Twist: a new player in the epithelial–mesenchymal transition of the peritoneal mesothelial cells

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Abstract

Background. The peritoneal membrane is a vital structure for peritoneal dialysis (PD) patients. It has been increasingly recognized that the transition of the peritoneal lining mesothelial cells into a more fibroblastic phenotype is a key step in peritoneal membrane injury.

Methods. Relevant literature was reviewed and summarized.

Results. Epithelial-to-mesenchymal transition (EMT) is a basic cellular process that occurs in a variety of physiologic and pathologic processes. The hallmark of this process is a loss of epithelial markers, and E-cadherin is a prototypical epithelial transmembrane protein. E-cadherin expression is suppressed at many levels and the gene is regulated by a family of transcription factors. Twist is one of the lesser studied E-cadherin regulatory factors, which belongs to a larger family of basic helix-loop-helix DNA-binding proteins. In this issue of Nephrology Dialysis Transplantation, Cuixiang Li reports on in vitro experiments where the expression of Twist led to a decreased expression of E-cadherin and the evidence of EMT. In an in vivo model of dialysate exposure, Li demonstrates that Twist expression is increased in the injured peritoneal tissues.

Conclusions. These important observations are the first to link Twist to mesothelial cell EMT and peritoneal membrane injury. Like most novel observations, this paper leaves many questions unanswered. Twist is only one of several transcription factors involved in EMT and how these factors interact will require further investigations. Furthermore, the question of whether Twist interacts at multiple levels in the EMT process, or simply gives an initial push to the process, is left unanswered. Finally, to bring these early significant findings to the bedside as potential therapies for PD patients will require further innovation.

Keywords: epithelial–mesenchymal transition; matrix metalloproteinase; peritoneal fibrosis; snail; Twist

The peritoneal membrane is a seemingly simple structure, which provides life support for patients with renal failure who rely on peritoneal dialysis (PD) as their renal replacement therapy. Over time, most PD patients develop fibrosis and angiogenesis of the peritoneal tissues which impacts negatively on the functional characteristics of this membrane. An emerging concept in organ fibrosis suggests that the cellular protagonist—the myofibroblast—can be derived from the transition of injured epithelial cells. This epithelial-to-mesenchymal transition (EMT) has been observed in animal models of peritoneal injury [1] and in the peritoneal tissue of patients on PD [2].

EMT is a cellular program consisting of a loss of cell–cell and cell–matrix interaction; loss of cellular polarity; cytoskeletal rearrangement with an increased expression of α-smooth muscle actin (α-SMA) and basement membrane degradation with subsequent migration or invasion. In a recent review article, Kalluri and Weinberg [3] suggested that EMT occurs in different settings. Type 1 EMT is an essential process in development. Type 2 EMT is the phenomenon we are interested in and describes a beneficial event in normal wound healing or pathologic event in fibrosis. Type 3 EMT occurs in the setting of metastatic transformation of cancer cells.
In the present edition of Nephrology Dialysis Transplantation, Cuixiang Li describes in vitro and in vivo experiments that provide further information about the role of the basic helix-loop-helix (bHLH) regulatory factor Twist in the induction of EMT in the peritoneal tissues (4). Li found that Twist inhibits E-cadherin expression and increases the expression of matrix metalloproteinase 9 (MMP9) in peritoneal mesothelial cells.

E-cadherin is a key epithelial transmembrane intercellular adhesion molecule and its loss is commonly used as an essential marker of EMT. E-cadherin expression is regulated at multiple levels including gene expression and both extracellular and intracellular protein cleavage. E-cadherin gene expression is suppressed by a family of regulatory proteins. These regulatory proteins fall into three broad families and include the zinc finger DNA-binding proteins Snail1 and Snail2 (Slug); the ZEB family of transcription factors (ZEB1 and ZEB2) and the bHLH family that includes Twist [5].

The bHLH family consists of over 100 evolutionarily conserved dimerized DNA-binding transcription factors. These factors share a common structure with two α-helices joined by a loop of varying length [6]. At the N-terminal end of this protein, there is a series of basic amino acids that form the binding domain and bind DNA at a particular sequence known as an E-box [7]. Together, this family of proteins controls a wide variety of developmental and regulatory functions.

Twist has actually two different proteins—Twist1 and Twist2—which share a high degree of homology [8]. Twist1 is the most studied partner and is the presumed target in the paper by Li [4]. There are up to 500 potential target genes regulated by Twist and its role in EMT has been carefully delineated in metastatic cancer [9] and development [6]. Its role in fibrosis and wound healing has perhaps been more recently identified, specifically in lung [10] and kidney fibrosis [11].

Although this paper by Dr Li [4] sheds light for the first time on the role of Twist in peritoneal membrane EMT and fibrosis, there are still many questions that remain unanswered. Li demonstrates that Twist expression is increased by high glucose concentration in mesothelial cell culture. It is unclear whether it is glucose directly, or a secondary mediator, that induces Twist expression. Twist expression is known to be induced by transforming growth factor (TGF)β [12] and is up-regulated by hypoxia inducible factor 1α in the hypoxic environment [13]. High glucose can induce TGFβ expression in mesothelial cells [14], suggesting a possible mechanism of glucose-induced Twist regulation. We have recently demonstrated that the fibrotic peritoneal membrane is hypoxic, and the hypoxic environment induces fibrogenic and EMT-like changes in the peritoneum [15]. Therefore, hypoxia may also be a stimulus for Twist expression, specifically in the in vivo experiment described by Li [4].

Li demonstrates that Twist appears to be essential for the EMT phenomenon in vitro and is up-regulated in the peritoneal tissues in an in vivo model of peritoneal membrane injury induced by exposure to PD fluid [4]. As Snail1 has a similar action on the suppression of E-cadherin expression and EMT in peritoneal tissue as Twist [1], it is not clear how these factors may interact. In cancer, these two factors may cooperate. Recent work by Dave et al. [12] has shed more light on this interaction. These researchers found that Snail1 effects are reliant on Twist expression and vice versa. Both were implicated in regulation in Zeb1 expression, the third family involved in E-cadherin expression and EMT (Figure 1). In future work, it will be interesting to see whether Twist is a unique mediator of EMT in peritoneal mesothelial cells, or if cooperation with other E-cadherin regulatory transcription factors is required.

Clearly, Twist down-regulates E-cadherin as a first step in the EMT process, but whether Twist has activity

Fig. 1. Transcriptional regulators such as Twist, Zeb1, and Snail1 respond to environmental stimuli and dictate the subsequent EMT effect.
on subsequent EMT-related pathways is not clear. Li demonstrates that overexpression of Twist leads not only to decreased E-cadherin expression, but also increased α-SMA and MMP9 expression. This suggests two possibilities that will require further research to sort out. First, Twist could have direct actions on the expression of EMT-related genes such as α-SMA and MMP9. Conversely, Twist down-regulation of E-cadherin may indirectly lead to these downstream effects. E-cadherin associates with the cytoskeletal network through the structural protein β-catenin. With E-cadherin down-regulation or cleavage, β-catenin accumulates in the cytosol and is phosphorylated by glycogen synthase kinase 3β (GSK3β). This targets β-catenin for ubiquitination and destruction. Growth factors, such as wnt and platelet-derived growth factor (PDGF) [16], phosphorylate and inactivate GSK3β thus protecting β-catenin from degradation. This allows β-catenin to translocate to the nucleus and induces gene transcription through the signal transduction pathway T-cell factor/lymphoid enhancer factor (TCF/LEF) [17]. TCF/LEF activation leads to several downstream effects including cell proliferation, inhibition of apoptosis and increased expression of growth factors [18]. MMP7 and possibly MMP9 have also been demonstrated to be a result of β-catenin signaling [19]. Recent evidence implicates β-catenin as having a role in regulating α-SMA in TGFβ-induced EMT [20].

A key finding in the paper by Cuixiang Li is the increased expression of MMP9 induced by Twist [4]. We have found that the full EMT response involves not only the loss of epithelial characteristics with a concomitant gain of mesenchymal markers and cellular mobility, but subsequent invasion of myofibroblasts into the interstitium where they secrete collagen, vascular growth factors and contribute to the wound healing response. Therefore, the mobilization and invasion of transformed epithelial cells is likely necessary for this full fibrogenic response. We have recently observed that PDGF is able to induce EMT in peritoneal mesothelial cells, but these transformed cells do not undergo invasion into the submesothelial tissue [21]. We ascribe this phenomenon to a lack of MMP2 and MMP9 induction by PDGF. MMP2 and MMP9 are gelatinases and are specific for type IV collagen which is a major component of the basement membrane.

Metalloproteinases are a family of enzymes characterized as zinc-dependent endopeptidases. This large and expanding family of MMPs is classified by their primary protein targets (collagenases, gelatinases and matrilysins) [22]. MMPs are either soluble mediators or membrane bound (i.e. membrane type 1 MMP—MT1-MMP also known as MMP14). MMPs are regulated at multiple levels including gene transcription, translation and protein expression [23]. Characteristically, MMPs are produced as inactive precursors and subsequently cleaved for activation. MMPs are also actively inhibited by a family of endogenous inhibitors—the tissue inhibitors of metalloproteinase [19]. Furthermore, MMPs have both extracellular matrix (ECM) and non-ECM targets. The non-ECM targets include cadherins, integrins, growth factors and receptors [22]. In the process of ECM degradation, the MMPs can also liberate matrix-bound growth factors. Finally, some MMPs are able to activate others; for example, MT1-MMP is known to cleave and activate MMP2 [24].

The data on the role of gelatinases in EMT and cellular invasion are contradictory. There are extensive data to suggest that MMP2 [25] and MMP9 [26] are directly involved in the cellular invasion. Opposing this, recent work by Orlichenko and Radisky [27] suggests that MMP7 (a matrilysin) is capable of inducing EMT and invasion in breast cancer cells, whereas MMP2 was not. Orlichenko and Radisky [27] also provided preliminary data to suggest that MMP9 can also induce EMT and cellular invasion. Further uncertainty in the role of gelatinases in EMT-related cellular invasion is raised by a series of studies where MT1-MMP (membrane bound, otherwise known as MMP14) is found to be essential [28].

MMPs have been studied in relation to PD. MMP2 and MMP9 are up-regulated in our TGFβ1-induced model of peritoneal fibrosis [1]. A non-specific MMP inhibitor was found to have some anti-fibrotic properties in a rodent model of peritoneal injury [29], and tissue plasminogen activator knockout mice have lower MMP2 expression and are resistant to peritoneal membrane injury [30]. In the studies of patients on PD, MMP2 concentration in the peritoneal effluent has been associated with increased peritoneal membrane solute transport and progressive peritoneal fibrosis [31]. MMP9 has been associated more with peritoneal inflammation and peritonitis [32].

The paper by Li adds significantly to our understanding of EMT in peritoneal tissues by inserting Twist as a key modulator of glucose-induced peritoneal mesothelial cell EMT and the regulation of the downstream EMT-related molecules α-SMA and MMP9. So what does this mean for patients reliant on the peritoneal membrane for life support? I think the key question that is so far unanswered is whether EMT is necessary for peritoneal fibrosis and peritoneal membrane injury. Transformed epithelial cells are certainly responsible for collagen production, and we have demonstrated that these cells also make vascular growth factor [33] and are therefore responsible for peritoneal angiogenesis. But would these processes occur without EMT? Inhibitors of regulatory proteins such as Snail1 have been described recently [34], and this may provide the answer by directly inhibiting EMT without affecting the overall TGFβ-induced profibrotic environment in the injured peritoneum. In the future, this type of targeted EMT inhibition, perhaps by locally blocking Twist in the peritoneum, will answer whether EMT is essential for peritoneal fibrosis and Twist may eventually be a novel therapeutic target to prevent peritoneal membrane injury.

Conflict of interest statement. P. Margetts has received research support from Baxter Healthcare, Roche Canada, and Pfizer Canada. (See related article by Li et al. Twist overexpression promoted epithelial-to-mesenchymal transition of human peritoneal mesothelial cells under high glucose. Nephrol Dial Transplant 2012; 27: 4119–4124.)
References


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