells leading to heavy proteinuria [5]. The phenotypic switch seen in the podocytes closely resembles type II EMT [6].

In diabetic nephropathy, high glucose medium upregulates the expression of MMP9, alpha-SMA and fibronectin and downregulates podocalyxin in podocytes resulting in podocyte EMT [7]

Endothelial Mesenchymal Transition.

Endothelial cells lining the vasculature of the renal parenchyma has been shown to have the propensity for the phenotypic switch through a process called endothelial-mesenchymal transition [8]. These cells lose their inherent endothelial characteristics and acquire the properties of fibroblast-like phenotype and gene expression [9]. This endothelial-mesenchymal transition has been demonstrated as an important pathological process in cardiac fibrosis [10], renal fibrosis [11], carcinoma-associated fibrosis [12] and atherosclerosis [13]. Any noxious stimuli to the renal parenchyma cause the endothelial cells to switch to mesenchymal cells as evidenced by the co-expression of CD31 (endothelial) and alpha-SMA and FSP-1 (mesenchymal) immunohistochemical markers in these lesions [9].

EMT and Biomarkers in Renal Fibrosis

In the normal kidney, renal tubules are lined by low cuboidal epithelial cells and are surrounded by the interstitium, which contains an occasional fibroblast in the quiescent state. These fibroblasts are formed during development with the help of type 1 EMT [14] and are responsible for maintaining the integrity of the interstitium [15].

Upon exposure to toxins, the tubular epithelial cells (TEC) undergo acute injury leading to death which activates surviving TEC to revert to its near normal structure and function. However, repeated TEC injury or chronic disease leads to prolonged release of stimulatory cytokines from the injured TECs as well as the

inflammatory cells in the vicinity leading to the production of activated myofibroblasts in the interstitium which contributes to fibrosis [4]. This process leads to the deposition of fibrotic tissue leading to progressive loss of renal function [16]. A wide range of pro-fibrotic cytokines plays an important role in chronic inflammation, which regulates the infiltration of inflammatory cells- neutrophils, macrophages and lymphocytes [17].

In chronic kidney disease, the three major signaling pathways which operate in the tubular and podocyte mesenchymal transition includes, TGFβ/Smad, integrin/ ILK, and Wnt/β-catenin signaling pathways [18]. Among these pathways, the TGFβ/Smad pathway is considered to be the prototype inducer of the tubular and podocyte mesenchymal transition [19]. Renal fibrosis is the consequence of the failed tissue repair following injury by noxious stimuli. This results in the secretion and gradual increase in various cytokines and proinflammatory cells in the tubular and capillary compartment of the renal parenchyma. This adversarial microenvironment makes the tubular epithelial cells to undergo phenotypic switching in order to escape apoptosis [18].

There are many factors in the tubular and capillary microenvironment which plays a vital role in promoting and suppressing EMT. The factors that promote EMT are cytokines (IL-1, Oncostatin M), growth factors (TGF-B1, FGF-2, connective tissue growth factor, angiotensin II), proteases (MMP-2, tissue-type plasminogen activator, Plasmin) and various environmental stresses. Suppressors of EMT on the other hand works in conjunction with injurious insults to balance the effect of EMT and they include growth factors (hepatocyte growth factor, bone morphogenic protein-7), nuclear receptor activator (vitamin D) and angiotensin II receptor blocker [18].

Studies have shown that experimental obstruction of one ureter followed by extraction of TECs and addition of transforming growth factor-beta-1 (TGF-β1) results in EMT [20]. The stages of this transformation result in the loss of epithelial cell adhesion, de-novo synthesis or cytoskeletal re-organization of alpha-smooth muscle actin (αSMA), disruption of the tubular basement membrane and migration of transformed cells into the interstitium [20]. TGFβ1 induces the transcriptional repressor gene Snail-1, which further downregulates the expression of E-cadherin in the renal tubular cells which is a cell adhesion molecule in renal tubular cells [19]. Other transcriptional factors that play a role in the downregulation of E-cadherin include Twist, Zeb 1, Zeb 2, and Slug [2]. (Figure 1) Additionally, this causes the tubular epithelial cells to acquire the morphological and functional features of myofibroblasts by de-novo expression of αSMA and re-organization of actin microfilaments [21]. This provides plasticity and mobility which are necessary properties in wound healing and fibrosis. These transformed cells express matrix metalloproteinase 9 (MMP 9) which has a major role in the dissociation of epithelial cells in EMT. Additionally, they have the capacity to degrade the basement membrane of the tubule and invade the interstitium, an inherent functional property of the myofibroblasts [22].

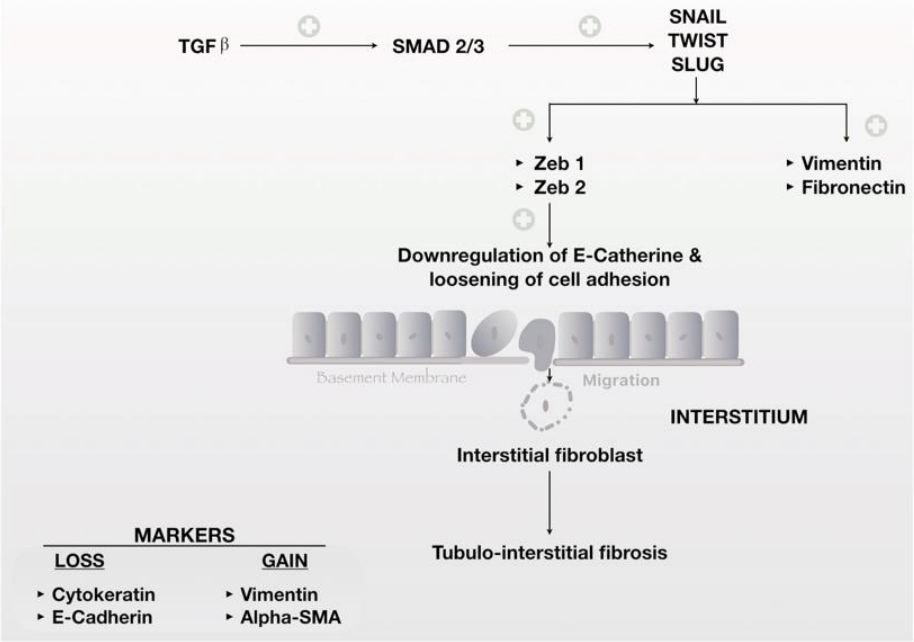

Figure 1. A simplified diagram showing the process of EMT where the transition of polarized epithelia cells transdifferentiates into mobile mesenchymal cells. TGF β induces the SMAD 2/3 which further upregulates the transcriptional repressor gene such as SNAIL/TWIST/SLUG to downregulate E-cadherin via activation of Zeb 1, 2 and also express vimentin and fibronectin in renal tubular cells

In murine models, it has been demonstrated that in the normal kidney, interleukin-15 (IL-15), maintains the homeostasis of the renal epithelial cells through an autocrine loop that protects the tubular epithelial cells from apoptosis and inflammation during nephritis [23]. Devocelle and his colleagues demonstrated a sharp depression of intra-renal IL-15 in different human inflammatory nephropathies resulting in fibrosis [24].

In an initial study by Strutz and coauthors, the authors used FSP1/S100A4 to demonstrate EMT. This protein is present on the fibroblast and not in the epithelial cells. In chronic kidney diseases (CKD) with renal fibrosis, FSP1/S100A4 was demonstrated in the interstitium and the tubular epithelial cells [25]. Subsequent studies showed that FSP1/S100A4 is not expressed in normal myofibroblasts. However, in diseased states, the expression of this marker is seen in fibroblasts and immune cells. Thus, making it a non-specific marker for myofibroblasts (26,27,28].

A common marker used to demonstrate the EMT process is vimentin. Studies have shown that injured tubular epithelial cells strongly express vimentin however, this is regarded as evidence of regenerating activity rather than proof of EMT [29,30,31]. Zheng and coauthors confirmed the importance of vimentin expression in the process of EMT as an inducer of allograft kidney failure in chronic allograft nephropathy [32]. These authors, established the role of vimentin in renal fibrosis via unilateral ureteral obstruction in vimentin knock out mice and compared the amount of fibrosis with the control group. A similar phenomenon was demonstrated in cultured human proximal renal tubular cells after vimentin expression was dimmed using lentivirus-driven inhibition of vimentin and subsequent treatment of the same with TGF-β which initiates the process of EMT. They concluded that inhibition of vimentin halts the process of fibrosis following unilateral ureteral obstruction, possibly by down streaming the signaling pathways [32].

A survey of the literature showed that α-SMA has also been suggested as a potential marker for EMT. Studies have shown that α-SMA positive cells are seen in the interstitium in injured tubules whereas the normal tubules were negative for this marker [33,34].

In a controversial paper by Humphreys BD and coauthors [35] and Iwano M and coauthors [36] regarding the origin of myofibroblasts in kidney fibrosis, the authors used high-resolution markers for renal tubular cells origin linked to nephrogenesis and demonstrated that interstitial cells differentiate into smooth muscle actin positive myofibroblasts during fibrosis, Concerning Humphreys BD and coauthors [35] and Iwano M and coauthors [36], Fragiadaki M and coauthors [4], suggested that to get an accurate picture of EMT it is essential to use two promoters along with different fluorescent proteins to confirm the presence of EMT in fibrosis and hence the original theory of EMT in renal fibrosis should not be rejected.

Thus a variety of immunohistochemistry (IHC) markers were used in the past as surrogates for direct detection of collagen synthesis by immunolabelling [37]. However, a definite marker for the demonstration of the EMT process is yet to be identified and this requires further research to identify definite markers to confirm the process of EMT.

EMT in Kidney Fibrosis- Clinical Perspectives

Chronic kidney disease (CKD) is characterized by the development of progressive renal tubular interstitial fibrosis, usually as a result of a primary renal disorder [33]. This process is irreversible which progressively leads to end-stage renal disease (ESRD). The initial insult is in the glomerulus, which then progresses to the tubulointerstitium, resulting in the replacement of the entire functional unit of the kidney to a fibrous scar. CKD has a high global prevalence which is estimated to be 11 to 13% [38]. In India, the age-adjusted incidence of ESRD due to CKD is 229 per million population with >100,000 new patients entering the renal transplantation program annually [39]. This increasing trend poses a major healthcare problem, especially in resource-limited countries.

Various surrogate markers such as albuminuria, doubling of serum creatinine and the evidence of a 50% reduction in eGFR are used in monitoring the progression of CKD to ESRD. However, the decline in renal function is not linear to the glomerular filtration rate (GFR) or proteinuria. Therefore, the prediction of a rapid or slow progression to chronic disease is completely dependent on either evidence in kidney biopsy or the use of newly developed biomarkers such as kidney injury molecule-1, neutrophil gelatinase-associated protein, apolipoprotein A-IV and soluble urokinase receptor [40].

There is limited evidence in support of EMT in vivo in hypertensive nephropathy [41,42]. In the rat cell culture models, angiotensin II was found to cause the transformation of epithelial cells to elongated, spindle-shaped mesenchymal cells [43], with loss of E-cadherin and gain of α-SMA in the transformed cells [44]. This supports the role of angiotensin II as a driving factor in EMT in hypertensive nephropathy.

Histopathological evaluation requires standardization of immunohistochemical markers, which can depict the progression of the disease in a phase-wise manner depending on the initiation or the level of EMT in the

renal biopsy during the various stages of CKD. Additionally, interstitial fibrosis and tubular atrophy (IFTA score) is used to quantify renal fibrosis which helps in the management of patients with CKD [45].

Rastaldi and coauthors [37] demonstrated EMT in 133 human renal biopsies from the spectrum of renal diseases with the help of IHC markers (ZO-1, α-SMA, CK, and vimentin) on renal tubular epithelial cells. With the progression of the disease, there was a loss of cytokeratin and ZO-1 and gain of α-SMA and vimentin thus showing a linear correlation with the severity of the disease. J. Yao and coauthors [46] studied 74 human renal biopsies in patients with IgA nephropathy and demonstrated EMT by using the IHC markers α- SMA and vimentin. They showed that there was an increased expression of tubular and interstitial α- SMA and vimentin with the severity of the disease. This proves that the process of EMT is responsible for the disease progression leading to CKD. Thus, early detection of EMT and targeted antifibrotic therapy is the key in the management of renal diseases associated with fibrosis.

The Current Trend in EMT Related Anti-Fibrotic Therapy

Numerous treatment modalities have been developed in the recent past to inhibit the progression of fibrosis. The three basic strategies used are a) Inhibition & Prevention of EMT, b) Removal of fibroblastic cells, and c) Re-transdifferentiation of myofibroblasts to epithelial cells [21].

a) Inhibition and prevention of EMT

To date, efforts have been made to inhibit EMT with the help of antagonists directed against EMT-inducing cytokines. The current important cytokine in the process of EMT-related tissue fibrosis is TGF-β. TGF- β has a wide range of biological functions that can affect all cellular functions involved in wound healing. Therefore, as a therapeutic agent the level of TGF- β should be adjusted to prevent its effects on other cells [47].

The targeted therapy is aimed at limiting the TGF-β signaling pathway as well as the reversal of established fibrosis [48]. Various molecular methods are available to disrupt the signaling pathway of TGF-β with the inhibitor of TGF-β mRNA expression. They include TGF- β1 anti-sense oligodeoxynucleotide and small molecule receptor kinase inhibitor [49]. Another method used is the sequestration of the ligand by soluble receptor ectodomain construct (ligand trap). These ligands occupy the same binding site of TGF-β receptors I and II and compete for receptor binding [47].

Hepatocyte growth factor (HGF) balances the effect of TGF- β and acts directly without interfering with the signaling pathway. Therefore, it has an antifibrotic property by reducing the levels of TGF- β in the lung [50].

Another chemical compound that can significantly inhibit the effects of TGF- β and reactive oxygen species production is zinc. Zinc inhibits the TGF- β/Smad pathway in the process of EMT in rat peritoneal mesothelial cells [51].

Other processes such as eliminating the local immune cell response or blocking the effects of inflammatory cytokines can effectively inhibit the EMT process [21].

In pathological stress, the levels of intra-renal IL-15 drops drastically and protects physiological function and thus leading to the survival of the renal epithelial cells [52]. The effectiveness of recombinant human IL-15 to inhibit TGF- β1-induced type 2 EMT has been demonstrated in a human cell model [24].

b) Removal of fibroblasts and/or myofibroblastic cells

Once fibrosis is established, it is practically impossible for the kidney to revert back to a normal function. Hence, novel strategies are aimed at preventing further damage by promoting apoptosis of fibroblast/myofibroblast in the damaged area via various signaling pathways like Rho/Rho-kinase and phosphatidylinositol-3-kinase/Akt signaling pathways. The end products of these pathways i.e. nerve growth factor and basic fibroblast growth factors respectively are used to stall the process of fibrosis [53, 54].

Carvalho and coauthors [55] demonstrated a novel method of removal of fibroblast through bone marrow mononuclear cell transplantation in cases of liver fibrosis secondary to bile duct ligation. The plausible pathogenesis includes apoptosis of fibroblasts and regeneration of hepatocytes through possible stimulation of cytokines.

c) Re-transdifferentiation of myofibroblast to epithelial cells

Myofibroblasts are not terminally differentiated cells and can easily revert to their origin in due course of time if the inducing signals are inhibited [21]. In an experimental model, TGF- β-induced EMT was reversed in humans and rat alveolar epithelial-like cell lines by using HGF which is an inhibitor of the TGF- β signaling

pathway, Smad7 [56]. Chemical compounds such as procyanidins and proanthocyanidins have anti-inflammatory and antioxidant features and are able to reverse the EMT process [57]. Human amniotic membrane stromal extracts and prostaglandins E2 also induces the trans-differentiation of myofibroblasts into fibroblasts [21].

Stem cells have a wide range of paracrine activity while maintaining the original cellular micro-environment. It has the capacity to replenish cells that are lost in tissue fibrosis [21]. Akram and coauthors [58] described the clinical application of intravenous and local administration of mesenchymal stem cells in the treatment of tissue fibrosis. Du and coauthors [59] demonstrated the use of Wharton's jelly-derived mesenchymal stem cells to delay the renal tubular EMT and thus alleviate renal fibrosis in an experimental model. Recent studies suggest that mesenchymal stem cell-derived from human adipose tissue play an important role in therapy for renal fibrosis. Adipose-derived mesenchymal stem cells (AMSC) through the autocrine and paracrine mediated release of angiogenic cytokines and growth factors improve the endogenous repair mechanism by increasing the peritubular capillary density and relieving tissue hypoxia [60].

Glial cell line-derived neurotrophic factor (GDNF) is known for its neuroprotective effects in hypoxic-ischemic encephalopathy [61]. Recent studies show that GDNF -/- mutant mice develop renal agenesis and dysgenesis suggesting that they play a critical role in renal development [62]. GDNF-modified AMSC helps in the repair of the injured microcirculation in the kidney by promoting more angiogenesis through the release of growth factors, thus protecting the kidney microenvironment from hypoxic injury [60].

CONCLUSION

The current knowledge on the role of EMT based on in vitro experiments shows that various cytokines including TGFβ play a major role in renal fibrosis. However, existing immunohistochemical markers have not been able to establish this mechanism. Multiple multidirectional in vitro experiments with in-vivo models are required to understand this process and the quest for appropriate IHC markers to demonstrate EMT in fibrogenesis should continue. This can aid in targeted antifibrotic therapy which reduces the burden of CKD.

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