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TUBULOEPITHELIAL - MYOFIBROBLAST TRANSDIFFERENTIATION - POSSIBLE PATHOGENIC MECHANISM OF INTERSTITIAL FIBROSIS IN BALKAN ENDEMIC NEPHROPATHY

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Summary. *Balkan endemic nephropathy (BEN) is a chronic tubulointerstitial disease of unknown etiology. The main morphological feature is interstitial fibrosis and tubular atrophy with absence of inflammatory infiltration. The pathogenesis of BEN is also obscure. Since tubular epithelial cells in the early phase of disease express vimentin as mesenchymal marker in addition to cytokeratin, we could speculate that transdifferentiation of tubular epithelial cells into myofibroblast could be responsible for causing interstitial fibrosis in BEN. Further studies are necessary to support this hypothesis*.

Key words: *Balcan endemic nephropathy, renal interstitial fibrosis, transdifferentiation, myofibroblast*

Balkan endemic nephropathy (BEN) is a chronic and fatal tubulointerstitial disease endemic to restricted areas of former Yugoslavia, Romania and Bulgaria, within the Danube Basin of unknown etiology. It was common in the past and it has become rare in recent years.

Morphology of BEN

Gross autopsy examination of BEN patients deceased due to the end-stage renal failure has revealed shrunken smooth surface kidneys. The kidneys are bilaterally and symmetrically involved with severe superficial cortex atrophy and sclerosis. They are usually very small and weight between 20 and 60 gr. Light microscopic findings in those kidneys show enormous interstitial fibrosis predominantly localized in the cortex associated with huge tubular atrophy. Absence of interstitial inflammation is characteristic morphologic feature of BEN. Glomerular sclerosis and hyalinization may be found in subcapsular areas, where the morphological changes are the most severe. Vascular lesions are usually mild.

Renal biopsy examination in the early stage of disease has predominantly shown changes of proximal tubules. Focal tubular atrophy has been accompanied by interstitial edema and fibrosis. In cases with less intensive involvement, scattered areas of interstitial fibrosis are usually observed in the superficial cortex. Rare interstitial infiltrating lymphocytes could be detected. Glomeruli are usually at that moment uninvolved. In the cases with more extensive involvement, the larger multifocal superficial interstitial areas of fibrosis became confluent extending into the deeper cortex. Interstitial inflammatory lymphocytes are still scattered. Glomerular findings are nonspecific and resemble those associated with aging.

The distribution of interstitial pathology more commonly in the superficial cortex is similar to that seen secondary to vascular involvement. Although renal vascular changes in BEN patients are relatively common, they are usually present as slight hyalinization of some small arteries or arterioles. However, classical morphological pictures of ischemic lesions due to vascular changes are different and at the final stage there is fine granular nephrosclerosis in contrast to smooth shrunken kidney in the case of BEN. All these vascular changes are found to be slightly more frequent in patients with low labile hypertension than in those who were normotensive.

Immunohistochemical investigations of renal tissue of BEN are unspecific and reveal in one third of biopsies focal segmental mesangial deposition of immunoglobulin M and component of complement C3. The most common finding is deposition of C3 in small extraglomerular vessels.

Changes visualized by electron microscopy are nonspecific, although osmiophilic bodies (0.1 to 0.5 mm in diameter) have been found in tubular epithelial cytoplasm.

Histological changes in BEN share similarities with renal injuries caused by toxic substances (lead, cadmium, lithium), low molecular proteins, cyclosporin as well as chronic radiation nephropathy.

Renal interstitial fibrosis

Renal interstitial fibrosis is the main morphological feature of BEN and in the same time the major cause of the chronic renal failure in those patients. Renal interstitial fibrosis is an abnormal process associated with the deposition of huge amounts of the extracellular matrix, which has a complex structure composed of different connective tissue elements such as collagen types I through VII, laminin, fibronectin, tenascein, elastin, proteoglycans etc. The architectural structure of the tubulointerstitium in healthy and nonfibrotic kidneys is based on the balance between different parts of the renal components: interstitial cells, the extracellular matrix, tubular and endothelial cells (1). Whenever any of these components are altered, the others are also involved in the disease process. Renal interstitial fibrosis will develop only when an imbalance between these components appears, and it seems that each of them could actively participate in triggering and developing fibrosis. Thus, there are several mechanisms that could induce renal interstitial fibrosis or scarification and the most common are interstitial inflammation or tissue necrosis. However, both mechanisms are not a dominant cause of renal interstitial fibrosis in BEN since inflammatory cells are very conspicuous at the beginning and almost absent in the developed disease. Very benign vascular changes seen at the early stage of BEN are not sufficient to induce serious ischemic or any necrotic lesions in the kidneys.

The resident interstitial cells of the kidney mainly involved in causing renal interstitial fibrosis are fibroblasts (2). These cells have traditionally been characterized by morphology describing the cell that is fusiform in appearance and related to the extracellular substance. Fibroblasts are able to respond to a variety of autocrine and paracrine factors released by cells infiltrating the renal tissue or by other renal cells. Fibroblasts produce components of extracellular matrix collagen I and III, fibronectin, tenascein within the interstitium.

In addition to fibroblast, myofibroblast could be also involved in developing renal interstitial fibrosis. It seems that these cells are more fibrogenic than fibroblasts and the precise origin of the interstitial myofibroblast remains obscure. A number of studies have suggested that renal interstitial myofibroblasts derive from the differentiation of fibroblasts or migration from perivascular smooth muscle cells. Recently, it has been clearly demonstrated that tubular epithelial cells are able to transdifferentiate into myofibroblasts and therefore could be responsible for inducing interstitial fibrosis.

Transdifferentiation

Transdifferentiation is the process of transformation of one type of tissue into another i.e. mesenchym into epithelium or *vice versa* epithelium into mesenchyme. The most important characteristic of transdifferentiation is changing of the tissue phenotypes (Table 1). So, intermediary filaments of epithelial cells such as cytokeratin could be changed into vimentin, which is a cytoskeletal component of mesenchymal cells. The predominant component of extracellular matrix produced by epithelial cells laminin could be changed with collagen types I or III synthesized by mesenchymal tissue. Expression of the most common adhesion integrin receptors $\alpha 6\ \beta 1$ is transformed into integrin $\alpha 5\ \beta 1$ expressed on mesenchymal cells. All these changes lead to morphological transformations that are manifested by loss of apico-basal polarity of epithelial cells.

Table 1. Transdifferentiation

	epithelium	mesenchym
Intermediary philaments	keratin	vimentin
extracellular matrix	laminin A	collagen I, III,
integrin receptors	$\alpha 6\ \beta 1$	$\alpha 5\ \beta 1$
polarity	apico-basal	forward-back

Transdifferentiation in the human kidney is a physiological process during embryonic and foetal development. Human kidneys arise from metanephros. During embryogenesis mesenchymal markers are expressed on nephrogenic mesenchyme. The ureteric bud induces the undifferentiated mesenchyme to convert to polarized tubular cells in the presence of laminin A with expression of $\alpha 6\ \beta 1$ integrin in addition to other epithelial markers (3).

Transdifferentiation could be also present in pathological conditions in adult kidneys. During malignant transformation tubular epithelial cells are able to reexpress some mesenchymal markers and thus renal cell carcinoma could coexpress cytokeratin and vimentin as well as other epithelial and mesenchymal markers (4). The hypothesis that transdifferentiation is involved in renal fibrogenesis was first postulated by Strutz (5) who has demonstrated that tubular epithelial cells are capable of *de novo* expression of a fibroblast specific protein (FSP1) *in vivo* and *in vitro* in a mouse model of anti tubular basement membrane nephritis. This has provided phenotypic evidence that tubular epithelial cells have the ability to transdifferentiate into fibroblasts in the progressive tubulointerstitial fibrosis. Further evidence that supports this hypothesis that transdifferentiation of tubular epithelial cells contributes to renal fibrosis was given in experimental progressive tubulointerstitial fibrosis in rat remnant kidneys (6). Here, the authors immunohistochemically showed *de novo* expression of α-smooth muscle actin by tubular epithelial cells indicating transdifferentiation of those cells into myofibroblasts and their participation in producing extracellular matrix in the interstitium. By electron microscopy they documented the presence of characteristic actin microfilaments and dense bodies within transdifferentiated tubular epithelial cells with an apparent morphological transformation from epithelial to mesenchymal cells.

Further research of pathogenesis of BEN

Recently, immunohistochemical analysis of vimentin expression in renal tissues at early stage of BEN has revealed that proximal tubular cells coexpressed vimentin and cytokeratin. The expression of vimentin on proximal tubular cells was corresponding to the degree of damage of those epithelial cells (7). In addition, very intense vimentin staining was found in the surrounding areas with marked interstitial fibrosis. Further studies are needed to clarify whether in BEN tubular epithelial cells could transdifferentiate into myofibroblast and thus participate in causing interstitial fibrosis.

Little is known of the mechanisms that may induce tubular epithelial - myofibroblast transdifferentation. It is still unclear what could activate the mesenchymal gene program leading to loss of tubular epithelial cell polarity, which facilitates the migration of transformed tubular epithelial cells into the peritubular interstitium. Maybe an investigation on the pathogenic mechanisms of interstitial fibrosis of BEN could get more input into renal fibrogenesis in general.

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