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\*Robert Świder, Magdalena Durlik, Agnieszka Perkowska

## Epithelial-mesenchymal transition in the context of chronic fibrosis and function of kidney transplants

### Przemiana nabłonkowo-mezenchymalna w kontekście przewlekłego włóknienia i czynności przeszczepionych nerek

Department of Transplantation Medicine and Nephrology, Prof. Tadeusz Orłowski Transplantation Institute, Warsaw Medical University  
Head of Department: prof. Magdalena Durlik, MD, PhD

#### Key words

epithelial-mesenchymal transition, interstitial fibrosis/tubular atrophy, extracellular matrix, chronic allograft dysfunction

#### Słowa kluczowe

przemiana nabłonkowo-mezenchymalna, włóknienie zrębu i zanik cewek nerkowych, macierz zewnątrzkomórkowa, przewlekła dysfunkcja przeszczepu

#### Summary

The principal cause of delayed renal failure after transplantation is interstitial fibrosis and tubular atrophy (IF/TA). Identification of all possible causes of IF/TA and improvement of the methods of specific treatment of such cases will be important issues for renal transplantation medicine in the future. Evidence to suggest that the epithelial-mesenchymal transition (EMT), alongside IF/TA, is a significant event in the process of damaging the patient's own and transplanted kidneys has recently been appearing in the professional literature. In the course of the EMT, renal tubular cells undergo a process of gradual transformation into myofibroblasts.

The presented review article discusses the molecular and cellular pathways of the EMT and the role they play in the progression of chronic fibrosis of the kidney transplant. The potential therapeutic options for the EMT are still a subject for discussion but many facts suggest that the EMT plays the principal role in the pathogenesis of chronic interstitial fibrosis and tubular atrophy (IF/TA), and as a consequence of chronic allograft dysfunction (CAD). The importance of the EMT involvement in kidney transplant fibrosis has not been elucidated. Many data are taken into account for the purpose of determining whether the EMT can be a useful marker in the assessment of chronic allograft dysfunction progression.

#### Streszczenie

Główną przyczyną późnej niewydolności nerek po przeszczepie jest włóknienie ich zrębu i zanik cewek nerkowych. Identyfikowanie wszelkich możliwych przyczyn IF/TA (ang. *interstitial fibrosis/tubular atrophy*) oraz doskonalenie metod specyficznego leczenia takich przypadków, to kwestie, które w przyszłości będą stać przed transplantologią nerek. W prasie fachowej pojawiają się dowody sugerujące, że przemiana nabłonkowo-mezenchymalna (EMT) jest ważnym wydarzeniem w uszkodzeniu własnych i przeszczepionych nerek łącznie z włóknieniem zrębu/zanikiem cewek nerkowych. Podczas przemiany nabłonkowo-mezenchymalnej (EMT) komórki cewek nerkowych ulegają przekształceniu w miofibroblasty przez stopniowy proces przemiany.

W prezentowanym artykule, o charakterze przeglądowym, rozpatrywane są molekularne i komórkowe szlaki przemiany nabłonkowo-mezenchymalnej (EMT) oraz rola, jaką pełnią w progresji przewlekłego włóknienia przeszczepionej nerki. Potencjalne możliwości terapeutyczne dotyczące przemiany nabłonko-mezenchymalnej nadal pozostają kwestią do dyskusji, jednak wiele faktów przemawia za tym, że EMT odgrywa główną w patogenezie przewlekłego włóknienia zrębu i zaniku cewek nerkowych (IF/TA), a w konsekwencji do przewlekłej dysfunkcji przeszczepu (ang. *chronic allograft dysfunction*, CAD). Wielkość udziału EMT w włóknieniu przeszczepu nerki pozostaje nierozpoznana. Wiele danych jest branych pod uwagę w celu określenia, czy przemiana nabłonkowo-mezenchymalna może być użytecznym markerem w ocenie progresji przewlekłej niewydolności przeszczepionych nerek.

#### Address/adres:

\*Robert Świder  
Department of Transplantation Medicine and Nephrology Prof. Tadeusz Orłowski Transplantation Institute  
Warsaw Medical University  
ul. Nowogrodzka 59, 02-005 Warszawa  
tel. +48 (22) 502-12-32  
medicusbonus@gmail.com

#### Abbreviations:

EMT – ang. *epithelial-mesenchymal transition* (przemiana nabłonkowo-mezenchymalna)  
IF/TA – ang. *interstitial fibrosis/tubular atrophy* (włóknienie zrębu i zanik cewek nerkowych)  
ECM – ang. *extracellular matrix* (macierz zewnątrzkomórkowa)  
CAD – ang. *chronic allograft dysfunction* (przewlekła dysfunkcja przeszczepu)

## INTRODUCTION

Interstitial fibrosis/tubular atrophy in kidney transplants is one of the principal causes of delayed dysfunction. IF/TA is a chronic progressive non-specific histopathologically irreversible nosocomial entity with an early onset after transplantation, associated with significantly increased morbidity and mortality of the allograft recipients. A new approach aimed at obtaining longer vitality of kidney transplants is identification of the IF/TA causes and development of a strategy of specific treatment of such cases. Interstitial fibroblasts are the main source of renal fibrosis. Under the effect of "stress", renal interstitial fibroblasts divide and spread, producing profibrotic molecules. More than 1/3 of the fibroblasts associated with renal fibrosis originate from renal tubular epitheliums, from the sites where a damaging factor was active as a result of the EMT. In the course of the EMT, renal tubular cells are gradually transformed into myofibroblasts, which includes the loss of links between tubular cells, the loss of E-cadherin expression, *de novo* expression of smooth muscle  $\alpha$ -actin, reorganisation of actins, abnormalities of the tubular basement membrane ultrastructure, and migration and invasion of fibroblast cells producing basic profibrotic molecules for the fibrosis process such as collagen type I and III and fibronectin.

Prolonged action of the factors damaging the kidney transplant is undoubtedly the main cause of chronic allograft dysfunction and consequential kidney transplant loss. Prolonged IF/TA and damage to renal blood vessels and glomeruli make kidney transplants lose their function over variable periods of time (several months to several or more than ten years after transplantation). The above-described situation is defined as chronic allograft dysfunction in the professional literature; it makes any further therapeutic options very limited (1). The incidence of chronic allograft dysfunction ranges from 23% at five years after transplantation to 60% at ten years after transplantation. It is necessary to effectively counteract this phenomenon, because loss of the transplanted organs not only deteriorates patients' health but also increases the number of patients awaiting transplantation (2).

## CHARACTERISTICS OF EPITHELIAL-MESENCHYMAL TRANSITION

The epithelial-mesenchymal transition is a biological process that enables polarised cells of the renal tubular epithelium, which normally interact between the basement membrane and their own parabasal surface, to obtain phenotypic characteristics of a mesenchymal cell: increased migration ability, invasiveness, increased resistance to apoptosis, and markedly increased production of extracellular matrix (ECM) constituents. The end of the epithelial-mesenchymal transition is signalled by damage to the basement membrane and formation of a mesenchymal cell which obtains ability to migrate from the monolayer renal tubular epithelium from which it originates to the renal in-

terstitium. Numerous molecular processes participate in EMT initiation and make its completion possible. They include activation of transcription factors, expression of specific surface proteins, reorganisation and expression of cytoskeleton proteins, production of enzymes breaking down extracellular matrix, and changes in genetic information expression. In many cases, the above-listed factors are used as biomarkers for confirmation of the completed epithelial-mesenchymal transition process of the tubular epithelial cells (3-5).

The groundbreaking study of Elizabeth Hay, who was the first to describe the process of "epithelial-mesenchymal transformation", was based on an animal model (notochord formation in chick embryos). Shortly after, the term "transformation" was replaced by "transition", reflecting partial reversibility of the process, which is in fact a separate form of neoplastic transformation (5, 6). Phenotypic plasticity of the EMT is manifested through a reverse process of mesenchymal-epithelial transition (MET) which engages a change of the mesenchymal cell phenotype to epithelial derivatives (7, 8).

There are three basic types of the epithelial-mesenchymal transition (8). Type I EMT is associated with implantation and gastrulation of the ovum, and it is a point of departure for differentiation of mesodermal and endodermal cells and migrating neural crest cells. The primitive epithelial cell, the epiblast, gives rise to primary mesenchyme via the EMT. Primary mesenchyme can be transformed to secondary epithelium via the MET. It is suggested that these secondary epithelial cells may differentiate further into different epithelium types and undergo subsequent EMT processes in connective tissue cells such as astrocytes, adipocytes, chondrocytes, osteoblasts and muscle cells (9). Type II EMT is associated with inflammation and fibrosis processes. In contrast to type I, type II EMT persists longer and may damage the kidney transplant if the primary inflammatory process is not eliminated or reduced (10). Secondary epithelial cells, present in many organs, may undergo transformation into cancer cells which subsequently undergo epithelial-mesenchymal transition making them invasive and metastatic; they represent type III EMT. Fibrosis of the organ where the epithelial tissue is present is indirectly caused by inflammation cells and fibroblasts, which signal inflammation symptoms and release ingredients of the extracellular matrix complex such as collagens, laminins, elastin and tenascin (9, 10).

The epithelial-mesenchymal transition occurs in the course of fibrosis of the kidneys, liver, lungs and intestine and is associated with the production of various molecules by inflammation cells and locally activated fibroblasts (myofibroblasts). These molecules cause disruption of epithelial layers, for example by degradation of their basement membranes. Epithelial cells lose their polarity or undergo apoptosis involving most of the cells or undergo the epithelial-mesenchymal transition involving a smaller number of cells. Fibroblast-specific proteins (FSP1, S100A4, MTS-1) belong to the

S100 protein class, which are cytoskeleton proteins;  $\alpha$ -SMA and type I collagen are markers of products of mesenchymal origin formed during the EMT (11, 12). These markers, along with vimentin and desmin, are usually used for identification of those epithelial cells of the kidneys, liver, lungs and intestine which are in the course of the epithelial-mesenchymal transition process associated with chronic inflammation. These cells still display the morphology typical for epithelial cells and protein markers such as cytokeratins and E-cadherin but also the morphology accompanying *de novo* expression of mesenchymal cell markers (FSP1) and  $\alpha$ -SMA. In fact, these cells represent an intermediate stage of the EMT, because epithelial cell markers are still expressed and new markers of mesenchymal cells are just being acquired. Eventually, these cells leave the monolayer renal tubule epithelium, crossing the basement membrane, and accumulate in the interstitium, where at the end of their journey, deprived of their own markers typical for epithelial cells, they obtain the full fibroblastic phenotype (11).

#### **EPITHELIAL-MESENCHYMAL TRANSITION AS A BIOMARKER OF CHRONIC ALLOGRAFT DYSFUNCTION**

As evidenced in professional literature, in subsequent protocol-specified biopsies of kidney transplants (at three and twelve months after transplantation) EMT intensity correlates with progressing IF/TA (13-15). Expression of EMT markers is detected in the first month after transplantation. Patients with confirmed EMT activity in the third month after transplantation experienced more rapid deterioration of kidney transplant function. However, the fact that these EMT markers are usually detected on the epitheliums of kidney transplant tubules makes it possible to detect them as an early indicator of renal fibrosis.

In an analysis of kidney transplant biopsy specimens obtained up to the third month after transplantation, molecular biology tools revealed inconsistent data on expression of the majority of genes associated with the EMT or MET: they were transcribed or were insignificantly transcribed in biopsy specimens with advanced IF/TA lesions (15, 16). Similar results were obtained in a study of surface and intracellular proteins, demonstrating that expression of EMT markers in the first month after transplantation was not related to IF/TA in the third month after transplantation. The epithelial-mesenchymal transition was defined as a loss of the surface marker E-cadherin and as  $\alpha$ -SMA or S100A4 expression on tubular epithelial cells (15). Baseline expression of E-cadherin is lowest on the epithelium of proximal renal tubules. In the third month after transplantation, rarely extensive expression of the S100A4 protein is detected in the fibrous tissue, which suggests that E-cadherin should be the marker of delayed epithelial-mesenchymal transi-

tion. One of the reasons for low detectability of EMT markers within three months after transplantation may be too early collection of the histopathological specimen (biopsies in the first and second months after transplantation), or an error in the adopted criteria of EMT diagnosis, or the fact that the EMT process has not yet started in the kidney transplants. The last stage of the EMT, i.e. migration of cells from kidney tubules outside the single-layer structure has been convincingly demonstrated *in vivo* in type II EMT.

The term "phenotypic changes of tubular epithelial cells" was proposed for the description of early stages of EMT observed in kidney transplants. Some tubular epithelial cells lose expression of typical surface markers of the renal tubular epithelium and abnormally express mesenchymal cell markers, which is what confirms the presence of the epithelial-mesenchymal transition. As long as the EMT is not considered a clinical therapeutic target, the role of the EMT in fibrosis of kidney transplants will still be disregarded (15, 16).

For many years IF/TA was considered to be a process of undetermined aetiology but closely correlated with clinical symptoms of chronic allograft dysfunction and having a high prognostic value, hence the identification of fibrosis markers was necessary. The presence IF/TA in a biopsy specimen of the kidney transplant rarely changes clinical therapeutic decisions. Although most kidney transplants are lost due to chronic fibrosis, it is rarely a consequence of organ rejection or relapse of the underlying disease in the transplant. The presence of advanced IF/TA is defined as one of the endpoints for taking clinical decisions (17-19).

For clinicians, the most important current application of the information from studies on the EMT is identification of endogenous factors that are able to inhibit phenotypic transformation of epithelial cells, suggesting a possibility of therapeutic intervention into the EMT. Two endogenous factors, namely TGF- $\beta$  and BMP-7, are presented as potential activators/inhibitor which effectively activate/block the EMT *in vitro* and *in vivo*. Retrospectively, these results are not surprising, because both antagonistic mediators: TGF- $\beta$  and BMP-7, play an important role in the early development and formation of renal tissue and preservation of the renal tubular epithelial cell phenotype in mature kidneys (15, 20). The consequence of the presence of both antagonistic mediators is a new idea explaining their role as that of internal factors which presumably protect the phenotype of renal tubular epithelial cells by preventing the EMT *in vivo*.

#### **CONCLUSIONS**

The article attempts to explain to clinicians the molecular and cellular pathways of the epithelial-mesenchymal transition and their role in the progression of chronic fibrosis of kidney transplants. The potential therapeutic options are still a subject for

assessment and discussion but many facts suggest that the epithelial-mesenchymal transition plays the principal role in the pathogenesis of chronic interstitial fibrosis and tubular atrophy, chronic allograft dysfunction, and as a consequence in the failure of a transplanted kidney (21, 22). The extent of the epithelial-mesenchymal transition in kidney trans-

plant fibrosis remains not fully elucidated and only a large clinical trial could resolve this issue. Moreover, a lot of additional information is taken into account for the purpose of determining whether the epithelial-mesenchymal transition can be a useful surrogate marker in the assessment of chronic allograft dysfunction progression (23).

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