Epithelial to Mesenchymal Transition and Peritoneal Membrane Failure in Peritoneal Dialysis Patients: Pathologic Significance and Potential Therapeutic Interventions

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In the past decades, peritoneal dialysis (PD) has become an established alternative to hemodialysis for the treatment of ESRD, and the number of patients who are in PD programs has increased progressively worldwide, especially in some Asian countries. One of the most important challenges in PD is the long-term preservation of the peritoneal membrane integrity.¹,² Damage to the peritoneum is a serious event in PD, because it may jeopardize the organ on which the whole treatment is based.¹–³ The morphology of the peritoneum is simple; a single layer of mesothelial cells (MC) covers a submesothelial region that is composed of connective tissue with few fibroblasts, mast cells, macrophages, and vessels.⁴ The nonphysiologic nature of dialysis fluids and the uremic status are considered the main etiologic factors that lead to the functional decline of the peritoneal membrane.¹ They induce a sustained situation of peritoneal chronic inflammation that can be exacerbated periodically by recurrent or acute episodes of peritonitis or hemoperitoneum. Closely linked to the inflammatory response is the reparative process. Its activation is responsible for many of the structural abnormalities of the peritoneal membrane, including loss of MC monolayer, submesothelial fibrosis, angiogenesis, and hyalinizing vasculopathy.⁵–⁸ Such alterations are considered the major cause of ultrafiltration failure and loss of the dialytic capacity of the peritoneum.

There are two different pathologic forms of PD-related fibrosis. The most common is simple peritoneal sclerosis, which appears in almost all patients. The degree of fibrosis is usually mild and shows a relation with time on dialysis.⁴ On the other end of the spectrum is encapsulating peritoneal sclerosis, which is an uncommon form of sclerosis that...
evolves rapidly with intense fibrosis, inflammation, and fibrin deposits. It is a life-threatening condition that in many cases evolves to visceral encapsulation and progresses even if the patient is removed from PD.\textsuperscript{1–3} It is well established, however, that fibrosis is not the unique structural alteration of the peritoneal membrane induced by PD. Besides this alteration, the peritoneum shows an increase of capillary number (angiogenesis) and vasculopathy.\textsuperscript{5} There is increasing evidence that fibrosis, angiogenesis, and probably augmented vessel permeability are key determinants of ultrafiltration dysfunction.\textsuperscript{1,5,10} The relationship between peritoneal fibrosis and angiogenesis has not been clearly defined. In animal models of PD, it has been shown that fibrosis and angiogenesis may be two separate responses to peritoneal injury.\textsuperscript{8} However, in PD patients, it is likely that fibrosis and angiogenesis are intimately and closely related in the response of the peritoneum to continuous injury.\textsuperscript{8}

Recent works have begun to identify the mechanisms that are involved in the pathogenesis of peritoneal membrane failure during long-term PD. Resident stromal fibroblasts and inflammatory cells have been classically considered the main cells responsible for structural and functional peritoneal alterations, whereas MC have been considered mere victims of peritoneal injury. However, more recently, it has been shown that MC also play an active role in peritoneal membrane alteration. It has been demonstrated that, soon after PD is initiated, peritoneal MC show a progressive loss of epithelial phenotype and acquire myofibroblast-like characteristics by an epithelial-mesenchymal transition (EMT).\textsuperscript{11} MC that have undergone an EMT acquire higher migratory and invasive capacities, which allow these cells to invade the submesothelial stroma, where they contribute to peritoneal fibrosis and angiogenesis and ultimately lead to peritoneal membrane failure.\textsuperscript{11–13} The myofibroblastic conversion of MC has been confirmed in an in vivo animal model based in the injection of an adenovirus vector that transferred active TGF-\(\beta\)1 in rat peritoneum.\textsuperscript{14} In these studies, EMT appears as the central point in the early pathogenesis of peritoneal damage associated with PD. Given that there is no definitive treatment for the progressive loss of the dialytic capacity of the peritoneum, the identification of the EMT of MC as a key process in the onset and progression of peritoneal fibrosis and angiogenesis opens new insights for therapeutic intervention.

**PATHOGENESIS OF PERITONEAL MEMBRANE FAILURE**

PD for ESRD treatment has been used for almost 30 yr.\textsuperscript{2} The two first decades of the modern era of PD were dedicated to catheter design, technical aspects, and peritonitis prevention. The third decade was devoted to improving the biocompatibility of the solutions with the expectancy of diminishing their adverse effects on peritoneal morphology and function.\textsuperscript{3} PD is a successful alternative to hemodialysis, and several studies have confirmed equivalent adequacy, mortality, and fluid balance status with both modalities, at least for the first 4 to 5 yr.\textsuperscript{15} However, the growth of PD continues to be limited by the membrane incapacity to perform adequate diffusive and/or convective transports in the long term.\textsuperscript{1,2} Peritonitis and ultrafiltration failure, with a clinical result of extracellular volume overload and an increased cardiovascular risk, are the major factors that contribute to technique dropouts.\textsuperscript{1,3,19}

Our overview of the morpho-functional relationship in peritoneal membrane failure has changed as a result of some recent experimental data. Some studies of peritoneal biopsies have suggested that hyalinizing vasculopathy and angiogenesis are the most characteristic features in PD-related peritoneal pathology, at least in patients with severe membrane failure.\textsuperscript{5,6} In contrast, a more recent report by Sherif et al.\textsuperscript{10} showed that in stable, uncomplicated PD patients, vascular density does not increase, whereas intact vessels decrease with time of treatment and severe vasculopathy predominate mostly in long-term PD. Other studies have demonstrated that before any other lesions are evident, human peritoneal biopsies show an EMT of the MC and the consequent environmental change, mainly represented by the accumulation of collagen I and fibronectin.\textsuperscript{12} In fact, the submesothelial thickening is the constant change found in peritoneal biopsies after a time on PD.\textsuperscript{3–7,12} In animal models that seem to reproduce the sequence of phenomena that occur in PD patients, Margetts et al.\textsuperscript{16} demonstrated that the transduction of TGF-\(\beta\) into rat peritoneum causes peritoneal sclerosis and angiogenesis, and both processes are followed by the EMT of MC.\textsuperscript{14}

It is worthy of remark that our understanding of peritoneal functional outcome in response to PD requires a deep knowledge of the starting point because of initial functional diversity of the human peritoneum. It can be hypothesized that the diversity in solute and water transports at PD initiation may represent different tissue responses to similar components of dialysates. Furthermore, different starting points followed by diverse reactions to PD at midterm can generate a wide spectrum of peritoneal morpho-functional scenarios.\textsuperscript{17} After molecular and cellular biology studies of the intimate processes that take place into the peritoneum, a refinement in therapeutic strategies is expected. Therefore, it can be proposed that the fourth decade of PD should be conducted to explore molecular aspects of the peritoneal response.

**PERITONEAL INFLAMMATORY RESPONSE TO INJURY AND INFECTION**

Exposure to bioincompatible fluids and episodes of bacterial and fungal infection or hemoperitoneum induce situations of acute and chronic inflammation that cause damage to the peritoneal tissue. The successful repair of injured tissue requires a tightly controlled response to limit the structural alteration. The peritoneal immune response to injury or infection involves, among other cells, MC and resident macrophages that work in a coordinated manner to recruit other infl...
Inflammatory cells, including mononuclear phagocytes, lymphocytes, and neutrophils. MC and infiltrating immune cells can produce a wide number of cytokines, growth factors, and chemokines to establish a complex network that feeds back, resulting in acute or chronic inflammation, which leads to membrane deterioration. Many of these inflammatory mediators, such as TNF-α, IL-1, IL-8, TGF-β, and fibroblast growth factor-2 (FGF-2), may have a role in peritoneal fibrosis by stimulating resident fibroblasts proliferation and extracellular matrix (ECM) component deposition and by inducing EMT of MC, which further increases the number of peritoneal fibroblasts. In addition, IL-8, FGF-2, and especially vascular endothelial growth factor (VEGF) may induce an increase of peritoneal capillary number and probably vessel permeability, causing an increase of small-solute transport, which characterize ultrafiltration failure.

Even in the absence of infection or hemoperitoneum, it is thought that PD patients have a low-grade chronic inflammation that promotes a progressive structural alteration of the peritoneal membrane. This could be due to the mechanical injury during PD fluid exchanges and also to nonphysiologic composition of the fluids. Prolonged exposure of the peritoneum to glucose, the most common osmotic agent in PD fluids, or to Amadori adducts, formed by the condensation between glucose and reactive amino groups of proteins, induces the synthesis of inflammatory, fibrogenic, and angiogenic factors by macrophages and MC. Glucose may also promote peritoneal inflammation through a leptin-dependent mechanism. In this context, adipocytes that are exposed to glucose produce leptin, which in turn promotes TGF-β production by MC. This evidence suggest that peritoneal inflammation is a key triggering process for peritoneal membrane failure; therefore, the knowledge of the mechanisms that are involved in its regulation may serve in the design of therapeutical interventions.

**MAJOR MEDIATORS OF PERITONEAL MEMBRANE DETERIORATION**

Chronic and acute inflammations are believed to be key determinants in the onset and progression of peritoneal membrane structural and functional alteration. Among the cytokines and growth factors produced during peritoneal inflammation, TGF-β, a strong profibrotic cytokine, is considered the master molecule in the genesis of peritoneal fibrosis, because its overexpression has been correlated to worse PD outcomes. The relevance of TGF-β in peritoneal fibrosis has been demonstrated in an in vivo rat model, in which TGF-β gene was transduced into the peritoneal cavity with an adenovirus vector, reproducing the structural and functional alterations that are observed in PD patients. Along with TGF-β, other proinflammatory and profibrotic cytokines as well as angiotensin II (AngII) have been shown to be upregulated during peritonitis episodes and may contribute in the setting of peritoneal fibrosis. In regard to peritoneal angiogenesis, it has been shown that local production of the proangiogenic and vasoactive factor VEGF during PD plays a central role in increased solute transport across the peritoneum and ultrafiltration failure. The most important factor of the PD solutions that are responsible for peritoneal deterioration seems to be glucose degradation products, which, through the formation of advanced glycation end products (AGE), stimulate the production of ECM components as well as the synthesis of cytokines and growth factors, including TGF-β and VEGF. Furthermore, AGE may also induce EMT of MC. Several studies have demonstrated the appearance of AGE in the peritoneal effluents of PD patients, which correlated with the time on PD treatment. Biopsy studies have confirmed the accumulation of AGE in the peritoneal tissues of PD patients. The intensity of AGE accumulation is associated with fibrosis and ultrafiltration dysfunction.

The uremic status also contributes to the accumulation of AGE and may affect the anatomy of the peritoneal membrane and its transport characteristics. In this context, the peritoneum of partially nephrectomized rats showed increased permeability associated with upregulation of nitric oxide synthase isoforms, VEGF, and FGF-2 and accumulation of AGE. Despite these findings in animal models, the effect of uremia itself on the peritoneum in humans is controversial. Two human peritoneal biopsy studies have shown a modest compact zone thickening and vasculopathy in predialysis renal patients. In contrast, in other studies, no significant fibrosis or vasculopathy was observed in uremic non-PD patients.

**SUBPOPULATIONS OF PERITONEAL FIBROBLASTS**

Fibroblasts represent a dynamic population of cells that show functional and phenotypic diversity. Among the various fibroblastic phenotypes, the myofibroblast is of the greatest importance. The term “myofibroblast” defines a cell with intermediate features between a fibroblast and a smooth muscle cell and is characterized by the expression of α-smooth muscle actin (α-SMA). Myofibroblasts have been reported as important protagonists of almost all situations of repair and fibrosis in various pathologies. Their capacity to synthesize ECM, growth factors, cytokines, and participation in the inflammatory response, as well as their contractile properties, convert them in the most important fibroblastic phenotype.

In normal peritoneum or in the peritoneum of uremic non-PD patients, myofibroblasts are not present, but the resident fibroblasts show an intense expression of CD34, an antigen characteristic of bone marrow stem cells (Figure 1). The expression of CD34 gradually disappears in PD patients during the onset of peritoneal fibrosis. The significance of the loss of CD34 expression is unknown but seems to correlate with the appearance of the myofibroblastic phenotype. Tissue CD34 fibroblasts are closely related to circulating CD34 fibroblasts.
brocytes and reflect a bone marrow origin. Fibrocytes were described as a small subpopulation of circulating leukocytes that express collagen I, CD45, CD13, CD11b, CD34, CD86, and MHC class II, which transform into myofibroblasts when exposed to TGF-β in vitro. However, peritoneal CD34+ fibroblasts do not express these fibrocyte markers, suggesting that they are simply residual embryonic mesenchymal cells that remained in the peritoneal tissue after organogenesis (Figure 1). In contrast to normal peritoneum, myofibroblasts can be easily detected in the peritoneal membrane of many patients who undergo PD treatment, even at very early stages of PD and preceding the appearance of fibrosis (Figure 1).

There is evidence that myofibroblasts may originate from different sources. First, they may differentiate from resident fibroblasts. Second, they may derive from circulating cells (fibrocytes) that are recruited to injured tissues. Finally, they can originate from nearby epithelial cells through an EMT. The contribution of EMT as an important source of myofibroblasts was initially described in animal models of renal fibrosis. In the peritoneum, the proportions of myofibroblasts that originated from resident fibroblasts, circulating fibrocytes, and MC remain to be established. Similarly, the dynamics in which EMT takes place has to be defined. Most probably, it is not a uniform process, and several factors may result in variations of its intensity. Among others, the episodes of peritonitis must have an influence on EMT.

**KEY CELLULAR AND MOLECULAR EVENTS DURING EMT**

Turning an epithelial cell into a mesenchymal cell is a complex and step-wise process that requires alterations in cellular architecture and behavior and a profound molecular reprogramming with new biochemical instruction. As illustrated in Figure 2, the EMT starts with the dissociation of intercellular junctions, as a result of downregulation of adhesion molecules such as E-cadherin, claudins, occludins, zona occludens-1, and desmplakin, and with the loss of microvilli and apical-basal polarity. Then, the cells adopt a front-back polarity, as a result of cytoskeleton reorganization, and acquire α-SMA expression and increased migratory capacity. In the latest stages of EMT, the cells acquire the...
capacity to degrade the basement membrane and to invade the fibrotic stroma by upregulating the expression of matrix metalloproteinases (MMP). Other commonly used molecular markers for EMT include the downregulation of cytokeratins; upregulation of vimentin, N-cadherin, and transcription factor snail; and increased production of ECM components.

Besides these classic markers, there are some other markers as well as a number of biochemical changes that define the EMT (summarized in Table 1). EMT can be easily engaged by combinations of a wide spectrum of extracellular stimuli, including cytokines, growth factors, AGE, MMP, and ECM components such as collagen I. It is worthwhile to point out that most of these EMT regulators have been identified by using in vitro cell culture system in which epithelial cells were treated with purified factors, either alone or in combination, at high concentra-

Table 1. Molecular and functional patterns of EMT$^a$

<table>
<thead>
<tr>
<th>Inducers/promoters of EMT</th>
<th>Proteins upregulated</th>
<th>Proteins in the nucleus</th>
</tr>
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<tbody>
<tr>
<td>TGF-β</td>
<td>N-Cadherin</td>
<td>β-catenin</td>
</tr>
<tr>
<td>FGF-2</td>
<td>Snail</td>
<td>LEF1/TCF</td>
</tr>
<tr>
<td>EGF</td>
<td>vimentin</td>
<td>Snail</td>
</tr>
<tr>
<td>AngII</td>
<td>TGF-β</td>
<td>Smad-2/3</td>
</tr>
<tr>
<td>PDGF</td>
<td>fibronectin</td>
<td>NF-κB</td>
</tr>
<tr>
<td>IL-1</td>
<td>collagen I/III</td>
<td>FGF-2/FGF-R1</td>
</tr>
<tr>
<td>AGE</td>
<td>α-SMA</td>
<td>Negative regulators</td>
</tr>
<tr>
<td>MMP-2 and -3</td>
<td>FGF-1 and -2</td>
<td>Smad co-repressors</td>
</tr>
<tr>
<td>collagen I</td>
<td>MMP-2 and -9</td>
<td>Smad-5</td>
</tr>
<tr>
<td>Proteins activated</td>
<td>FSP-1</td>
<td>Smad-7</td>
</tr>
<tr>
<td>ILK</td>
<td>PAI-1</td>
<td>Cellular changes</td>
</tr>
<tr>
<td>Wnt</td>
<td>Proteins downregulated</td>
<td>front-back polarity</td>
</tr>
<tr>
<td>MAPK</td>
<td>E-cadherin</td>
<td>increased migration</td>
</tr>
<tr>
<td>PI3-K</td>
<td>cytokeratins</td>
<td>increased invasion</td>
</tr>
<tr>
<td>Src</td>
<td>claudins</td>
<td>increased scattering</td>
</tr>
<tr>
<td>Ras/Rho GTPases</td>
<td>occludins</td>
<td>degreased fibrinolysis</td>
</tr>
<tr>
<td>ROCK</td>
<td>desmoplakin</td>
<td>growth arrest</td>
</tr>
<tr>
<td>Proteins inhibited</td>
<td>ZO-1</td>
<td>survival</td>
</tr>
<tr>
<td>GSK-3β</td>
<td>mucin-1</td>
<td></td>
</tr>
<tr>
<td>tPA</td>
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</tr>
</tbody>
</table>

$^a$AGE, advanced glycation end products; AngII, angiotensin II; EMT, epithelial-to-mesenchymal transition; FGF, fibroblast growth factor; FGF-R1, fibroblast growth factor receptor-1; FSP-1, fibroblast-specific protein-1; GSK-3β, glycogen synthase kinase-3β; ILK, integrin-linked kinase; LEF1/TCF, lymphoid enhancer factor/T-cell factor; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; PAI-1, plasminogen activator inhibitor-1; PI3-K, phosphatidylinositol-3-kinase; ROCK, Rho-activated kinase; α-SMA, α-smooth muscle actin; tPA, tissue plasminogen activator; ZO-1, zona occludens-1.
tions. These fractionated studies, although necessary to define the potential role of each individual factor in regulating EMT, did not mimic the real situation in vivo, in which the EMT regulators are present at low concentrations. Therefore, an EMT in vivo may result from an integration of diverse signals triggered by multiple factors, being difficult to assign priorities or hierarchy.

Receptor-mediated signaling in response to these factors triggers the activation of a complex network of intracellular effector molecules, such as Ras/Rho GTPases, Rho-activated kinase, tyrosine-kinase Src, integrin-linked kinase (ILK), Wnt-1, Smad 2 and 3, the mitogen-activated protein kinases p38 and extracellular–signal regulated kinases, and phosphatidylinositol-3-kinase (Table 1). These effectors orchestrate the dissociation of intercellular adhesion complexes, the changes in cytoskeletal organization, and the acquisition of migratory and invasive capacities that occur during EMT.

A central target of some of these signaling pathways is glycogen-synthase kinase-3β (GSK-3β), which has been shown to phosphorylate β-catenin and the transcriptional repressor Snail, leading to their ubiquitination and degradation via the proteasome. The phosphorylation of GSK-3β by extracellular–signal regulated kinases, ILK, Wnt-1, or phosphatidylinositol-3-kinase leads to its functional inhibition. As a result, β-catenin is stabilized and localizes to the nucleus, where it feeds into the Wnt signaling pathway by interacting with lymphoid enhancer factor-1/T cell factor. In addition, the inhibition of GSK-3β drives the stabilization and nuclear translocation of Snail, a potent transcriptional repressor of E-cadherin and other intercellular adhesion molecules, and inducer of cell growth arrest and survival. The regulation of signaling pathways also results in the activation and nuclear translocation of other transcription factors, such as Smads 2 and 3, lymphoid enhancer factor-1/T cell factor, and Snail, repress the expression of epithelial markers and engage the EMT transcriptome. It is important to point out that EMT is a reversible process, at least in the early stages. Therefore, molecules that negatively regulate EMT and that promote mesenchymo-epithelial transition must exist (Table 1). In this context, two endogenous factors, namely hepatocyte growth factor (HGF) and bone morphogenetic protein-7 (BMP-7), have been demonstrated to block and reverse EMT by inducing the expression of the transcriptional co-repressors, in the case of HGF, or by activating Smad-5, in the case of BMP-7, which interfere with TGF-β-activated Smad-2/3. Furthermore, Smad-7 is another molecule that negatively regulates EMT by blocking Smad-2 phosphorylation and activation.

**Table 2. Molecular markers of mesothelial cells and fibroblasts**

<table>
<thead>
<tr>
<th>Molecular Marker</th>
<th>Omentum</th>
<th>Peritoneal Dialysis Effluent</th>
<th>Fibroblast</th>
</tr>
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<tbody>
<tr>
<td>E-cadherin</td>
<td>+ + +</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>N-cadherin</td>
<td>–</td>
<td>+</td>
<td>+ +</td>
</tr>
<tr>
<td>Snail (mRNA)</td>
<td>–</td>
<td>+ / –</td>
<td>+ + +</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>+ + +</td>
<td>+ +</td>
<td>+ +</td>
</tr>
<tr>
<td>Cytokeratins</td>
<td>+ + +</td>
<td>+ +</td>
<td>+ / –</td>
</tr>
<tr>
<td>Vimentin</td>
<td>+</td>
<td>+</td>
<td>+ + +</td>
</tr>
<tr>
<td>Calretinin</td>
<td>+ + +</td>
<td>+ / –</td>
<td>–</td>
</tr>
<tr>
<td>CA125</td>
<td>+ + +</td>
<td>+</td>
<td>+ + +</td>
</tr>
<tr>
<td>VEGF</td>
<td>+</td>
<td>+</td>
<td>+ + +</td>
</tr>
<tr>
<td>COX-2</td>
<td>+</td>
<td>+</td>
<td>+ + +</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>–</td>
<td>+</td>
<td>+ + +</td>
</tr>
<tr>
<td>Collagen I</td>
<td>–</td>
<td>+</td>
<td>+ + +</td>
</tr>
<tr>
<td>α-SMA</td>
<td>–</td>
<td>+ / –</td>
<td>+ + +</td>
</tr>
</tbody>
</table>

aICAM-1, intercellular adhesion molecule-1; CA125, cancer antigen 125; VEGF, vascular endothelial growth factor; COX-2, cyclooxygenase-2.
bIn activated fibroblasts.

**EVIDENCE FOR EMT OF MC IN PD PATIENTS**

The presence of EMT in the peritoneum of PD patients was first demonstrated in a landmark paper that published in 2003. It was described that confluent MC cultures from PD effluents may show epithelioid and nonepithelioid (fibroblast-like) morphologies. The frequency of nonepithelioid MC was associated with time on PD and with episodes of peritonitis or hemoperitoneum. MC from effluents showed high expression of intercellular adhesion molecule-1 independent of their morphology. In contrast, this mesothelial marker was negligible on cultured peritoneal fibroblasts, supporting that effluent nonepithelioid cells had a mesothelial origin and were not fibroblast contaminations. The analysis of the epithelial markers cytokeratins and E-cadherin was also important to determine more precisely the nature of effluent-derived cells. High expression of cytokeratins and E-cadherin was observed only in omentum-derived MC, whereas effluent-derived cells showed a progressive reduction in the expression of these molecules, although even fibroblast-like MC maintained a small population of positive cells. Fibroblasts were completely negative for these two markers. The phenotype changes in effluent MC were indicative of an EMT. Additional evidence that the PD-induced changes of the MC were due to an EMT came from the analysis of the expression of the transcription factor Snail, a potent inducer of EMT. Omentum-derived MC did not express Snail mRNA, whereas effluent-derived cells showed a progressive expression of this mRNA was observed in effluent MC preparations along the transdifferentiation process. Besides these molecules, there are some other markers that may help to distinguish effluent MC from contaminating fibroblasts as well as to establish the various stages of transdifferentiation of MC (summarized in Table 2).
EMT of MC in vivo results from integrated signals that are induced by multiple stimuli. These include elevated glucose and glucose degradation products (GDP) and concentration of PD fluids, which through the formation of advanced glycation-end products (AGE) stimulate the transdifferentiation of MC. The formation of AGE may also be due to the uremic status of the PD patients. The low pH of the dialysates and the mechanical injury during PD fluid exchanges can cause tissue irritation and contribute to chronic inflammation of the peritoneum, which promote EMT of MC. Episodes of bacterial or fungal infections or hemoperitoneum cause acute inflammation and upregulation of cytokines and growth factors such as TGF-β, IL-1, FGF2, TNF-α, and angiotensin II (AngII), among others, which are strong inducers of EMT. The therapeutic strategies may be designed either to prevent or to reverse the EMT itself or to treat its effects such as cellular invasion, fibrosis, or angiogenesis. The diagram illustrates six steps related to the EMT process of the MC that can be clinically managed, alone or in combination, to prevent peritoneal membrane failure. See text for details.

The mesenchymal conversion of MC could also be observed in vivo in the peritoneum as a response to PD. Immuno-histochemical analysis of peritoneal biopsies from PD patients revealed the presence of fibroblast-like cells embedded in the compact zone expressing mesothelial markers. In addition, these peritoneal biopsies showed expression of α-SMA in the fibrotic stroma, especially in the upper submesothelial level, and in many cases, these myofibroblasts showed coexpression of cytokeratins (Figure 1).12 These results indicated that new myofibroblastic cells may arise from local conversion of MC by EMT during the repair responses that take place in PD. Recently, the myofibroblastic conversion of MC was confirmed in vivo by injection of an adenovirus vector that transferred active TGF-β1 in rat peritoneum.14

**PATHOLOGIC SIGNIFICANCE OF EMT IN PERITONEAL FIBROSIS AND ANGIogenesis**

It has been shown that during the progression of EMT, MC acquire higher ex vivo and in vitro ability to synthesize components of the matrix, such as fibronectin and collagen I.13 In addition, MC that undergo an EMT express high levels of cyclooxygenase-2 (unpublished data, Aroeira et al.), which has been implicated in tissue remodeling and fibrosis processes.40 Furthermore, it can be observed that the grade of peritoneal fibrosis and small-solute transport rate of PD patients correlate with the expression of mesothelial markers within the fibrotic stroma (unpublished data, del Peso et al.). These results support the notion that transdifferentiated MC play an essential role in the initiation of fibrosis and subsequent peritoneal functional decline.

Besides fibrosis, an increased number of capillaries is related to peritoneal membrane failure.1,8 It was proposed that local production of VEGF during PD plays a central role in the processes that lead to peritoneal angiogenesis and functional decline.30,31 However, the main source of VEGF in the PD patients as well as the mechanisms that are implicated in VEGF upregulation during PD remained elusive. A number of studies have shown that MC from omentum have the capacity to produce VEGF in vivo in response to a variety of stimuli.13,41 A recent study demonstrated that transdifferentiated MC are an important source of VEGF in PD patients and that the underlying mechanism of VEGF upregulation in MC is the mesenchymal conversion of these cells.13 It was shown that MC from effluents with fibroblast-like phenotype produce much more VEGF ex vivo than MC with epithelial phenotype and that patients who drain fibroblast-like cells have higher blood VEGF levels than patients with MC with epithelial phenotype in their effluents. Moreover, a correlation between ex vivo and in vivo VEGF levels and the rate of peritoneal transport in patients who were on PD could be demonstrated.13 These results suggest a direct and active role of MC not only in fibrosis but also in peritoneal angiogenesis. In addition, these studies indicated that the EMT of MC reflects peritoneal functional decline better than previous tests, such as measurement of effluent cancer antigen 125 (CA125).13 Although CA125 has been classically used as an index of MC mass and peritoneal health in PD patients, the value of effluent CA125 has been questioned recently because it correlates with peritoneal transport rate only in short-term but not in long-term PD.42

**THERAPEUTIC INTERVENTION ON EMT**

The insights of the molecular mechanisms that regulate chronic inflammation that is induced by peritoneal injury
will allow the identification of potential therapeutic targets. However, from the clinical nephrologist’s point of view, perhaps the most important aspect is the identification of the EMT of MC as a key event in peritoneal membrane failure, because this process can be manipulated with a wide range of agents and pharmaceutical products. The therapeutic strategies may be designed either to prevent or to reverse the EMT itself or to treat its effects such as cellular invasion, ECM accumulation, or angiogenesis factor synthesis.

It can be proposed that at least six steps that are related to the EMT process of the MC can be clinically managed, alone or in combination, to prevent peritoneal membrane failure (Figure 3). Because EMT is reversible, treatments may be tailored either to prevent (step 1) or to reverse (step 2) this process. These therapeutic approaches have been proved to be effective in animal models of renal fibrosis. The endogenous factors HGF and BMP-7 have been demonstrated to block EMT both in vitro and in vivo and to prevent renal interstitial fibrosis. In addition, these two proteins reversed the phenotypic conversion of tubular epithelial cells that was induced by TGF-β by restoring E-cadherin expression and downregulating the expression of mesenchymal markers such as α-SMA, vimentin, and fibronectin. The reversion from mesenchymal to epithelial phenotype by BMP-7 has also been demonstrated for transdifferentiated MC. Mechanistically, HGF interferes with TGF-β–mediated EMT by inducing the expression of the transcriptional co-repressors such as SnoN and TG interacting factor that interact with activated Smad-2/4 complex and block the expression of Smad-dependent genes, including ILK. The underlying mechanism of BMP-7 blockade of EMT is by activation of Smad-5 protein that counteracts with TGF-β–activated Smad-2/3.

Besides targeting EMT by supplementation of endogenous factors, this process can be disrupted by using small molecule inhibitors. For instance, the inhibition of Rho-activated kinase, a downstream effector kinase of RhoA, resulted in suppression of α-SMA expression and renal interstitial fibrosis in a mouse model of ureteral obstruction. Pharmacologic inhibition of AngII, a potent EMT promoter, also attenuated EMT and reduced renal fibrosis. More recently, it was shown that paricalcitol, a vitamin D analogue, blocked the mesenchymal conversion of tubular epithelial cells and ameliorated renal fibrosis in animal models of obstructive nephropathy.

Whereas these endogenous factors and pharmacologic compounds have been widely demonstrated to block transdifferentiation of tubular epithelial cells and to ameliorate renal fibrosis, their effects on EMT of MC and in the prevention of peritoneal membrane failure are just starting to be tested in vitro and in animal models of PD. In this context, it has been demonstrated that AngII type 1 receptor attenuate the production of VEGF in these cells. In addition, it was previously shown that intraperitoneal administration of an inhibitor of angiotensin-converting enzyme and AngII type 1 receptor attenuate the production of VEGF in these cells. In addition, it was previously shown that intraperitoneal administration of an inhibitor of angiotensin-converting enzyme attenuated structural and functional alteration of the peritoneum in a rat PD model.

It should be taken into consideration that EMT of MC is a physiologic process that is necessary for wound healing during the progression of the peritoneal membrane induced by PD; therefore, it is plausible that chronic blockade of EMT would result in inefficient tissue repair. Therefore, alternative therapeutic approaches may be addressed to treat the consequences of the EMT of MC such as cellular invasion (step 3), fibrosis (steps 4 and 5), or angiogenesis (step 6; Figure 3).

Induction of EMT of MC is accompanied by upregulated expression of MMP such as MMP-2 and MMP-9, which would degrade the basal membrane and the connective tissue, allowing the submesothelial invasion by the transdifferentiated cells. It can be expected that MMP inhibitors may prevent the accumulation MC-derived myofibroblasts in the submesothelial compartment, which in turn would diminish the structural alteration of the peritoneal membrane.

MC that have undergone an EMT produce higher amounts of ECM components, including fibronectin and collagen I, and display less fibrinolytic capacity as a result of unbalanced ratio between tissue plasminogen activator and plasminogen activator inhibitor-1. Therapeutic interventions of peritoneal fibrosis may be designed either to prevent ECM synthesis (step 4) or to increase fibrinolysis (step 5). A number of antifibrotic drugs, including pentoxifylline, dipyridamole, troglitazone, diltiazem, and emodin, have direct inhibitory effects on ECM protein synthesis or on TGF-β expression and activity in MC.

Finally, it has been demonstrated that during the progression of EMT, MC produce higher amounts of VEGF, which correlated with increased peritoneal transport. Therefore, therapeutic intervention may also be directed to prevent peritoneal angiogenesis and vessel permeability, via interrupting VEGF expression or its effects on endothelial cells (step 6). For instance, the inhibitors of angiogenesis TNP-470 and endostatin have been shown to suppress the progression of peritoneal deterioration in mouse experimental models.

**CONCLUSION**

Recent studies using ex vivo cultures of effluent-derived MC, in conjunction with immunohistochemical analysis of peritoneal biopsies, have allowed the identification of the EMT of MC as a key process in peritoneal membrane failure. Although it can be argued that effluent MC are not representative of the mesothelium because they might be cells that are committed to detach from the
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DISCLOSURES

None.

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