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The hidden secrets of the renal tubular epithelial cell – an overview of fibrogenesis in chronic renal disease

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INTRODUCTION

Regardless of the aetiopathogenesis, glomerular or tubulointerstitial related chronic renal failure is characterised by fibrotic disorganisation of the kidney's architecture with a progressive decline in renal function. The nephropathy can be a consequence of an injury caused by different pathogenic mechanisms, including gene mutations (either inherited or non-inherited) and the aging process, which sooner or later undermine the normal functioning of the cells. In a general perspective, and at a pathological level, kidney disease is truly an ominous tissue transformation that can progress quickly and dramatically, but more often, in a slow and silent way, and appears to follow a coordinated sequence of complex events. These events can depend on the nature, point of impact, and duration of the insults on the kidney, spreading thereafter from the glomeruli to the interstitium and *vice versa*. The quality and intensity of the injury can finally be regulated by the target cell's genome that orchestrates the entire cell behaviour.

Progress in biotechnology, applied to both basic and clinical research, has led to important advances in the understanding of pathophysiological mechanisms in renal disease, and particularly the final event – fibrosis. The old, reductive, concept of fibrosis saw it as an invariably monotonous, terminal phenomenon, ignoring the true magnitude of the biological changes observed in the tissue. In this context, the fundamental questions are: what types of cells produce large amounts of ECM proteins under pathological conditions, and how are they regulated? Intensive research in the areas of histopathology, genomics and proteomics, has provided important advances in this subject, including the synthesis of the extracellular matrix and its modifications, as well as the identification of the cells responsible for its production in the normal and pathological kidney. Today, it is known that the synthesis of the extracellular matrix, especially collagen, is not the exclusive responsibility of native fibroblasts. In fact, it has been proved that other cells may, at some point in their evolution, develop the capacity to produce an abnormal extracellular matrix and contribute in a significant way to fibrosis in the kidney[1]. This is of particular importance for the opening up of new lines of research in the development of molecules capable of blocking and/or inhibiting fibrosis in renal tissue.

In the last three decades, research has demonstrated the extraordinary importance of the tubular epithelium, not only in renal physiology (water metabolism, electrolytes and acid-base balance), but also in the changes in the extracellular matrix that characterise

fibrogenesis[2]. About 80% of total kidney volume is composed of tubular epithelial cells and cells within the interstitial space. Most of the non-epithelial cells are associated with the rich vascular network of the kidney, and there are also a small number of resident mononuclear cells and fibroblasts[3].

In the sixties, the first study was published demonstrating a positive correlation between tubulointerstitial fibrosis and renal dysfunction in patients with persistent glomerulonephritis[4]. These observations were later confirmed by other studies exploring other nephropathies, where the decline in renal function did not correlate with glomerular sclerosis, but instead with the obliteration of the post-glomerular capillaries caused by interstitial fibrosis. In these cases, there was also a significant inverse correlation between the capillary area in the renal cortex and the blood concentration of creatinine[5,6].

In the light of present knowledge, the component of interstitial fibrosis in the kidney reveals itself as the most prominent clinical-pathological factor in determining the prognosis in both tubulointerstitial and glomerular diseases.

CONSIDERING A BASIC CONCEPT OF PATHOGENESIS APPLIED TO RENAL DISEASE

There are several pathogenic mechanisms involved in glomerular and tubulointerstitial nephropathy which, if we look to the teachings of biology, are very similar to those involved in defence and protection mechanisms against the injury. In this context, the importance of inflammation comes into play, mediated by the innate and/or acquired (immune and non-immune) response and the potential of cells to regenerate the tissue, to heal it and towards a potential recovery of the functioning of the organ.

In spite of this rescue action, it seems clear that when the disease occurs something has gone wrong. In a general view, three critical events could be involved: 1 – the initial impact of the insult is devastating and destroys any defence mechanisms; 2 – the defence mechanisms exceed their function, and trigger other pathways, causing a vicious and damaging circle; 3 – the potential of the tissue to heal and repair the injury, or even to regenerate the tissue, is imperfect and or incomplete. This last capacity is dependent on the organ and animal species, as in the example of the human kidney, where cells do not have the potential to fully regenerate, and rebuild the integrity of the organ.

These general concepts are essential to understand the pathophysiology of chronic kidney disease, as well as the chronic failure of any organ. They stress the need to consider the tissue destruction as a consequence of an imperfect and poorly adaptive physiological mechanism, triggered by the injury.

Biologically, fibrogenesis is a late response of the tissue to the injury, developing with a gradual emergence of a scar and an imperfect replacement of the original structures, irreversibly compromising its function. In this phenomenon, we can find many similarities between the mechanisms that promote tissue repair and the pathogenesis of the disease[1]. From this dual perspective, the fibrosis can be either friend or foe, depending on the circumstances in which the injury occurs.

It is interesting to note that the more mature the tissue is and the more differentiated the cells are, the more imperfect the repair process will be. Furthermore, mature cells continue to keep latent in the genome their potential to express new phenotypes of molecules, waiting for the opportunity to be transcribed whenever the tissue suffers any insult. These phenotypes were once important in the early stages of embryonic development, and particularly in the ontogenesis of the kidney. The reactivation of these molecules in disease, and particularly in fibrosis, suggests a sort of recapitulation of the critical events involved in organogenesis, with an awakening of deepest cellular secrets[7,8].

THE ROLE OF THE EXTRACELLULAR MATRIX IN KIDNEY FIBROSIS

In general, the extracellular matrix (ECM) is a complex network of various glycoproteins, polysaccharides and other macromolecules secreted from the cell into extracellular space. The ECM provides a supportive framework, directly influencing various cellular characteristics, including shape, motility, strength,

flexibility, and adhesion. In this exceptional biological environment, several cytokines and chemokines can interact with native and migrant receptor cells in an intense and dynamic circle which further promotes the signalling of other molecules. The extracellular matrix is therefore a structure with an intrinsic metabolism, which both agglutinates and interacts with the cells consolidating the renal structure.

The extracellular matrix has different characteristics at the level of the glomerulus and interstitium. In developing fibrosis, overproduction and increased deposition of ECM materials can result in the thickening and malformation of various membranous and cellular components, reducing local flexibility and surface area of the affected site, and impairing a number of bodily processes at the glomerular and tubulointerstitial level[8,9]. Matrix homeostasis in normal tissues is a balance between matrix production and its degradation. It is generally believed that the excessive matrix accumulation seen in the fibrotic kidney results from both overproduction of the components of the matrix and the defects in its degradation[10]. In fact, renal tissue produces a number of proteases, in which the plasminogen/plasmin and matrix metalloproteinase systems constitute a proteolytic network that is capable of degrading all components of the proteins of the matrix[11].

Glomerular sclerosis

Within the glomeruli, the injury is followed by damage to resident cells, with the release by endothelial cells of pro-inflammatory cytokines, chemokines and growth factors which attract inflammatory cells and initiate a micro-inflammatory process[12]. The infiltration of the glomerular capillaries by monocytes/macrophages leads to their interaction with all glomerular cell lines, stimulating the proliferation of endothelial and mesangial cells, and also to the synthesis of extracellular collagenous matrix by most (endothelial, mesangial and epithelial) cells[13]. Mesangial cells can transdifferentiate in response to injury from a mature, adult, pericyte phenotype to an embryonic myofibroblastic one (the "mesangioblast"), characterised by proliferation and contraction, as well as by the expression of cytoskeletal cell markers such as α-smooth muscle actin (α-SMA)[14]. The mesangioblast is capable of releasing interstitial collagens type I and III, which are not normally detected within healthy glomeruli. Collagen IV and laminin are the normal constituents of the mesangial and glomerular ECM, but after the injury the deposition of collagens, type I and III, is irreversible as glomeruli are devoid of collagenases (metalloproteinases) capable of breaking down such collagens[15].

In addition to mesangial cells, there are also phenotypical changes within the epithelial cells in response to injury. Glomerular epithelial cells react to injury by acquiring an embryonic phenotype similar to that of the mesangioblast/myofibroblast with the expression of α-SMA[16]. Glomerular parietal epithelial cells acquire an intracellular network of stress fibres and the morphological ultrastructural appearance of myofibroblasts. These changes are also associated with crescent formation and excessive production of ECM.

There are numerous reports demonstrating the role of TGFβ in glomerulosclerosis. Generally, TGFβ induces deposit of ECM by stimulating the production of matrix proteins, decreasing synthesis of ECM-degrading proteinases and upregulating the synthesis of proteinase inhibitors[17]. But, curiously, other reports have shown that TGFβ can also have a protective function against glomerular injury through inhibition of glomerular cell proliferation[18], opposing the proinflammatory actions of IL-1, IL-2, IL-3, CSF, interferon α, γ and TNFα[19], and promoting apoptosis[20]. This quite intriguing and ambiguous behaviour of the TGFβ molecule shows the complexity of the fibrogenic environment.

Most nephropathies that begin with glomerular sclerosis will sooner or later develop a progressive interstitial fibrosis. Extensive studies trying to understand this observation have demonstrated that glomerular degenerative and inflammatory lesions cause cell bridges and fibrous adhesions between the glomerular tuft and Bowman´s capsule, which cause a diversion of part of the glomerular filtrate toward the periglomerular areas. This misdirected urine, as well as the protein leakage in the tubular urina, cause interstitial inflammation, fibrosis and finally, nephron loss[21,22].

Tubulointerstitial fibrosis

The extracellular matrix in renal interstitium is comprised of several molecules, especially collagen,

a precious amorphous material establishing special links with the epithelial cells. As in glomerular sclerosis, the tubulointerstitial fibrosis is the final result of an imbalance between ECM synthesis and degradation. The pathological tubulointerstitial ECM is composed of collagen I, III, IV, V, VII, XV, laminin and fibronectin[9]. ECM turnover is thought to be largely dependent on a balance between the plasminogen system (plasminogen, plasminogen activators, and plasmin), primarily via the activation of latent metalloproteinases and their tissue inhibitors (tissue inhibitor metalloproteinases – TIMP)[23,24].

The principal cells involved in collagen deposition are fibroblasts transformed into myofibroblasts. The profibrotic role of myofibroblasts in renal fibrosis is widely accepted and three hypotheses have been advanced regarding the origin of these cells: 1 – native (resident) cells; 2 – bone-marrow-derived cells; and 3 – tubular epithelial cells transformed in myofibroblasts, by a complex process of transdifferentiation/transition[10,25].

Several studies have also demonstrated the importance of TGFβ as a key molecule in promoting kidney interstitial fibrosis. TGFβ is strongly induced by angiotensin II and its profibrotic actions are thought to involve reduction of metalloproteinases expression, increased TIMP and PAI-1 expression, and increased synthesis of matrix proteins[26].

TUBULOINTERSTITIUM – A PECULIAR FIBROGENIC ENVIRONMENT

(Teachings from a unilateral urethral obstruction model)

The progression of tubulointerstitial fibrosis, even when the peritubular capillaries are essentially intact, severely impairs tubular oxygen supply. The chronic hypoxia hypothesis, proposed by *Fine et al.*, emphasises chronic ischaemia damage in the tubulointerstium as a final common pathway in end-stage kidney injury[27].This important concept enhances tissue hypoxia as one of the main insults capable of inducing an extraordinary constellation of cellular responses, and gives a basic support to these following considerations:

In basic research, the experimental model of unilateral urethral obstruction (UUO) is an excellent way to study tubulointerstitial disease. The obstruction of urine outflow results in progressive renal parenchymal changes and makes it possible to analyse in a dynamic way the main mechanisms that promote extracellular matrix remodelling[28].

Some studies, using UUO models lasting for a few days, have emphasised the role of arteriolar vasoconstriction in response to the release of vasoactive compounds from interstitial macrophages and damaged tubular cells[29]. In fact, a substantial vasoconstriction of the renal vascular bed (cortical and medullar) is the predominant alteration observed after urethral obstruction. The changes in blood flow are mediated by portioned, angiotensin II and nitric oxide, but the most vasoconstrictive substances are the angiotensin II, thromboxane A2 and the antidiuretic hormone[30,31]. Increasing levels of angiotensin II may, in turn, upregulate the expression of other factors, such as transforming growth factor β (TGFβ1), tumour necrosis factor α (TNFα), osteopontin, vascular cell adhesion molecule (VCAM-1), endothelin, and nuclear factor B (NF-kB)[32]. According to some studies, it seems that angiotensin II formation can account for 50-60% of the molecular and cellular changes that occur in urethral obstruction, whereas TNFα accounts for ~20%[33,34].

The urinary obstruction triggers a sequence of cellular responses at the tubulointerstitium with the infiltration of inflammatory cells, proliferation, cell (des)differentiation, necrosis and apoptosis[10,35].

In the UUO model, the early inflammatory cell infiltration is followed by peritubular capillary loss, tubular atrophy, and later fibrosis, characterising the pathological picture of hydronephrosis[36]. Cells of monocyte/macrophage lineage are always present and have been considered to be key players that actively promote fibrosis[37]. Although macrophages constitute the predominant infiltrating cell population in the acutely obstructed kidney, increased numbers of T lymphocytes are also evident after 24h of obstruction, though neither B lymphocytes nor neutrofils are observed[38]. Multiple molecules on the endothelial cell surface and in the subendothelial environment direct circulating monocytes to inflamed sites and these include selectins and integrins that mediate monocyte rolling and adhesion, followed by diapedesis[39].

Some studies have indicated an inverse correlation between the number of interstitial macrophages and

the degree of fibrosis, also suggesting a potential renoprotective role of those cells[40,41]. This apparent paradoxical finding may reflect the differential regulation of inflammation and fibrosis by two functionally distinct phenotypes of macrophages M1 (anti-fibrosis) and M2 (pro-fibrosis) pointing out to an interaction between marrow cells and the kidney[39,42]. The renin-angiotensin system can have an important and direct influence on this behaviour, regulating the macrophage function, namely the migratory and phagocytic capacity, but also influencing the ability to stimulate or inhibit fibrosis[40,43]. Macrophages can be also a source of HGF, which seems to block the conversion of renal epithelia to mesenchymal cells [44,45].

Renal cells can die through either necrosis or apoptosis. Necrosis predominates in a few processes, such as in renal infarct or in acute tubular necrosis. However, cell death in the normal kidney and in most renal diseases, such as in chronic obstructive nephropathy, occurs mainly through apoptosis[46]. Previous studies have demonstrated apoptosis in tubular and interstitial cells during obstructive nephropathy, which plays a significant role in tubular atrophy and renal weight loss[47]. Curiously, these studies also demonstrate an intriguing temporal relationship between tubular cell apoptosis and proliferation, with the latter occurring immediately before apoptosis[35,46]. The continuous increase in interstitial cell apoptosis and proliferation, at a time when these processes subside in the tubules, clearly reflects the high turnover of interstitial cells and the dynamic role of the interstitium in the late phase of the obstruction model[48]. Several cytokines and growth factors, triggered by the hypoxic environment, have been identified as major contributors to obstruction-induced fibrosis, and also to apoptotic cell death, most notably transforming growth factor β1 (TGFβ1), angiotensin II, nuclear factor – kB (NF-kB), and tumour necrosis factor-α (TNFα)[3,49]. These assumptions point to a close relationship between apoptosis and extracellular matrix overproduction in the interstitium.

THE ROLE OF THE EPITHELIAL TUBULAR CELL IN FIBROGENESIS

Tubulointerstitial fibrosis invariably accompanies the course of chronic renal failure towards end-stage renal disease. Tubular epithelial cells become activated very early, either by glomerular ultrafiltrate from their apical side, or by mononuclear cells from their basolateral side. They initiate the scarring process by secreting chemokines, which in return attract mononuclear cells, as well as growth factors that stimulate interstitial fibroblasts[50]. Tubular epithelial cells can react through proliferation or hypertrophy to initial stimuli, and may undergo apoptosis or transdifferentiate into fibroblasts, thus contributing to tubular atrophy in the later stages of progressive renal disease[51].

Since the original observations of *Cohnheim*, investigators have debated the origin of tissue fibroblast, considered the master collagen secretor cell[52]. As we mentioned above, there are three notions regarding their origin: the longest-held concept is that fibroblasts are simply residual embryonic mesenchymal cells left over from organogenesis[1]; a second notion argues that fibroblasts emerge from the bloodstream after release from the bone marrow[53]; and a third view suggests that fibroblasts derive locally in tissues following epithelial-mesenchymal transition (EMT) (or transdifferentiation) of the tubular epithelial cells[54]. According to the most recent findings and when renal fibrogenesis sets in, about 36% of new fibroblasts come from local EMT, about 14-15% from bone-marrow, and the rest from local proliferation, reinforcing the notion that fibrogenesis is a local epithelial event[52-54].

EMT was first demonstrated by developmental biologists in the early 1980s, and was considered a fundamental process which governs morphogenesis in multicellular organisms during embryonic development[55]. The growing interest regarding its significance in oncology, nephrology and other areas of medicine is because EMT represents a reactivation of a fundamental, embryonic process in a variety of diseases, including fibrosis and in the progression of carcinomas[56].

According to the third hypothesis, tubular epithelial cells, the predominant cell type in the tubulointerstitium, have the capacity to regress from an adult, mature phenotype to an embryonic one, in response to injurious stimuli. This cellular regression is characterized by the neo-expression of a variety of cytoskeletal markers including α-SMA, vimentin, desmin, and prolyl 4-hydroxylase, a key enzyme in collagen synthesis[57-59]. These findings confirm that

tubular epithelial cells acquire the capacity to produce collagen through the change to a fibroblast-like phenotype.

Some authors argue that the concept of epithelial-mesenchymal transition (EMT) is not the same as epithelial-mesenchymal transdifferentiation[60]. According to them, this last designation classically refers to differentiated cells changing into other differentiated cells, as in the example observed in endothelial cells that become vascular smooth muscle cells. Therefore, epithelial-mesenchymal transition must be considered as a variant of transdifferentiation.

The injury to the kidney is associated with many inflammatory cells which can incite epithelial-mesenchymal transition, through a combination of cytokines (TGFβ, EGF, IGF-II or FGF-2), as well as through the proteolytic digestion of basement membranes by metalloproteinases[61,62]. Degradation of the basal tubular membrane results in disruption of tubular nephrons, and delaminated epithelial cells either fall off into the tubular fluid or migrate towards the interstitium, under the influence of increasing growth factor gradients and chemoattractants[63].

FIBROSIS – A POINT OF NO RETURN?

Contrary to the kidney, regression of fibrosis is well-established in other tissues, such as the heart, liver, and skin[64-66]. Renal fibrosis appears irrespective of the underlying disease, and it is generally believed to be under the control of a common final physiopathological pathway, independent of the primary cause[10]. In the meanwhile, what causes the difference between a healthy wound-healing and a fibrotic response remains quite a fascinating question, although one obvious distinction is the duration of the injury. In fact, an acute, transient renal injury may trigger similar responses to those in chronic kidney disease, including inflammatory infiltration, secretion of fibrogenic factors, and fibroblastic activation, but the damage is eventually repaired via tubular regeneration and matrix remodelling[67]. As the duration of the injury prolongs, wounded tissues react in some kind of a maladaptative way that is characterised by the overproduction of the extracellular matrix causing a fibrous scar. It is interesting to consider why the same tissue interprets the injurious signal differently in acute versus chronic conditions. One potential explanation could be that in the chronic situation, after repeated injury, the fibrogenic signal is continuously present and increasingly amplified owing to the progressive loss of anti-fibrotic signals[68]. The fibrogenic signals not only stimulate fibroblast activation, but also initiate epithelial-mesenchymal transition, a critical event that leads to the destruction of renal parenchyma, and to the point of no return, if left untreated. In this sense, tubular EMT is a unique cellular event that distinguishes a fibrogenic consequence in chronic kidney disease from a reparative injury response after acute insult, and thereby determines the divergent fates of the kidney when afflicted with a transient or sustained injury[52].

In recent years several studies have permitted a better understanding of the pathophysiological mechanisms that cause fibrosis, opening up new lines of research for the application of drugs that may intervene in fibrogenesis. The main targets have been: 1 – the inhibition of inflammation-oxidative stress[69]; 2 – the blockage of vasoconstrictor peptide action, through the inhibition of the renin-angiotensin system[70], and endothelin[71]; 3 – the inhibition of TGFβ action[72] and 4 – the inhibition of collagen biosynthesis[73].

One of the most challenging questions is: is it enough to eliminate the excessive accumulation of extracellular matrix protein to make nephrons work well again? Or, is there a point of no return? Many investigators agree that the point of no return is directly associated to podocyte loss, but there is no clear definition of this irreversible stage in terms of the quantity of podocytes (number), quality (kind of functional or phenotype alteration), and exclusivity (interaction with other renal cells)[74].

Another challenging area deals with the potential biological effects of stem cells (SCs), as has already been demonstrated in acute kidney disease. While it is clear that renal remodelling in health and disease involves the migration of haematopoietic stem cells into the kidneys expressing various glomerular and tubular epithelial phenotypes[75,76], the recent identification of resident progenitor/SC populations in the adult kidney supports the hypothesis that resident SCs may play a critical role in the repair of renal injury[77]. Therefore, therapeutic strategies to exploit the regenerative potential of SCs may be

based on the administration of *ex vivo* expanded SCs, or on the stimulation or expansion and differentiation of local progenitor/SC populations[77].

In the next few years, a better understanding of some of these key events in renal remodelling may open the way to new interventions and new strategies to control or inhibit kidney fibrosis.

Conflict of interest statement. None declared.

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