We are IntechOpen,
the world's leading publisher of
Open Access books
Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

BOOK CITATION INDEX INDEXED
CLARIVATE ANALYTICS

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

The Mesothelial to Mesenchymal Transition a Pathogenic and Therapeutic Key for Peritoneal Membrane Failure

Abelardo Aguilera, Jesús Loureiro,
Guadalupe Gónzalez-Mateo, Rafael Selgas and
Manuel López-Cabrera

Additional information is available at the end of the chapter

1. Introduction

Peritoneal dialysis (PD) is a form of renal replacement therapy that is growing progressively, possibly because of the freedom it offers to the patients and the undoubted improvement in the PD technique. Parallel the PD-related complications have also increased. In PD, the peritoneal membrane (PM) is exposed to bio-incompatible dialysis solutions, rich in glucose, which can cause peritoneal injury when associated with peritoneal incidents like repeated episodes of peritonitis or hemoperitoneum [1]. Progressive fibrosis, angiogenesis and ultimately, ultrafiltration failure, are some characteristics of the so-called sclerotic peritonitis syndromes (SPS) [2].

Several pathologic factors, such as inflammatory mediators, high glucose content, the presence of glucose degradation products, and low pH can induce peritoneal mesothelial cells (MCs) to lose certain epithelial characteristics, and they progressively acquire a fibroblast-like phenotype soon after initiation of PD [3]. This so-called mesothelial-to-mesenchymal transition (MMT) serves as a trigger for peritoneal fibrosis and angiogenesis, via up-regulation of transforming growth factor-β (TGF-β1 and vascular endothelial growth factor (VEGF), respectively. As such, MMT is considered an important potential therapeutic target in sclerotic peritonitis syndromes [4]. Encapsulating peritoneal sclerosis (EPS) is a severe form of peritoneal fibrosis characterized by intestinal encapsulation through the formation of excessive matrix components that subsequently may lead to obstruction of the intestinal tract [5]. Although rare, EPS is a serious complication of PD for which no specific and definitive

treatment exists [6]. However, peritoneal resting, steroids, immunosuppressive agents and Tamoxifen have been used previously as therapeutic approaches with divergent results [4]. Herein, we review in detail the effect of PD liquids and other peritoneal accidents like peritonitis and hemoperitoneum on the MCs physiology, the trandifferentiation in fibroblast-like cells (MMT), its clinical correlation with data from peritoneal and morphologic function (peritoneal biopsies), the initiation and perpetuation of peritoneal fibrosis (SPS) and his eventual rise to EPS. We also purpose therapeutic alternatives, ranging from the improvement in the biocompatibility of the liquids of DP, the use of drugs available on the market today, or even the use of molecular strategies, as blockades or stimulation of genes involved in the peritoneal damage.

2. Morphologic and phenotypic MCs characterization

The mesothelium is a continuous superficial layer of MCs formed by flattened, polygonal, mononuclear, squamous epithelial cells. This monolayer shows remarkable fibrinolytic properties and is thought to be involved in the prevention of fibrous adhesion formation in the peritoneum. MCs cells have vast biosynthetic capacity and secrete phospholipids and phosphotidylcholine in the form of lamellar bodies that provide a lubricating surface for the movement of abdominal viscera [7]. Besides this function, the mesothelium also modulates peritoneal microcirculation by secreting vasodilators (eg, prostaglandin E2 and nitric oxide), as well as vasoconstrictors (eg, endothelin) [8]. The luminal aspect of MCs plasmalemma has numerous cytoplasmic extensions called "microvilli" which play a significant role in the transperitoneal transfer of anionic macromolecules such as proteins. Microvilli are extremely sensitive and easily lost due to injury [9].

MCs can be isolated from healthy omentum donors of elective abdominal surgeries and the effluent from patients with PD. The analysis of the effluent drained peritoneal MCs has allowed us to assess the health status of peritoneal. The purity of effluent and omentum-derived MC cultures are determined by the expression of standard mesothelial markers: ICAM-1, cytokeratins, and calretinine. MCs cultures remain stable, without any evident sign of senescence, for at least two to three passages [10, 11].

The analysis of cytokeratins and E-cadherin expression, that are typical epithelial markers and highly expressed in MC, is important to determine more precisely the nature of effluent-derived cells. High expression of cytokeratins and E-cadherin is only observed in omentum-derived MC, whereas effluent-derived cells show a progressive reduction in the expression of these molecules, although even fibroblast-like MC may maintain a small population of positive cells (Figure 1). In mixed populations the expression of cytokeratins and E-Cadherin is normally bimodal. Fibroblasts are completely negative for these two markers. Previous studies had characterized the cobblestone-like MC from effluents as indistinguishable from omentum-derived MC. However, already in this early stage a loss of apico-basolateral polarity as well as down-regulation of cytokeratins and E-Cadherin is observed *ex vivo*, even though cells still show a morphologically epithelioid appearance [10-12].
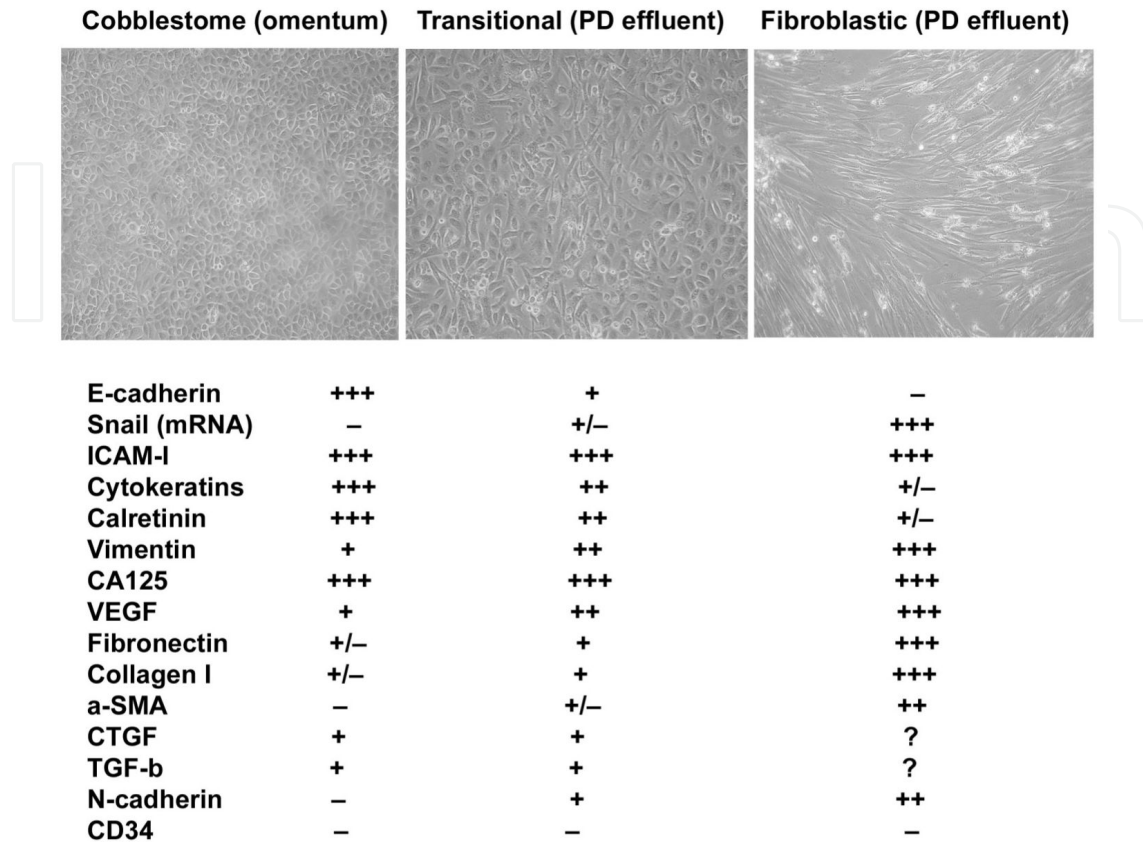
MCs Phenotype

	Cobblestome (omentum)	Transitional (PD effluent)	Fibroblastic (PD effluent)
E-cadherin	+++	+	–
Snail (mRNA)	–	+/–	+++
ICAM-I	+++	+++	+++
Cytokeratins	+++	++	+/–
Calretinin	+++	++	+/–
Vimentin	+	++	+++
CA125	+++	+++	+++
VEGF	+	++	+++
Fibronectin	+/–	+	+++
Collagen I	+/–	+	+++
a-SMA	–	+/–	++
CTGF	+	+	?
TGF-b	+	+	?
N-cadherin	–	+	++
CD34	–	–	–

Figure 1. Morphology and gene expression of MCs. Panel "A" shows a culture of MCs isolated from omentum donor. Cells show the typical cobblestone phenotype. Panel "B" shows MCs isolated from PD effluent with transitional phenotype, and panel "C" shows a fibroblastoid phenotype these cells were isolated and cultured from the PD effluent. The genetic pattern of each phenotype is described below.

The morphological changes and down-regulation of cytokeratin and E-Cadherin in effluent-derived MC are indicative of an MMT. MMT is a complex and generally reversible process that starts with the disruption of intercellular junctions and loss of apical–basolateral polarity, typical of epithelial cells, which are then transformed into fibroblast-like cells with increased migratory, invasive and fibrogenic features. The objective of this process is to repair tissue wounds by promoting the recovery of ancestor capabilities of epithelial cells. Cell migration, production of extracellular matrix and induction of neoangiogenesis are the main activities [11, 12]. This process is conducted by the transforming growth factor-β (TGF-β) and the representative cell form is the myofibroblast (Figure 2). TGF-β synthesis may be stimulated by glucose, and infections, via peritoneal leucocyte-derived factors. TGF-β has been found to be up-regulated in peritoneal inflammatory processes and its over-expression has been correlated to worse PD outcomes [13]. Moreover, the injection of an adenovirus vector that transferred active TGF-β1 in rat and mice peritoneum induces myofibroblastic conversion of MC. [14, 15]. TGF-β is a growth factor that has been implicated as the causal agent in fibrosis of different tissues and organs [16].
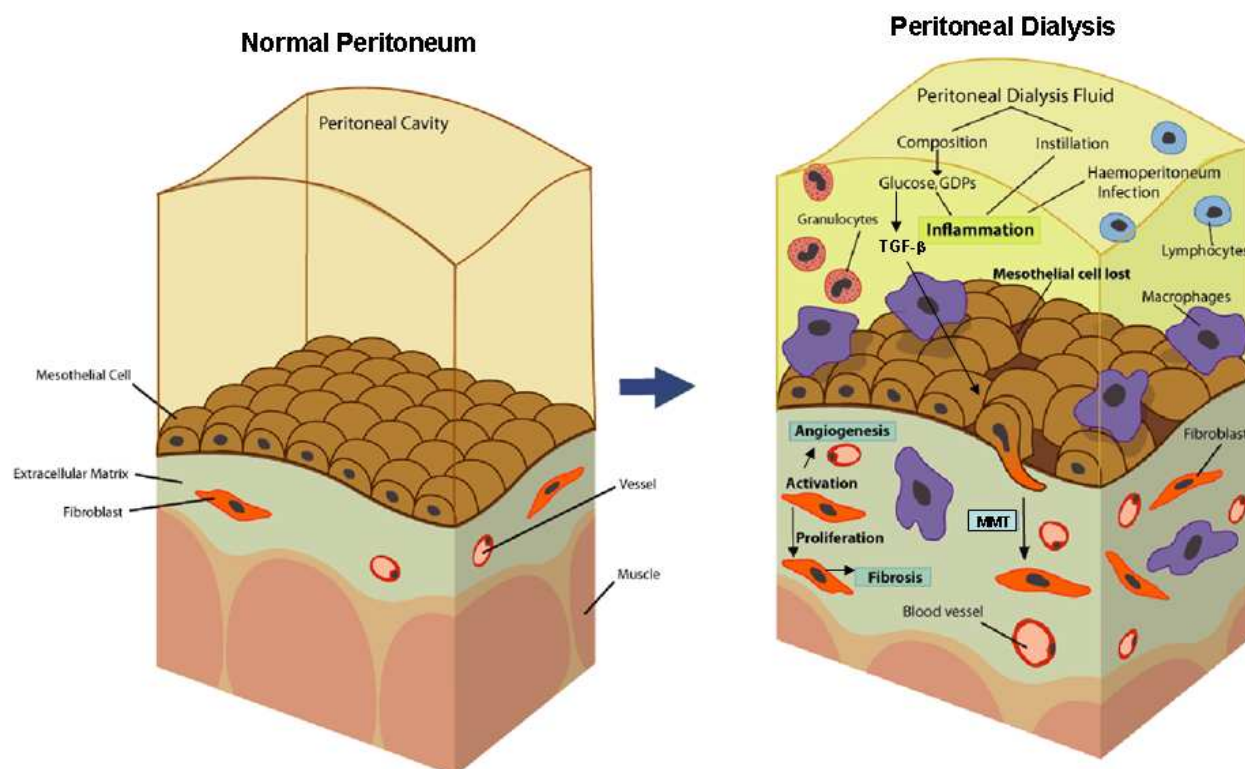
Figure 2. From a normal peritoneum to a PD peritoneum suffering MMT. Panel "A" shows a normal peritoneun without fibrosis, angiogenesis or MMT (3D image). Panel "B" shows a PD paritoneum with MCs exposed to PD fluids. MCs lose their microvilli, suffer MMT and invade submesothelial area, where synthesize VEGF, angiogenesis, proliferation, migration and EMC production. Both glucose (GDPs and AGEs) from PD fluids and inflammatory molecules stimulate TGF-β production which trigger MMT.

3. MMT signalling

Figure 3 shows the signaling cascade of MMT which begins with the activation of TGF-β which is considered the master molecule in peritoneal injury during PD. Activation of TGF-β receptors triggers smads-dependent and smads-independent signaling. Smads depending pathway include integrin-linked kinase, GSK-3, β-catenin, Lef-1/Tcf and AP gene cascade. Smads independing include RhoAp160ROCK and H-Ras/Raf/ ERK pathways [17-32].

4. Clinical implication of MMT in PM failure

We have described three major morphologies of MCs cultures from PD effluents: cobblestone-like, similar to omentum-derived MC, transitional and fibroblast-like MC which remained stable for at least two to three cell passages. After analyzing more than two hundred MC cultures with growth capacity, from more than 100 PD patients, we determined that the frequencies of the different effluent-derived MC cultures are approximately 53 percent for
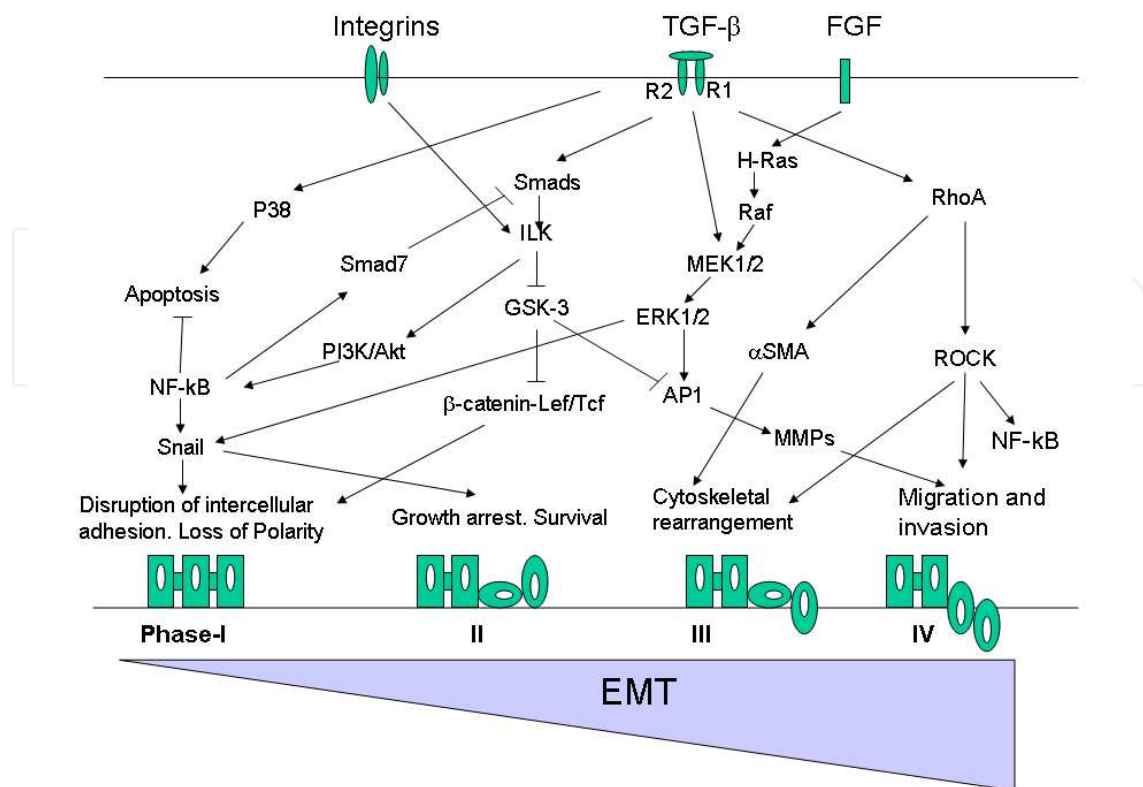
Figure 3. TGF-β signaling. Glucose, low pH from PD fluids, advanced glycation end products (AGEs), glucose degrada‐
tion products (GDPs), peritonitis and haemoperitoneum stimulate TGF-β synthesis, and possibly FGF which in turn trig‐
gers the healing processes that ultimately lead to tissue fibrosis and angiogenesis. The increase in total VEGF
production might increase the VEGF-C levels, which are directly implicated in lymphogenesis. TGF-β receptor I phos‐
phorylates Smad 2 and 3 inducing their association with the common partner Smad 4, and then they translocate into
the nucleus, where they control the expression of TGF-β-responsive genes, such as that encoding integrin-linked kin‐
ase (ILK). The activation of up-regulated ILK by β1 integrins results in strong phosphorylation of Akt and glycogen syn‐
thase kinase-3 (GSK-3). Phosphorylated-Akt triggers NF-kB activation, which in turn induces the expression of Smad 7,
an inhibitory Smad molecule that interferes with the phosporylation of Smad 2 and 3, and of snail, a key regulator of
MMT. The transcription factor snail regulates MMT by inhibiting the expression of E-cadherin, and by inducing growth
arrest and survival, which confer selective advantage to migrating trans-differentiated cells. The phosphorylation of
GSK-3 by ILK results in its inhibition and subsequent stabilization of β-catenin, released from the adherens junction,
and of AP-1. Stabilized b-catenin, in conjunction with Lef-1/Tcf, may per se induce MMT, and AP-1 activates MMP-9
expression inducing the invasion of ECM. One of the main Smad-independent signalling cascades triggered by TGF-β
receptor I ligation, includes the RhoAp160ROCK pathway that regulates cytoskeleton remodelling and cellular migra‐
tion/ invasion. In addition, RhoA induces the expression of α-SMA in a ROCK-independent manner. Another signal
transduction stimulated by TGF-β is the H-Ras/Raf/ ERK pathway, which is also necessary for the induction of snail ex‐
pression and MMT.

cobblestone-like, 24 percent for transitional, and 17 percent for fibroblast-like MC. The
prevalence of non-epitheliod MC cultures (transitional or fibroblast-like) is associated with the
time the patients have been subjected to PD and with the episodes of acute or recurrent
peritonitis or hemoperitoneum [3, 10, 11]. We have also described a less frequent (less than 6
percent) cell culture with mixed morphologies [3, 10]. Effluent mesothelial cells were isolated
from 37 PD patients and analyzed for mesenchymal conversion. Mass transfer coefficient for
creatinine (Cr-MTC) was used to evaluate peritoneal function. VEGF concentration was

measured by using standard procedures. Patients whose drainage contained nonepithelioid mesothelial cells had greater serum VEGF levels than those with epithelial-like mesothelial cells in their effluent. VEGF production ex vivo by effluent mesothelial cells correlated with serum VEGF level. In addition, Cr-MTC correlated with VEGF levels in culture and serum. Cr-MTC also was associated with mesothelial cell phenotype. VEGF expression in stromal cells, retaining mesothelial markers, was observed in peritoneal biopsy specimens from high-transporter patients. These results suggest that mesothelial cells that have undergone epithelial-to-mesenchymal transition are the main source of VEGF in PD patients and therefore may be responsible for a high peritoneal transport rate [3].

In a clinical study performed by our group, we studied the peritoneal anatomical changes during the first months on PD, and to correlate them with peritoneal functional parameters. We studied 35 stable PD patients for up to 2 years on PD, with a mean age of 45.37 years. Seventy-four percent of patients presented loss of the mesothelial layer, 46% fibrosis and 17% in situ evidence of MMT (submesothelial cytokeratin staining), which increased over time. All patients with MMT showed myofibroblasts, while only 36% of patients without MMT had myofibroblasts. The myofibroblasts represent a dynamic population of cells showing functional and phenotypic diversity. During the last years, numerous different molecules have been reported to be expressed by tissue fibroblasts including peritoneal ones [10]. The origin of tissue fibroblasts has been largely overlooked, so that their lineage is not fully elucidated. There is now evidence supporting that fibro-myofibroblasts might originate from different sources. Firstly, they may differentiate from resident tissue stem cells or fibroblasts. Secondly, they can originate from nearby epithelial cells through a process known as MMT. Finally, the bone marrow and circulating cells may be responsible for the production of fibro-myofibroblasts circulating in the blood stream to their final tissue destination [33].

In PD, emergent evidence points that fibroblasts may arise from local conversion of epithelial cells by MMT or from CD34 + cells (fibrocytes) of the bloodstream after being recruited from bone marrow. In the case of renal fibrosis models, it has been shown that 36% of new fibroblasts derive from MMT, 15% from bone marrow and the rest comes from local proliferation of resident fibroblasts. In PD-related fibrosis, we have demonstrated the expression of mesothelial markers in stromal spindle-like cells, suggesting that they stemmed from local conversion of MC. In contrast, we did not observe a significant contribution of CD34+ cells from bone marrow to the submesothelial fibroblast population in the fibrotic peritoneal tissue [33].

In regard to angiogenesis, the number of peritoneal vessels did not vary when we compared different times on PD. Vasculopathy was present in 17% of the samples. Functional studies were used to define the peritoneal transport status. Patients in the highest quartile of mass transfer area coefficient of creatinine (Cr-MTAC) showed significantly higher MMT prevalence but similar number of peritoneal vessels. In the multivariate analysis, the highest quartile of Cr-MTAC remained as an independent factor predicting the presence of MMT after adjusting for fibrosis [34]. These findings indicate that MMT is a frequent morphological change in the peritoneal membrane. These myofibroblastic cells with submesothelial localization may arise from local conversion of MC during the repair responses and the high solute transport status is associated with MMT.

5. Are MMT, SPS and EPS part of the same process?

From MMT to SPS. Peritoneal fibrosis (or sclerosis) is a term that comprises a wide spectrum of peritoneal structural alterations, ranging from mild inflammation to severe sclerosing peritonitis and its most complicated manifestation, encapsulating peritonitis sclerosis (EPS) [6, 35, 36]. Simple sclerosis (SS), an intermediate stage of peritoneal fibrosis, is the most common peritoneal lesion found in the patients after few months on PD, and could represent the initial phase of sclerosing peritonitis syndrome (SPS). Rubin et al [5] described a normal thickness of the peritoneum of 20 μm, but after a few months on PD could reach up to 40 μm (SS). The SP is a progressive sclerosis that is characterized by a dramatic thickening of the peritoneum (up to 4000 μm) and is accompanied by inflammatory infiltrates, calcification, neo-vascularization and dilatation of blood and lymphatic vessels, being the thickening the most commonly used pathological criterion for differential diagnosis [6, 35, 36].

The importance of establishing a connection between MMT, SPS and EPS is the potential therapeutic and preventive effect of blocking this axis. Also emerging evidence suggests that partial or total blockage of the MMT prevents early stages of PM fibrosis and angiogenesis and preserves the PM function. Moreover, current studies show TGF-β is probably the most important molecule in the PM failure development, so act on a single molecule, the TGF-β, facilitates therapeutic approach. In fact we have shown that blockade of TGF-β significantly attenuated PM failure induced by PD fluids.

One of the biggest problems to establish the definitive connection between SPS and EPS is that the EPS animal model has not been fully and properly developed. While in our mice PD model in 4 or more weeks reaches the typical changes induced by PD fluids on humans, the peritoneal fibrosis model with chlorhexidine results artificial and extremely aggressive. The experimental development of an appropriate EPS model is mandatory. Possibly the most appropriate EPS mice model would be to maintain long-term (months) in PD according to our model of SPS. Once accepted this limitation, the current data suggest that MMT and SPS are part of the process. We have analyzed serially PM pieces of mice in PD at baseline, 15 and 30 days and we found a linear correlation between time on PD, the thickness of the PM and the number of MCs cytokeratin (+) and FSP-1 (+) in the area submesothelial. This phenomenon was accompanied by progressive loss of the mesothelial monolayer which indicates an important participation of the MMT in the development of peritoneal fibrosis (our unpublished results). Using a TGF-β adenovirus model, we found early MMT at 4 day after stimuli intraperitoneal injection that was correlated with PM fibrosis [14]. Similar finding was found by others [15]. Clinically, in MCs serially isolated and cultured from PD effluents, the MMT was present progressively over time in PD and is associated with solute transport disorders and ultrafiltration failure [37]. In PM biopsies from 35 PD stable patients performed during the first 2 years on PD, we demonstrated that the first morphological change in peritoneum that appears as a consequence of PD is submesothelial thickening partially caused by the MMT. This phenotype change is associated with an increase in peritoneal solute transport independent of the number of capillaries present in the tissue [1, 3]. Reaching this point, the following questions arise, as follows: could have peritoneal fibrosis without MMT?, or more specifically,

could have MMT without the participation of TGF-β?. Experimental data by us [14] and others [15] indicate that blocking MMT in different degrees result in a significantly attenuation of structural and functional changes of PM. Using the adenovirus (TGF-β) and our PD mice model, the double submesothelial staining for cytokeratin (+) and FSP1 (+) was positive in approximately 37% of activated fibroblasts, indicating its epithelial origin [14]. However, the peritoneal fibrosis is inhibited in more than 50% indicating that direct inhibition of TGF-β with anti-TGF-β peptides inhibited other effects of this molecule as the activation of regional fibroblasts. Promising results have also obtained acting on immune system [38], on AGEs accumulation [39] or on renin-angiotensin system (ACE, AR-II, Paricalcitol) and BMP-7 which also modulate directly or indirectly the TGF-β [40]. These arguments lead us to conclude that TGF-β is a key in the initiation and possibly perpetuation of an uncontrolled MMT, which leads to fibrosis and SPS.

From SPS to EPS. The next question is as follows: at which point the SPS becomes an irreversible process to become EPS? The "two-hit" hypothesis explains the EPS as the result of the PD injury. Two factors are required for the onset of EPS: a predisposing factor, such as peritoneal deterioration from persistent injury caused by peritoneal dialysis (the first "hit"), and an initiating factor, such as inflammatory stimuli superimposed on the chronically injured peritoneum (the second "hit"). Peritoneal deterioration (consisting of mesothelial denudation, interstitial fibrosis, vasculopathy, and angiogenesis) leads to an increased tendency toward plasma exudations that contain fibrin and coagulation factors. The fibrins in the exudates contribute to the intestinal adhesions and formation of fibrin capsule. Inflammatory stimuli caused by infectious peritonitis are superimposed on the damaged peritoneum and act as an initiating factor to trigger the onset of EPS. Inflammatory cytokines also induce activation and proliferation of the peritoneal fibroblasts, promoting peritoneal fibrosis and intestinal adhesions. The relationship between the extent of the first and second "hits" can be demonstrated. The extent of peritoneal damage (the first "hit") increases with the duration of peritoneal dialysis [41, 42].

The onset of EPS depends on the total intensity of both lesions: peritoneal damage and inflammatory stimuli. For the onset of EPS, the total intensity must be greater than a given threshold. The extent of the inflammatory stimuli (the second "hit") are required for the onset of EPS [41, 42].

In both cases (acute and chronic peritoneal injury), the TGF-β is activated with subsequent initiation and perpetuation of MMT and its deleterious effects (fibrosis, angiogenesis, etc.). However, it is very difficult to establish the point of no return in peritoneal lesions clinically because patients with type-I PM failure usually recover functionality and possibly tissue damage with rest peritoneal [43]. In experimental animals, data about fibrosis reversibility are not available. Unfortunately, the initial degree of PM fibrosis has been determined in very few cases (peritoneal biopsies not available). Finally a genetic component cannot be ruled [44, 45].

From MMT to EPS. In both, experimental animals [14, 46] and human peritoneal biopsies from patients within 2 years in PD [34], it seems clear that MMT is an early phenomenon able to determine the degree of peritoneal fibrosis and the future of the PM. But no information about MMT in patients with long term in PD or diagnosis of EPS is available. It is possible that MMT

may be an initial phenomenon and few signs of it are in severe stages of fibrosis. However, in bridles and postsurgical adhesions, we have found MMT signs (unpublished data by us), and Bowel adherences may represent an intermediate degree between the SPS and EPS (our unpublished data by us), which encourages to conduct studies aimed to find MMT peritoneum with EPS.

These findings represent important evidence linking both processes, but indirect evidence may also be marked. In human studies [3, 11] and in experimental animals [47], our studies demonstrated a direct relationship between MMT and time on PD. Similarly, the several studies showed a parallel between EPS and time on PD [2, 48]. Another important fact is that peritoneal function studies also show a parallel between high frequency ofMMT ofMCs, high Cr-MTC, and low ultrafiltration. Indeed we observed a higher frequency of mesothelial fibroblastoid phenotype in patients with type Cr-MTC >11 mL/min [3]. Furthermore, as is well known, patients with EPS even displayed these with SPS showed similar functional PM deterioration [35, 49, 50]. Another indirect association between these two events is peritonitis. Yañez-Mo and coworkers [11] found that the frequency of nonepithelioidMCwas associated with episodes of peritonitis, thismeans that peritonitis leads to theMMT. In the case of theEPS, there are some studies in the literature that correlate it with peritonitis events. Previous studies suggest that peritonitis may predispose to EPS, especially if this is caused by *Staphylococcus aureus*, fungi, and/or *Pseudomonas* [9, 51]. There is also an association between persistent infections such as tuberculosis peritonitis and EPS [52]. Although peritonitis and EPS are highly associated in several studies it is also known that, especially in a long-term case, EPS may occur without peritonitis. Moreover, patients that have suffered from more events of peritonitis have a higher incidence of MMT and EPS, which suggest again that these processes are related. Finally, we have analyzed more than 10 peritoneal biopsies from patients with EPS where we had found a significant amount of mesothelial cells (CK +) in the peritoneal submesothelial area, which indicates that despite the significant denudation of the peritoneal MCs monolayer.

6. The MMT as therapeutic target

Based on the concept, that MMT, fibrosis and angiogenesis may be part of the same process of peritoneal membrane failure, therapeutic approaches may be addressed to prevent either MMT of the MC or its deleterious effects such as ECM synthesis and/or VEGF production. In this context, *in vitro* and *ex vivo* cultures of MC may be useful for testing pharmacological agents with potential effects on MMT of the MC. Two molecules with expected preventive effect on the MMT of MC are hepatocyte growth factor (HGF) and bone morphogenic protein-7 (BMP-7). It has been demonstrated that these molecules are able to inhibit and reverse MMT and renal fibrosis in animal models. [53-54]. Other strategies that would open new avenues of therapeutic intervention to prevent or reverse MMT of MC may include the inhibition of ILK, RhoA-ROCK or Akt-mediated signaling cascades [25-33, 55]. In this context, the administration of the ROCK inhibitor Y-27632 resulted in suppression of α-SMA expression and renal interstitial fibrosis in a mouse model of ureteral obstruction. [55]
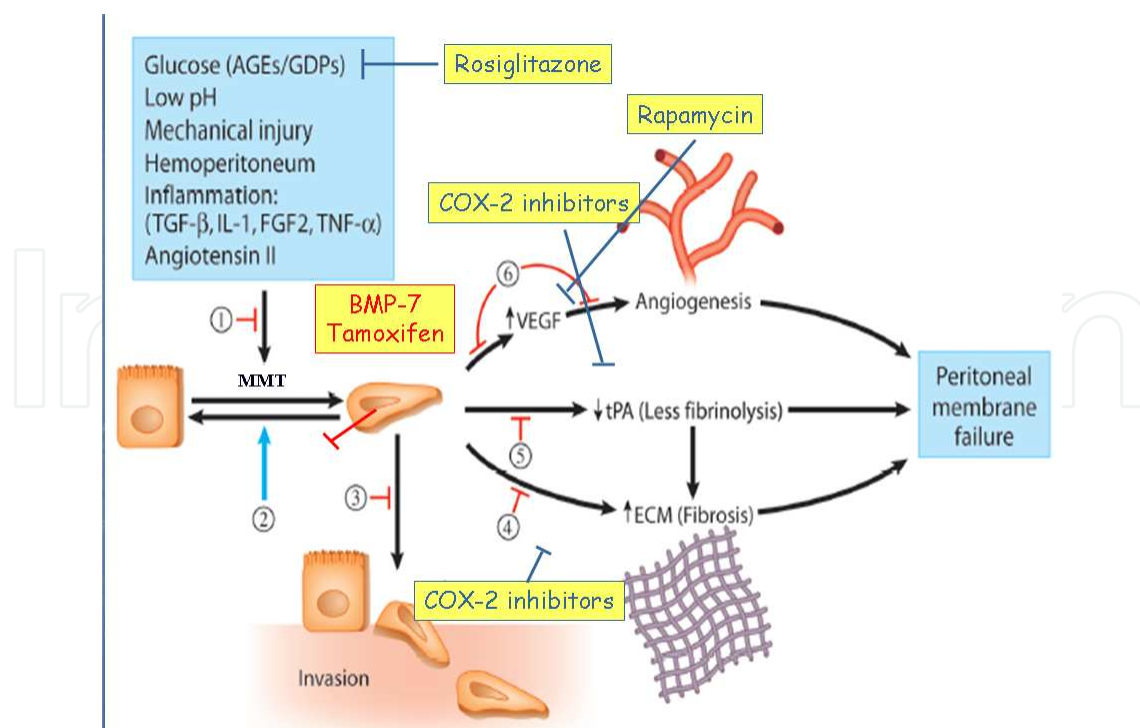
Figure 4. Therapeutic approach to MMT. MMT *in vivo* results from integrated signals that are induced by multiple stimuli. These include elevated glucose and glucose degradation products (GDP) and concentration of PD fluids, which through the formation of advanced glycation-end products (AGE) stimulate 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 may cause tissue irritation and contribute to chronic inflammation of the peritoneum, which promote MMT. Episodes of bacterial or fungal infections or hemoperitoneum cause acute inflammation and upregulation of cytokines and growth factors such as TGF-β, IL-1, fibroblast growth factor-2 (FGF-2), TNF-α, and angiotensin-II, among others, which are strong inducers of MMT. The therapeutic strategies may be designed either to prevent or to reverse the MMT itself or to treat its effects such as cellular invasion, fibrosis, or angiogenesis. The diagram illustrates six steps related to the MMT process of the MC that can be clinically managed, alone or in combination, to prevent peritoneal membrane failure. The numbers represent the steps where different drugs or molecules can act. 1: Tamoxifen, AGEs accumulation inhibitors (Rosiglitazone), BMP7 and HGF; 2: BMP7 and HGF; 3: Invasion Inhibitors (MMPs blocked); 4: Antifibrotic i, e: rapamycin; 5: Tamoxifen and heparin; 6: Angiogenesis inhibitors (rapamycin, inhinitors CO2, etc.)

We performed our studies using testing different drugs or anti-MMT strategies. We have developed a PD mouse model, this consist in the intra-abdominal catheter implantation with a subcutaneous chamber localized in the top of mouse back. After, we injected a daily intra-peritoneal injection of PD solution (1.5-2 mL) at least for 4 weeks. In-vitro we used MCs isolated from omentum and from PD peritoneal effluent. We managed to inhibit the MMT and its adverse effects from using rBMP7 [40]. With Rapamycin got a specific inhibition of fibrosis and the vessels formation specifically lymphatic vessels (56, 57, our unpublished results). Rosiglitazone showed an inhibitory effect on the accumulation of submesothelial AGEs, also anti-inflammatory action mediated by T-cells was observed [39]. Similarly, celecocib inhibited the peritoneal fibrosis by an anti-cox2 effect [38]. MMT also was prevented by tamoxifen. This drug inhibited the peritoneal fibrosis and increased MCs fibrinolytic capacity [47]. Clinically, tamoxifen also improved survival in patients diagnosed of EPS [58]. Paricalcitol acted on

smads cascade inhibiting the MMT (our unpublished results). Finally, we were able to inhibit specifically TGF-β with anti-TGF-β specific peptides demonstrating the role of TGF-β in the initiation and perpetuation of MMT [14]. In this context, other promising substances are pentoxifylline, dipyridamole, and emodin [59-61]. Figure 4 summarizes the sites where we may act by blocking the MMT or adverse effects. Some of these drugs and / or therapeutic strategies have been described by our group.

7. Conclusion

Recent findings suggest that in the peritoneum new fibroblast-like cells arise from local conversion of MMT during the repair responses that take place in long-term PD. These trans-differentiated MC may invade the submesothelial tissue and may contribute to peritoneal fibrosis and angiogenesis, which ultimately lead to peritoneal membrane failure. MMT appears as the central point in the pathogenesis of peritoneal damage associated to PD. Current data support a connection between MMT and SPS. However, the jump from SPS to EPS and the connection between MMT and EPS have not been fully established. MMT can be a therapeutic target the blockade of which could benefit especially in initial stages of the process.

Acknowledgements

Author details

Abelardo Aguilera[1*], Jesús Loureiro[1], Guadalupe Gónzalez-Mateo[2], Rafael Selgas[2] and Manuel López-Cabrera[3]

*Address all correspondence to: abelardo.aguilera@salud.madrid.org aguileraa@terra.es

1 Unidad de Biología Molecular and Servicio de Nefrología. Hospital Universitario de la Princesa, Instituto de Investigación Sanitaria Princesa (IP), Madrid, Spain

2 Hospital Universitario La Paz, Instituto de Investigación Sanitaria la Paz (IdiPAZ), Madrid, Spain

3 Centro de Biología Molecular-Severo Ochoa, CSIC-UAM, Cantoblanco, Madrid, Spain

References

[1]   Krediet RT, Lindholm B, Rippe B. Pathophysiology of peritoneal membrane failure. Perit Dial Int Suppl 2000; 20: S22-S42.

[2]   Rigby RJ, Hawley CM. Sclerosing peritonitis: The experience in Australia. Nephrol Dial Transplant 1998; 13:154-159.

[3]   Aroeira LS, Aguilera A, Selgas R, Ramírez-Huesca M, Pérez-Lozano ML, Cirugeda A, Bajo MA, del Peso G, Sánchez-Tomero JA, Jiménez-Heffernan JA, López-Cabrera M. Mesenchymal conversion of mesothelial cells as a mechanism responsible for high solute transport rate in peritoneal dialysis: role of vascular endothelial growth factor.Am J Kidney Dis 2005; 46: 938-48.

[4]   Aguilera A, Yáñez-Mo M, Selgas R, Sánchez-Madrid F, López-Cabrera M. Epithelial to mesenchymal transition as a triggering factor of peritoneal membrane fibrosis and angiogenesis in peritoneal dialysis patients. Curr Opin Investig Drugs. 2005; 6: 262-268.

[5]   Rubin J, Herrera GA, Collins D. An autopsy study of peritoneal cavity from patients on continuous ambulatory peritoneal dialysis. Am J Kidney Dis 1991; 18:97-102.

[6]   Di Paolo N, Garosi G. Peritoneal sclerosis. J Nephrol 1999; 12:347-361.

[7]   Di Paolo N, Sacchi G, Garosi G,Taganelli P, Gaggiotti E. SPS and sclerosing peritonitis: related or distinct entities? Int J Artif Organs 2005;28:117-28.

[8]   Nagy JA. Peritoneal membrane morphology and function, Kidney Int Suppl 1996;50: 56: S2-S11.

[9]   Gotloib L, Shostak A,Wajsbrot V. Functional structure of peritoneum as dialyzing membrane. In: The text book of peritoneal dialysis, 2nd ed: London: Kluwer Academic Publishers; 2000:37-106.

[10] López-Cabrera M, Aguilera A, Aroeira LS, Ramírez-Huesca M, Pérez-Lozano ML, Jiménez-Heffernan JA, Bajo MA, del Peso G, Sánchez-Tomero JA, Selgas R. Ex vivo analysis of dialysis effluent-derived mesothelial cells as an approach to unveiling the mechanism of peritoneal membrane failure. Perit Dial Int 2006; 26: 26-34

[11] Yáñez-Mó M, Lara-Pezzi E, Selgas R, Ramírez-Huesca M, Domínguez-Jiménez C, Jiménez-Heffernan JA, Aguilera A, Sánchez-Tomero JA, Bajo MA, Alvarez V, Castro MA, del Peso G, Cirujeda A, Gamallo C, Sánchez-Madrid F, López-Cabrera M. Peritoneal dialysis and epithelial-to-mesenchymal transition of mesothelial cells.N Engl J Med 2003; 348: 403-413.

[12] Aroeira LS, Aguilera A, Sánchez-Tomero JA, Bajo MA, del Peso G, Jiménez-Heffernan JA, Selgas R, López-Cabrera M. Epithelial to mesenchymal transition and perito-

neal membrane failure in peritoneal dialysis patients: pathologic significance and potential therapeutic interventions. J Am Soc Nephrol 2007; 18: 2004-2013.

[13] Freser D, Wakefield L, Phillips A. Independent regulation of transforming growth factor beta 1 transcription and translation by glucose and platelet-derived growth factor. Am J Pathol 2002; 161: 1039-1049.

[14] Loureiro J, Aguilera A, Selgas R, Sandoval P, Albar-Vizcaíno P, Pérez-Lozano ML, Ruiz-Carpio V, Majano PL, Lamas S, Rodríguez-Pascual F, Borras-Cuesta F, Dotor J, López-Cabrera M. Blocking TGF-β1 protects the peritoneal membrane from dialysate-induced damage. J Am Soc Nephrol. 2011; 22: 1682-95.

[15] Margetts PJ, Kolb M, Hoff CM, Gauldie J. The role of angiopoietins in resolution of angiogenesis resulting from adenoviral mediated gene transfer of TGF-β1 or VEGF to the rat peritoneum. J Am Soc Nephrol. 2001; 12: 2029-2039.

[16] Border WA, Noble NA. Transforming growth factor β in tissue fibrosis. N Engl J Med 1994; 331: 1286-1292.

[17] Massagué J, Wotton D. Transcriptional control by the TGF-β/Smad signaling. EMBO 2000; 19: 1745-1754.

[18] Fan JM, Ng YY, Hill PA, Nikolic-Paterson DJ, Mu W, Atkins RC, Lan HY. Transforming growth factor-beta regulate tubular epithelial-myofibroblast transdifferenciation in vitro. Kidney Int 1999; 56: 1455-1467.

[19] Tan C, Mui A, Dedhar S. Integrin-linked kinase regulate inducible nitric oxide synthase and cyclooxygenase-2 expression in an NF-κB-dependent manner. J Biol Chem 2002; 277: 3109-3116.

[20] Bitzer M, Von Gersdorff G, Liang D, Dominguez-Rosales A, Beg AA, Rojkind M, Böttinger EP. A mechanism of supression of TGF-β/SMAD signalling by NF-κB/Re1A. Genes and Development 2000; 14: 187-197.

[21] Cano A, Pérez-Moreno MA, Rodrigo I, Locascio A, Blanco MJ, del Barrio MG, Portillo F, Nieto MA. The transcription factor snail controls epithelial-mesenchymal transitions by repressing E-cadherin expression. Nat Cell Biol 2000; 2: 76-83.

[22] Poser I, Dominguez D, de Herreros AG, Varnai A, Buettner R, Bosserhoff AK. Loss of E-cadherin expression in melanoma cells involves up-regulation of the transcriptional repressor Snail. J Biol Chem 2001; 276: 24661-24666.

[23] Vega S, Morales AV, Ocaña OH, Valdés F, Fabregat I, Nieto MA. Snail blocks the cell cycle and confers resistance to cell death. Genes and Development 2004; 18: 1131-1143.

[24] D'Amico M, Hulit J, Amanatullah DF, Zafonte BT, Albanese C, Bouzahzah B, Fu M, Augenlicht LH, Donehower LA, Takemaru K, Moon RT, Davis R, Lisanti MP, Shtutman M, Zhurinsky J, Ben-Ze'ev A, Troussard AA, Dedhar S, Pestell RG. The integrin-linked kinase regulates the cyclin D1 gene through glycogen synthase kinase 3β and

cAM-responsive element-binding protein-dependent pathways. J Biol Chem 2000; 275: 32649-32657.

[25]   Kim K, Lu Z, Hay ED. Direct evidence for a role of beta-catenin/LEF-1 signaling pathway in induction of EMT. Cell Biol Int 2002; 26: 463-476.

[26]   Troussard AA, Costello P, Yoganathan TN, Kumagai S, Roskelley CD, Dedhar S. The integrin linked kinase (ILK) induces an invasive phenotype via AP-1 transcription factor-dependent upregulation of matrix metalloproteinase 9 (MMP-9). Oncogene 2000; 16: 5444-5452.

[27]   Masszi A, Di Ciano, Sirokmany G, Arthur WT, Rotstein OD, Wang J, McCulloch CA, Rosivall L, Mucsi I, Kapus A. Central role for Rho in TGF-beta1-induced alpha-smooth muscle actin expresion during epithelial-mesenchymal transition. Am J Physiol Renal Physiol 2003; 284:F911-F924.

[28]   Benitah SA, Valeron PF, Lacal JC. ROCK and nuclear factor-kappaB-dependent activation of cyclooxygenase-2 by Rho GTPases: Effects on tumor growth and therapeutic consequences. Mol Biol Cell 2003; 14: 3041-3054.

[29]   Huber MA, Azoitei N, Baumann B, Grünert S, Sommer A, Pehamberger H, Kraut N, Beug H, Wirth T. NF-κB is essential for epithelial-mesenchymal transition and metastasis in a model of breast cancer progression. J Clin Invest 2004; 114:569-581.

[30]   Barberà MJ, Puig I, Domínguez D, Julien-Grille S, Guaita-Esteruelas S, Peiró S, Baulida J, Francí C, Dedhar S, Larue L, García de Herreros A. Regulation of snail tran-scription during epithelial to mesenchymal transition of tumor cells. Oncogene 2004; 23: 7345-7354.

[31]   Peinado H, Quintanilla M, Cano A. Transforming growth factor β-1 induces snail transcription factor in epithelial cell lines. J Biol Chem 2003; 278: 21113-21123.

[32]   Li Y, Yang J, Dai C, Wu C, Liu Y. Role for intergrin-linked kinase in mediating tubular epithelial to mesenchymal transition and renal interstitial fibrogenesis. J Clin Invest 2003; 112: 503-516.

[33]   Del Peso G, Jimenez-Heffernan JA, Bajo MA, Hevia C, Aguilera A, Castro MJ, San-chez-Tomero JA, Lopez-Cabrera M, Selgas R. Myofibroblastic differentiation in simple peritoneal sclerosis. Int J Artif Organs 2005; 28: 135-140.

[34]   Del Peso G, Jiménez-Heffernan JA, Bajo MA, Aroeira LS, Aguilera A, Fernández-Perpén A, Cirugeda A, Castro MJ, de Gracia R, Sánchez-Villanueva R, Sánchez-To-mero JA, López-Cabrera M, Selgas R. Epithelial-to-mesenchymal transition of mesothelial cells is an early event during peritoneal dialysis and is associated with high peritoneal transport. Kidney Int Suppl 2008; 108: S26-33.

[35]   Nomoto Y, Kawaguchi Y, Kubo H, Hirano H, Sikai S, Kurokawa K. Sclerosing encap-sulanting peritonitis in patients undergoing continuous ambulatory peritoneal dialy-

sis: a report of sclerosing encapsulating peritonitis group. Am J Kidney Dis 1996; 28: 420-427.

[36] Schneble F, Bonzel KE, Waldherr R, Bachmann S, Roth H, Schärer K. Peritoneal morphology in children treated by continuous ambulatory peritoneal dialysis. Pediatr Nephrol 1992; 6: 542-546.

[37] Do JY, Kim YL, Park JW, Chang KA, Lee SH, Ryu DH, Kim CD, Park SH, Yoon KW. The association between the vascular endothelial growth factor-to-cancer antigen 125 ratio in peritoneal dialysis effluent and the epithelial-to-mesenchymal transition in continuous ambulatory peritoneal dialysis. Perit Dial Int Suppl. 2008; 28: S101-S106.

[38] Aroeira LS, Lara-Pezzi E, Loureiro J, Aguilera A, Ramírez-Huesca M, González-Mateo G, Pérez-Lozano ML, Albar-Vizcaíno P, Bajo MA, del Peso G, Sánchez-Tomero JA, Jiménez-Heffernan JA, Selgas R, López-Cabrera M. Cyclooxygenase-2 mediates dialysate-induced alterations of the peritoneal membrane. J Am Soc Nephrol 2009; 20: 582-592.

[39] Sandoval P, Loureiro J, González-Mateo G, Pérez-Lozano ML, Maldonado-Rodríguez A, Sánchez-Tomero JA, Mendoza L, Santamaría B, Ortiz A, Ruíz-Ortega M, Selgas R, Martín P, Sánchez-Madrid F, Aguilera A, López-Cabrera M. PPAR-γ agonist rosiglitazone protects peritoneal membrane from dialysis fluid-induced damage. Lab Invest 2010; 90: 1517-1532

[40] Loureiro J, Schilte M, Aguilera A, Albar-Vizcaíno P, Ramírez-Huesca M, Pérez-Lozano ML, González-Mateo G, Aroeira LS, Selgas R, Mendoza L, Ortiz A, Ruíz-Ortega M, van den Born J, Beelen RH, López-Cabrera M. BMP-7 blocks mesenchymal conversion of mesothelial cells and prevents peritoneal damage induced by dialysis fluid exposure. Nephrol Dial Transplant. 2010; 25: 1098-1108

[41] K. Honda K Oda H, "Pathology of encapsulating peritoneal sclerosis," Perit Dial Int Suppl 2005; 25: S19–S29

[42] Barini G, Schuinski A, Moraes TP, Meyer F, Pecoits-Filho R, "Inflammation and the peritoneal membrane: causes and impact on structure and function during peritoneal dialysis," Mediators Inflamm. 2012; 2012: 912595.

[43] de Alvaro F, Castro MJ, Dapena F, Bajo MA, Fernandez-Reyes MJ, Romero JR, Jimenez C, Miranda B, Selgas R. Peritoneal resting is beneficial in peritoneal hyperpermeability and ultrafiltration failure. Adv Perit Dial. 1993; 9: 56-61.

[44] Gillerot G, Goffin E, Michel C, Evenepoel P, Biesen WV, Tintillier M, Stenvinkel P, Heimbürger O, Lindholm B, Nordfors L, Robert A, Devuyst O. Genetic and clinical factors influence the baseline permeability of the peritoneal membrane. Kidney Int. 2005; 67: 2477–2487.

[45] Numata M, Nakayama M, Hosoya T, Hoff CM, Holmes CJ, Schalling M, Nordfors L, Lindholm B. Possible pathologic involvement of receptor for advanced glycation end

products (RAGE) for development of encapsulating peritoneal sclerosis in Japanese CAPD patients. Clin Nephrol 2004; 62: 455–460.

[46] Margetts PJ, Bonniaud P, Liu L,.Transient overexpression of TGF-β1 induces epithelial mesenchymal transition in the rodent peritoneum. J Am Soc Nephrol 2005; 16: 425–436.

[47] Loureiro J, Sandoval P, del Peso G, Gónzalez-Mateo G, Fernández-Millara V, Bajo MA, Sánchez-Tomero JA, Guerra-Azcona G, Selgas R, López-Cabrera M, Aguilera AI.. Tamoxifen Ameliorates Peritoneal Membrane Damage by Blocking Mesothelial to Mesenchymal Transition in Peritoneal Dialysis. Plos One 2013; 8: e61165.

[48] H. Kawanishi H, Moriishi M. Epidemiology of encapsulating peritoneal sclerosis in Japan. Perit Dial Int Supp 2005; 25, 4: S14–S18.

[49] Kawaguchi Y, Hasegawa T, Kubo H, Yamamoto H, Nakayama M, Shigematsu T. Current issues of continuous ambulatory peritoneal dialysis. Artif Organs, 1995; 19: 1204–1209.

[50] Afthentopoulos IE, Passadakis P, Oreopoulos DG, Bargman J. Erratum: sclerosing peritonitis in continuous ambulatory peritoneal dialysis patients: one center's experience and review of the literature, Adv Ren Repl Ther 1998; 5: 157-167.

[51] Slingeneyer A. Preliminary report on a cooperative international study on sclerosing encapsulating peritonitis. Nephrol Dial Transplant 1988; 3: 66–69.

[52] Bentley PG, Higgs DR. Peritoneal tuberculosis with ureteric obstruction, mimicking retroperitoneal fibrosis. B J Urol 1976; 48: 170-1976.

[53] Yang J, Liu Y. Blockage of tubular epithelial to myofibroblast transition by hepatocyte growth factor prevents renal interstitial fibrosis. J Am Soc Nephrol 2002; 13: 96-207.

[54] Zeisberg M, Hanai J, Sugimoto H, Mammoto T, Charytan D, Strutz F, Kalluri R. BMP-7 counteracts TGF-β1-induced epithelial-to-mesenchymal transition and reverses chronic renal injury. Nat Med 2003; 9: 964-968.

[55] Nagatoya K, Moriyama T, Kawada N, Takeji M, Oseto S, Murozono T, Ando A, Imai E, Hori M. Y-27632 prevents tubulo-interstitial fibrosis in mouse kidneys with unilateral ureteral obstruction. Kidney Int 2002; 61: 1684-1695.

[56] Sekiguchi Y, Zhang J, Patterson S, Liu L, Hamada C, Tomino Y, Margetts PJ. Rapamycin inhibits transforming growth factor β-induced peritoneal angiogenesis by blocking the secondary hypoxic response. J Cell Mol Med. 2012; 16: 1934-45.

[57] Aguilera A, Aroeira LS, Ramirez-Huesca M, Perez-Lozano ML, Cirugeda A, Bajo MA, Del Peso G, Valenzuela-Fernandez A, Sanchez-Tomero JA, Lopez-Cabrera M, Selgas R. Effects of rapamycin on the epithelial-to-mesenchymal transition of human peritoneal mesothelial cells. Int J Artif Organs 2005; 28: 164-169.

[58] del Peso G, Bajo MA, Gil F, Aguilera A, Ros S, Costero O, Castro MJ, Selgas R. Clini-
cal experience with tamoxifen in peritoneal fibrosing syndromes. Adv Perit Dial
2003; 19: 32-35.

[59] Fang CC, Yen CJ, Chen YM, Shyu RS, Tsai TJ, Lee PH, Hsieh BS. Pentoxifylline inhib-
its human peritoneal mesothelail cell growth and collagen synthesis: Effects in TGF-
β. Kidney Int 2000; 57: 2626-2633.

[60] Hung KY, Chen CT, Huang JW, Lee PH, Tsai TJ, Hsieh BS. Dipyrodamole inhibits
TGF-beta-induced collagen gene expression in human peritoneal mesothelial cells.
Kidney Int 2001; 60: 1249-1257.

[61] Chan TM, Leung JK, Tsang RC, Liu ZH, Li LS, Yung S. Emodin ameliorates glucose-
induced matrix synthesis in human peritoneal mesothelial cells. Kidney Int 2003;
64:519-533.