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Original contribution

Tubular nuclear accumulation of Snail and epithelial phenotypic changes in human myeloma cast nephropathy ☆

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Summary The transcription factor Snail is an important repressor of E-cadherin gene expression. It plays a key role in the induction of epithelial-mesenchymal transition, an essential process important not only in embryonic development and tumor progression but also in organ fibrogenesis. We studied the expression of Snail by immunohistochemistry, along with several epithelial phenotypic changes suggestive of epithelial-mesenchymal transition, in 14 patients with multiple myeloma cast nephropathy. This nephropathy is characterized by a rapid progression toward fibrosis. As controls, we used normal kidneys and kidneys from patients displaying an idiopathic nephrotic syndrome, a syndrome unassociated with renal fibrosis. We discovered that, in all patients with multiple myeloma nephropathy, a drastic accumulation of Snail is seen in the nuclei from tubular epithelial cells showing epithelial phenotypic changes. In contrast, normal and idiopathic nephrotic syndrome kidneys did not exhibit either of these markers. Snail, a major player in the process of epithelial-to-mesenchymal transition, is highly expressed by tubular epithelial cells during multiple myeloma nephropathy. It is, therefore, a potential target to prevent multiple myeloma kidneys from fibrosing. Intranuclear accumulation of Snail is a characteristic in phenotypically altered tubular cells from multiple myeloma kidneys. The epithelial-mesenchymal transition pathway could, therefore, be involved in the rapid renal fibrogenesis observed in this setting.

1. Introduction

Epithelial-mesenchymal transition (EMT) is a complex biologic process defined by a profound phenotypic switch: cells lose important epithelial features and acquire

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mesenchymal-like properties. Activation of the EMT program is essential to embryonic development and tumor progression, notably because EMT promotes cell movement and thereby allows "transformed" epithelial cells to invade adjacent tissues or to migrate even further [1-3]. In mice, it was also reproducibly observed that EMT was contributing to the swelling of the fibroblast population in injured adult organs and promoted tissue fibrogenesis [4]. For example, in the kidney, phenotypically altered tubular epithelial cells were found to migrate into the interstitial space and deposit extracellular matrix [5,6]. Whether EMT is instrumental in human fibrosing diseases is at present vividly debated. Regardless, our group has reported that the presence in tubular cells from human renal grafts of 2 early epithelial phenotypic change (EPC) markers, highly suggestive of EMT (viz, the intracellular translocation of β-catenin and the de novo expression of vimentin), performed well to predict occurrence as well as of progression of fibrosis [7,8]. Similar EPC have been reported in native kidneys, and they are also associated with interstitial fibrosis [9,10]. Thus, although the evidence that injured tubular cells will become interstitial fibroblasts is lacking in humans, they undoubtedly exhibit EMT-like changes, which correlate with fibrogenesis.

The Snail superfamily of zinc finger transcriptional factors plays a pivotal role in the initiation of EMT and cell movement. Kidney tubules from transgenic mice expressing a high level of a tamoxifen-inducible Snail nuclear translocation construct exhibited profound phenotypic alterations that are highly reminiscent of EMT, and Snail activation was sufficient to induce kidney fibrosis [10]. By binding to the E-box, Snail family members repress the transcription of characteristic epithelial genes such as *E-cadherin*, *epithelial mucin muc 1*, *desmoplakin*, and *cytokeratin 18*. They may also indirectly disrupt epithelial homeostasis by repressing hepatocyte nuclear factor 1 β (HNF-1β, a key kidney differentiation transcription factor responsible for the transcription of cadherin 16 [10]. Conversely, they activate mesenchymal genes such as *vimentin* and *fibronectin* [2]. Interestingly, the many signaling molecules that are capable to induce EMT, such as transforming growth factor β (TGFβ), epidermal growth factor (EGF), fibroblast growth factor (FGF), hepatocyte growth factor (HGF), WNT signaling proteins and Notch, all induce the expression of Snail [11]. The expression and the nuclear accumulation of Snail are also known to be highly regulated at the posttranscriptional level. In physiological conditions, Snail is rapidly routed to the proteasome and degraded, but several events may counteract its ubiquitination and perpetuate its activity in the nuclei: a glycogen synthase kinase 3 β–mediated phosphorylation [12], downstream of Wnt signaling; a lysine oxidation by Loxl2 in hypoxic condition [13]; or the activation of nuclear factor κ B triggered by tumor necrosis factor α in inflammatory situations [14].

An increase in the transcription of Snail was demonstrated in human fibrotic kidneys [10], but to the best of our knowledge, the nuclear accumulation of the protein Snail has never been studied in human kidneys (in particular in the tubular epithelial cells in which EMT should be looked for). Herein, we studied the expression of Snail in the kidneys from patients with multiple myeloma (MM) cast nephropathy, a kidney disease well known for its rapid progression to fibrosis, as opposed to idiopathic nephrotic syndrome (iNS) kidneys (a disease with overt albuminuria but little fibrosis) and normal kidneys. We found that, in contrast with these 2 latter control groups, MM kidneys were characterized by a strikingly high level of intranuclear Snail and by an abundant expression of other mesenchymal markers such as vimentin, β-catenin, and HSP47 (a chaperone protein for collagen synthesis).

2. Concise methods

2.1. Patients

Fourteen patients with MM nephropathy have been included in the present study who met the following criteria: (1) admission in our renal intensive care unit between 1997 and 2004 because of renal insufficiency (serum creatinine >1.3 mg/dL), (2) diagnosis of MM based on the criteria established by the International Myeloma Working Group [15] (ie, presence on a bone marrow aspirate of >10% of plasma cells, showing a neoplastic phenotype), (3) proteinuria primarily consisting of immunoglobulin light chains (LCs), and (4) a renal biopsy showing MM cast nephropathy.

Fifteen patients with overt albuminuria due to an iNS were also studied. Unaffected areas from kidneys removed because of a malignant tumor (3 patients) were used as normal kidneys. The patients included in this study all gave a written informed consent.

2.2. Immunohistochemistry

Paraffin sections were deparaffinized and hydrated. The antigens were retrieved by boiling for 20 minutes in 10-mmol/L citric acide solution (pH 6). The sections were incubated overnight at 4°C with the following primary antibodies: 1:1/2000 anti-Snail1 (rabbit polyclonal antibody; Abcam, Cambridge Science Park, UK), 1 μg/mL anti–β-catenin (rabbit polyclonal antibody; Santa Cruz Biotechnology, Tebu, Le-Perray-en-Yvelines, France), 1:1000 antivimentin (mAb V9; Zymed, In Vitrogen, Cergy-Pontoise, France), 1:1/5000 anti–Heat-Shock protein 47(HSP47) (mAB M16.10A1; Stressgen, Biotechnologies, Victoria, BC, Canada), or 1/10000 anti–Tamm-Horsfall (TH) protein (mouse monoclonal antibody, home made [16]). The sections were then incubated with antirabbit or antimouse antibody conjugated with peroxidase-labeled polymer (Dako, Trappes, France). Immunoreactive proteins were visualized with a 3-amino-9-ethylcarbazole–containing peroxidase kit (Dako) and counterstained with