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Renal studies provide an insight into cardiac extracellular matrix remodeling during health and disease

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Abstract

The remodeling of a heart ventricle after myocardial infarction involves numerous inflammatory mediators that may trigger a long-lasting and a highly fibrogenic process. Likewise, in the kidney, acute and chronic injuries may lead to abnormal extracellular matrix deposition and eventually lead to the loss of renal function. Major breakthroughs have emerged during the last ten years with respect to the pathophysiology of matrix remodeling. Epithelial and endothelial cells are plastic, and able to engage in epithelial (or endothelial)-to-mesenchymal transition (EMT or EndMT), thus actively contributing to the fibrogenesis. Members of the fibrinolytic system were demonstrated to possess unsuspected properties and interact with receptors and integrins on endothelial and epithelial cells. Finally, a notion that stem cells could integrate into damaged tissue has recently emerged, which likely contributes to the tissue repair. In many aspects, the kidney and the heart share many common injury mechanisms. We envision that some of them will be accessible as common therapeutic targets in the future.

Keywords

Extra-cellular matrix; Heart remodeling; Acute kidney injury; Fibrosis; Epithelial to mesenchymal transition; Endothelial to mesenchymal transition; Integrin; tPA; PAI-1; Transforming growth factor beta

Introduction

Combined renal and cardiac insufficiency is frequent [1] and [2]. In patients with heart condition, superimposed kidney failure has severe prognostic implications [3] and, vice-versa, the majority of patients with kidney disease will die from cardiovascular complications [4]. Obviously enough, the sodium and water retention that accompanies severe renal failure represents a burden for the ventricles, and even earlier in the course of kidney diseases, the altered glomerular filtration rate is responsible for the accumulation of

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many undesirable substances that will eventually increase the risk for cardiovascular diseases (Fig. 1). Still, the possibility that more fundamental pathways are at stake, including at the subcellular level, is rarely considered. Considerable data have however accumulated over the last two decades that support the idea that, in both organs, the beneficial effect of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, as an example, goes well beyond their hemodynamic properties, and also relates to their interference with mutually shared intra-cellular signaling pathways [5] and [6]. Our goal here is to outline that the mechanisms involved in the remodeling of extra-cellular matrix that occurs after acute or chronic kidney injury, are of interest for the understanding of the remodeling of the left ventricle after myocardial infarction. It is indeed probable that the pharmaceutical or cellular tools that are currently designed to temper the fibrogenic response to a chronic condition will not be organ (kidney) specific.

Ischemia and inflammation trigger an abnormal epithelial response in both organs

Nowadays, on the short term, most patients will survive a myocardial infarction as long as they reach the hospital. However, ischemia leads to the activation of an inflammatory reparative process, which eventually leads to heart fibrosis and organ dysfunction in a persistently high proportion of cases [7]. In the kidney world, ischemic events are also very frequent but two major differences should be mentioned: the acute tubular necrosis usually follows a transient drop in blood pressure and not the clotting of a renal artery, and second, the tubular epithelium shows a high quality of repair [8], so that in the absence of fatal multi-organ dysfunction, the majority of patients will have recovered their anterior renal function by a couple of weeks. In the long-lasting inflammatory context of kidney transplantation, the cold ischemia time is of very bad prognostic value, though [9]. The superimposed inflammation, if chronic and unopposed, might thus be critical after an ischemic insult, either in the heart or in the kidney, through the liberation of many mediators that will harm the tissue with time. In the heart, pro-inflammatory cytokines like $\text{TNF}\alpha$, IL-1 and IL-6 are activated even earlier than the renin angiotensin system following the stroke [10]. Based on in vitro evidence, these cytokines supposedly account for the concomitant increase in matrix metalloproteinase (MMP) synthesis and activity by myocytes, fibroblasts and inflammatory cells. Since several animal models reported that MMPs are central in ventricle remodeling (and the same is true in the kidney [11] and [12]), the pathway from ischemia to fibrosis could be seen as trivial. The reason why this healing and highly fibrogenic response persists in the heart and goes unopposed with time is however unclear. The quality of the underlying vascular bed could be key in the control of the repairing process after a brutal drop in blood flow: in the heart, how invasive they are, in 2009 surgical bypasses still provide the best outcome in patients with severe coronary heart disease [13], maybe because it allows a better revascularization, and in the kidney, benign chronic ischemic nephropathy is increasingly recognized as a cause of end-stage renal disease [14]; also, aging hampers the chances to recover from acute tubular necrosis [15]. Thus, while the heart and the kidneys probably have the potential to recover ad integrum, the fibrogenic tonus in both tissues could be critically influenced by the residual ischemia following (or not) an acute injury. To better understand this ongoing fibrogenesis in the heart as in the kidneys, 1) the origin of the fibroblasts has to be elucidated, 2) the reason(s)

for their activation should be apprehended and 3) the events occurring at the surface of tubular epithelial cells should be further studied.

The origin(s) of interstitial fibroblasts

The extra-cellular matrix proteins that progressively invade the tissues and compromise their function are produced by activated fibroblasts, called myofibroblasts [16]. Their origin has been vividly debated during the last decade [17], and the belief that they were only resident, activated, fibroblasts has been challenged in many renal models. Observing microscopic lesions in the biopsies from patients with chronic renal disease, a striking feature is almost invariably associated with interstitial fibrosis: around renal tubules, basement membranes often appear thickened. This so-called “tubular atrophy” is difficult to reconcile with the patchy localization of fibroblasts scattered in the tissue. The circular matrix is more probably laid down by the tubular epithelial cells themselves and this implies that the normal function of epithelial cells (in tubules, the transport of water and electrolytes) has been diverted to mesenchymal tasks, such as collagen synthesis. The concept that adult epithelial cells could undergo a transition towards a mesenchymal phenotype is still being debated. Such an epithelial to mesenchymal transition (EMT) exists however during development: under appropriate stimuli, intra-cellular adhesion complexes are disrupted in primary epithelial cells, and some major cytoskeletal changes occur, conferring migratory properties that allow for the dispersion of the cells [18]. This generates the first set of mesenchymal cells, which will, subsequently, give rise to secondary epithelia. For example, in the midline of the embryo, cells in the epithelial neural plate undergo EMT to become migratory neural crest cells, and along their way these cells will form many different structures, owing to a reverse phenomenon of mesenchymal to epithelial transition (MET). Of note, this biological route is also taken by epithelial cancer cells, allowing them to migrate out of the primary tumor and disseminate metastatic nodules [19].

In the setting of organ fibrosis, we and other groups have demonstrated that adult tubular epithelial cells are also a significant source of fibroblasts in animal models of glomerular [20] and tubulo-interstitial [11] diseases. Thus, transformed epithelial cells leave the tubular structure [21] through tubular basement defects induced by the synthesis of MMPs [11], and reach the interstitium to fully contribute to ECM synthesis and thereby, to scarring (Fig. 2). Taking into consideration the different circumstances of EMT, we have proposed a new classification, according to which “primary” EMT occurs during development, “secondary” EMT is responsible for organ fibrosis after injury in adult tissues, and “tertiary” EMT is a mechanism for epithelial cancer dissemination [22].

In human beings, features of type 2 EMT (i.e. the loss of the epithelial phenotype and the *de novo* acquisition of a mesenchymal one) have been observed in renal biopsies, both in native [23] and in transplanted kidneys [24] and [25], and we have recently demonstrated that in renal grafts they were associated with the progression of interstitial fibrosis with time [26].

Likewise, in the heart, excessive ECM may be seen in peri-vascular and sub-endothelial areas. We tested the provocative hypothesis that the endothelium, which is basically an epithelium, could as well provide the adult myocardial tissue with fibroblasts (in a variant of secondary EMT, a process we called EndMT for endothelial to mesenchymal transition).

Using genetically recombinant mice, endothelium of which irrevocably expresses the LAC-Z gene, we demonstrated in two different models of heart fibrosis that endothelium was indeed a relevant source of fibroblasts, and that this EndMT process was accessible to a therapeutic intervention [27]. The phenotype of myocardial endothelium, or of tubular epithelium, is thus much less stable than previously thought: cells are not terminally differentiated, and, mesenchymal in origin, if solicited to contribute to the repairing process by appropriate transcription factors, they may adapt and profoundly alter their genetic program [28]. Conceptually, such plasticity implies that, on the other hand, once the danger is out, another transition should occur and have these cells go back to their “normal” phenotype. The reason why this process goes unopposed, and why the matrix overwhelmingly accumulates, is not known, neither in the heart nor in the kidney, but it should be connected to the persistence of some form of injury. To our opinion, in the majority of the cases, low but chronic ischemia and/or inflammation are causal.

How ischemia further fuels the fibrogenesis process

Whether they are resident or epithelium-derived, fibroblasts need to be activated to make scars. As we have seen before, inflammation is sufficient to trigger this activation, but hypoxia could take over in a context of chronic vascular disease. Among the mediators involved in the response of cells to low-oxygen, hypoxia inducible factor-1 α (HIF-1 α) is key. This transcription factor is physiologically ubiquitinated because by inducing its hydroxylation, oxygen allows the interaction of HIF-1 α with the von Hippel Lindau protein, routing it to the proteasome [29]. But in insufficient oxygen conditions HIF-1 α is not degraded and may promote the transcription of numerous genes, some very fibrogenic, such as connecting tissue growth factor. This is not the topic of this manuscript, but it should be reminded that other factors than hypoxia may stabilize HIF-1 α . In the inflammatory and rapidly fibrogenic model of unilateral ureteral obstruction, it was recently found that HIF-1 α deficiency was protective, reducing the number of fibroblasts and the collagen content of the kidneys[30]. Mechanistically, the authors provided evidence that HIF-1 α was implicated in the induction of EMT of tubular epithelial cells, through its induction of a lysyl oxidase that, inside the cells, will in turn deregulate E-cadherin expression, allowing the cells to lose their epithelial phenotype. Together, we may thus propose that even when the inflammatory event is over, a persistent and pernicious stimulation of fibroblasts (or the induction of EMT) can be seen because of hypoxia-related mediators, in a chronically compromised vascular bed [31].

Adhesive and de-adhesive molecules play a crucial role

An important contribution of the kidney research has been to enlarge our comprehension of the agents responsible for adhesion of cells to their environment, or for their de-adhesion. Integrins, agents of the fibrinolytic system, and their ligands have been shown to play a very important role in the context of kidney fibrosis, in a way that was not always expected (Fig. 3). As for integrins, the role of $\alpha 1\beta 1$ integrin, first revealed by a gene expression analysis approach in a model of alport renal disease, is major [32], and links inflammation with renal fibrogenesis. Thus, its expression by monocytes/macrophages is critical for the invasion of injured tissues and for the subsequent activation of myofibroblasts. Coherently, genetic ablation of $\alpha 1$ gene was found to reduce inflammation and induce a marked decrease in the

TGF β signaling cascade. The expression of β 1 by interstitial fibroblasts is also very important because *in vivo* this integrin may interact with – and activate – integrin-linked kinase (ILK) [33], a highly fibrogenic kinase. The mechanism through which β 1 is activated was recently found to involve the fibrinolytic system: tPA, the main plasminogen activator in the vascular compartment, turned out to be an important ligand for the LDL receptor-related protein 1 (LRP-1) [11] and [34]. In contrast with the working hypothesis that tPA would protect from interstitial fibrosis, it was found, iteratively, in the common animal model of unilateral ureteral obstruction, that absence of tPA was protective. The interaction of tPA with LRP-1 which is independent from its serine-protease activity, actually facilitates the recruitment of β 1 integrin at the cell surface and subsequently activates ILK and the TGF β pathway; more recent studies also identified an anti-apoptotic effect of this interaction for myofibroblasts [35]. These kidney studies thus revealed an unsuspected, cytokine-like, property of tPA, which should not only be seen as a plasmin generator, but also as a profibrogenic molecule, a somewhat unexpected finding considering that plasmin is supposed to protect mice from interstitial fibrosis by the direct or indirect destruction of ECM proteins. As a matter of fact, the anti-fibrogenic property of plasmin was never conclusively demonstrated [36]. Again, in the context of vascular diseases with fibrin deposition, these properties of tPA should be considered when chronic and not acute disorders are explored. For instance, the main inhibitor of tPA *in vivo*, type 1 plasminogen activator inhibitor (PAI-1), is seen as an important molecule to target in the context of fibrin-dependent vascular diseases: PAI-1 inhibitors would thus help fibrinolysis. But as for chronic diseases, it may well be that the chronic inhibition of PAI-1 could eventually be equivalent to reinforce the properties of tPA and paradoxically exert a fibrogenic role. At least two animal models, one in the aorta [37] and another one in the kidney [38], have shown that a deficiency of PAI-1 was associated with a huge activation of TGF β . Whether tPA also confers a fibrogenic impact in the myocardium is unknown, but in the rat, evidence was brought that these systems were maintained activated months after a myocardial infarction [39].

Associated with this tPA/LRP-1/ILK pathway is indeed TGF β signaling, one of the most highly fibrogenic molecules. TGF β is also activated by another integrin, probably at the surface of epithelial cells which highly express it under pathological conditions, namely integrin α v β 6. This integrin not only permits the cells to adhere to fibronectin or tenascin, but also binds to the “latent-associated protein”, inducing conformational changes that eventually convert TGF β into its active form. In the same model of alport renal disease, the role of α v β 6 was apprehended in a double approach, genetic ablation of the gene and antibody administration[40]. These two manipulations significantly alleviated renal fibrosis similar to TGF β blocking agents. This reduction of interstitial fibrosis was not associated with an improvement in proteinuria or renal function, though, and so far, no study described the role of α v β 6 in ventricle remodeling.

Facts and doubts

In sum, following an acute injury, the following sequence is anticipated: hypoxia-related and inflammatory mediators are released that first trigger a repair signal. Cells that survive the aggression are thus not only solicited to proliferate and reconstitute a tubular structure, but

also to contribute more generally to a program of tissue healing, which, as we have learned from phylogenesis, is typical for primates. Placed in an affluent cytokinetic bath, tubular epithelial cells will interrupt their epithelial program, and undergo EMT. A whole orchestra of mediators push together in this direction:

- HIF-1 α , stabilized by hypoxia, leads to E-cadherin repression, which has two consequences: the dissociation of epithelial cells from adjacent cells, and the liberation of β -catenin into the cytoplasm; this event is critical, in that β -catenin is theoretically able to enter the nucleus and considerably alter the transcriptome. A firewall is however present: in the cytoplasm, a kinase (GSK-3) phosphorylates β -catenin and provokes its ubiquitination by the proteasome.

- Enter TGF β and tPA, two abundant mediators in an inflammatory context. TGF β , activated by proteases (plasmin or MMPs), and/or by integrin $\alpha_v\beta_6$, increases the production of ILK, which, further activated by integrin β_1 , suppresses the function of GSK3: the firewall is switched off, and β -catenin is free to go into the nucleus, consolidating the transition to a mesenchymal program.

- The matrix metalloproteinases (MMP2 and MMP9, now synthesized both by inflammatory and epithelial cells) disrupt basement membranes, an essential step for the cells to reach interstitium and swell the ranks of interstitial fibroblasts. There, the program consists mostly in the synthesis of collagens and other ECM proteins. Hence, tubular atrophy and onset of interstitial fibrosis.

Importantly, this repair program should be transient and, as long as some viable tissue persists, stop once the aggression has ended. The facts, however, show that in adults these inflammatory systems may go persistently activated long after the acute injury has occurred.

From this sequence of events, four major questions arise (Table 1): 1. What is the cause of this major evolutionary move that turned repair into scarring? We should focus on the kinetic of these biological events, and, although we anticipate that many of them overlap, we should establish what differentiates a transient repair (leading to recovery) from a persistent repair program (leading to fibrosis). Here, the differences between the physiological repair in the kidney and the pathological repair in the heart, as the increase in the number of elderly patients incompletely recovering from acute tubular necrosis, probably point to the (micro)vascular bed. Animal models should integrate this, and take into account the poor-quality of the underlying vasculature in patients with myocardial infarction (e.g. the production of tPA has not the same biological consequences, depending on whether it is acute in a young tissue, or persistent in conditions of subintra-arterial ischemia).

2. Is fibrogenesis really a generic phenomenon or is it eventually organ-specific? While we have observed that tubular epithelial cells and endothelial cells share a capacity to undergo EMT after acute injury, it may be that this switch has some subtle but important specificities in the heart, as in the kidney. We should keep in mind that fibroblasts harvested from different tissues have distinct gene expression profiles [41], and further study the differences between organs. For instance, while the β_1 /ILK pathway plays a key role in the activation of the EMT program in the kidney, loss of ILK or disruption of β_1 signaling in the

heart induces a dilated cardiomyopathy [42] and [43]. If we aim at counteracting fibrogenesis systemically, we will need to know the specific properties each of these major mediators exert in the main organs.

3. When one organ, heart or kidney, is injured, is there a systemic fibrogenic signal that may affect the other organ? Such a signal may very well be generated: as an example, it was shown in a model of unilateral ureteral obstruction, that Kielin/Chordin Like Protein (KCP), an enhancer of BMP signaling, was protecting the control-lateral, erstwhile normal, kidney [44]. Mice deficient in KCP thus showed the onset of a fibrotic response in the unobstructed kidney, a finding that had never been reported so far. If cytokines find their way to the other kidney, then they also reach the heart, and future research should determine this very important point. We may have had a too narrow approach, either in our time frames, or in our obsession with studying one organ in particular. Thus, we should determine whether the ligation of an ureter, or the constriction of an aorta, as examples, affect or not the transcriptome of other organs, and if so, how soon, and how much.

4. Assuming such a systemic approach is both efficient and safe, how late will it be accessible? We have learned, from developmental biology, that primary EMT was not terminal: MET follows, and pushes mesenchymal cells to switch back to an epithelial phenotype. Similarly, metastatic cells reform epithelial nodules far from the primary tumor. Will secondary EMT show such plasticity? Probably. From a theoretical point of view, the chromatin of newly formed interstitial fibroblasts should obviously be able to respond to beneficial signals and switch back, through a MET process, into epithelial cells. Our experimental data, in the heart [27], in the kidney [45], and in the liver [46], show that fibrosis may regress after administration of BMP-7, a key transcriptional factor for embryonic MET. In human beings, using iterative renal biopsies from patients with diabetic nephropathy who had undergone a pancreas transplantation, Fioretto et al. demonstrated that the amount of matrix could decrease with time [47]. All these data concur to discard the widespread opinion that fibrosis is a definitive lesion and that epithelial cells are terminally differentiated. The concept has eventually emerged that fibrosis may be reverted and transformed epithelial cells, hopefully, diverted again to their epithelial phenotype. Not all circles are vicious.

Regeneration: The hope in stem cells

In the worst case scenario, though, for which the fibrogenic pathways will not be easy to manipulate, there is still some hope that diseased tissues will be open to a colonization by pluripotent stem cells. Here, the heart world showed the example and human studies have validated this approach [48] and [49], but it was also found to be dramatically helpful to prevent the progression of renal disease in mice, in the Alport model [50]. It should be mentioned, however, that nothing is known as of the mechanisms by which stem cells incorporate diseased structures. It could be spontaneous and independent from the disease, or related to some distress signals that we will have to discover. Moreover, once in the tissues, these cells may alternatively differentiate into new epithelia, or fuse with altered epithelial cells to rescue them and literally repair the cells. Sophisticated tools will have to be employed to improve our knowledge of these biological events: to demonstrate a

differentiation of stem cells, they can be tagged with a marker to be exclusively expressed under the influence of an organ-specific promoter; to demonstrate fusion, the recipient mice can be genetically altered so as to express a marker spontaneously silent, but brought up by an intra-cellular enzyme only present in stem cells (using the CRE/LOX system). Many exciting findings will undoubtedly emerge from this field.

Conclusion

Assuming that we will progressively have to deal with more and more “chronic” patients, now that many acute vascular or inflammatory diseases have had their immediate prognosis improved, the control of matrix remodeling is a major challenge for the next decade, not only in heart and kidney, but also in liver, lung, in fact in almost every organ. A very dynamic vision of matrix remodeling and epithelial phenotype has recently emerged, according to which plasticity prevails. Although it will need confirmation in human beings, we envision that a modern therapeutic approach will not be organ-specific and rather devoted to the systemic antagonization of fibrogenesis in those patients who will have a repairing process ongoing, and be at high risk for tissue scarring. In every injured organ, in the long-term, fibroblasts are the arch enemy. More specifically in the heart, the endothelial to mesenchymal transition process will deserve a special attention.

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Fig. 1. Pathophysiological interactions between heart and kidney

Heart and kidney failures impact on each other, but beyond the well-known mechanical and metabolic interactions depicted here, endothelial and epithelial cells could share a predisposition to engage into a fibrogenic process, at the intra-cellular level. The possibility also exists that, after an injury of one organ, systemic signals are being transmitted to the others.

Fig. 2. Acute injury directly (or indirectly, via the inflammatory process) insults epithelial/endothelial cells

Those which survive may undergo a transition to a mesenchymal phenotype, and provide new fibroblasts in a process called epithelial (or endothelial) to mesenchymal transition (EMT), thus contributing to repair, just like resident fibroblasts, activated by inflammatory mediators. If the process is unopposed with time, the end-result is tissue scarring.

Fig. 3. Biological events occurring outside and inside the cells after injury

Extra-cellular mediators (TGF beta activated by integrin alpha v beta 6, or tPA that binds to LDL related receptor 1 and facilitates the recruitment of integrin Beta1) play a critical role in the activation of integrin-linked kinase (ILK). This leads to the loss of E-cadherin, a major epithelial adhesion molecule, and subsequently to the translocation of the intermediate protein beta-catenin into the cytoplasm. Beta catenin normally connects E-cadherin to the cytoskeleton, but, in the absence of E-cadherin, associates with the tcf/lef transcription factor which activates the transcription of a mesenchymal program, in the nucleus. Ubiquitination of beta-catenin is also prevented by ILK.

Table 1

Key questions and experiments that need to be done.

• Kidney and heart respond differently to injury. Complete recovery from acute tubular necrosis is the rule. By contrast, heart remodeling is frequent and harmful after myocardial infarction: why? More specifically, what is the role of subintra-vascular ischemia? Experimental models should take into account the chronic vascular lesions often present in patients when studying ECM remodeling in the heart or in the kidneys.
• Is there a critical time point, in either organ, after which repair is no more physiological? What is the kinetic of the production of the mediators involved in ECM remodeling and E(n)dMT, and when should we try to interrupt it in order to prevent pathological scarring without compromising physiological repair?
• Will kidney and heart share biological targets in the antagonization of the EMT process? Is tPA facilitating EndMT? Is ILK or β 1 integrin inhibition safe in the context of heart remodeling?
• In response to injury of any organ, is there a systemic signal that could, if persistent, drive injury and fibrogenesis in other organs? What is the transcriptome of the supposedly unaffected organs, regarding ECM remodeling?
• How late will stem cells be able to regenerate a new tissue, and will they have to be injected periodically? How and why do they incorporate a diseased structure?

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