Fibrosis, regeneration and cancer: what is the link?

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Abstract
Tubulo-interstitial fibrosis constitutes the final common pathway for all pathological conditions that evolve towards chronic kidney disease, and transforming growth factor-β1 plays a key role in this process. Furthermore, neutrophil gelatinase-associated lipocalin appears not only to be a simple marker of renal injury but also an active player in disease progression. We are not yet able to control and modulate this phenomenon. Therefore, a better understanding of fibrogenic molecular mechanisms is necessary to detect possible therapeutic strategies that interfere with fibrosis and then stop the progression of renal disease. The line of research called ‘regenerative medicine’ works toward this.

According to many authors, the formation of a fibrotic extracellular matrix disrupts the cells’ polarity and stimulates their proliferation, creating conditions for cancer development.

However, there is another plausible hypothesis: is it possible that fibrosis provides a sort of ‘protection’ from the development of a cancer as a consequence of the intense proliferation that characterizes any inflammatory process? In superior organisms, and also in humans, regeneration may have been selected negatively and replaced by fibrosis in the course of evolution, to warrant species survival: in fact, unchecked pluripotent cell production and proliferation can lead to tumour development and the potential death of a single individual.

Hence, tumours might be the outcome of the failure of fibrotic processes, most likely due to some mediators predominating over others. So, valid experimental models are necessary to understand the interactions that exist between fibrosis and tumours and to evaluate the real advantage of therapies that aim to inhibit the fibrotic process at the renal level or that of other organs. The ideal approach would be to limit fibrosis and then organ function loss but without exposing the patient to risks of developing a tumour, starting from as early as the drugs prescribed.

Keywords: anti-fibrotic drugs; NGAL; TGF-β1; tubulo-interstitial fibrosis

Tubulo-interstitial fibrosis
The term ‘fibrosis’ indicates a pathological process characterized by mesenchymal cell infiltration and proliferation in the interstitial space, which occurs in order to repair epithelial injuries leading to the formation of granulation tissue.

In this context, a key role is played by the differentiation of fibroblasts to myofibroblasts, which act through the production of metalloproteinases (MMPs) [1] and other enzymes that degrade the extracellular matrix (ECM), of type 1 collagen, fibronectin, hyaluronic acid and other ECM components; furthermore, they are able to re-model granulation tissue producing the so-called stress fibres which contain actin and confer to myofibroblasts themselves a notable contractile ability which spreads to the ECM through specialized adhesion sites [2].

Under normal conditions, the repair process that follows an injury is completed by provisional ECM degradation and by myofibroblasts apoptosis, throughout mechanisms subjected to close control. However, in some cases, a sustained myofibroblast activation and then a repair mechanism dysregulation occur; this results in the accumulation...
of fibrotic ECM, rich in collagen fibres that coalesce forming fibrous bundles resistant to degradation [3].

At renal level, the progressive tubulo-interstitial fibrosis constitutes the common final way for all pathological conditions that evolve toward chronic kidney disease (CKD) (Figure 1); we can schematically divide it into three phases [4]:

(1) Inflammatory phase: in response to the initial injury, the resident renal cells produce pro-inflammatory cytokines, chemokines and adhesion molecules that induce a chronic phlogosis with massive infiltration of inflammation cells, overall represented by macrophages [5, 6].

(2) Phase of appearance of cells secreting the ECM components: activated macrophages produce cytokines, such as transforming growth factor-β (TGF-β) and connective tissue growth factor (CTGF), which stimulate myofibroblast formation from resident interstitial fibroblasts. Another important source of myofibroblasts is represented by tubular epithelial cells and vascular endothelial cells which, under the action of the two cytokines, undergo a phenotypic transition process [respectively defined as ‘epithelial–mesenchymal transition (EMT)’ and ‘endothelial-mesenchymal transition’], so they lose their initial phenotype, change into mesenchymal cells and invade the renal interstitial compartment, contributing to myofibroblasts accumulation [7]. However, very recent studies seem to question the real role played in vivo by the EMT process [8, 9].

(3) Matrix accumulation phase: myofibroblasts, under the action of TGF-β and CTGF, synthesize a large amount of matrix proteins, such as fibronectin and Type 1 and 3 collagen; moreover, they prevent degradation of these proteins synthesizing protease inhibitors like plasminogen activator inhibitor-1 (PAI-1) and tissue inhibitor of metalloprotease (TIMP).

Many other molecular factors take part in the fibrotic process, some of which speed up the tubulo-interstitial fibrosis progression [e.g. chemokine (C-C motif) ligand 2 (CCL2), platelet-derived growth factor (PDGF), retinoblastoma 1 (RB1), fibroblast growth factor 2 (FGF2), angiotensin II (Ang II)] [10, 11]; on the contrary, others delay it [e.g. hepatocyte growth factor (HGF), bone morphogenetic protein 7 (BMP7), retinoblastoma-like 2 (RB2)] [12]. It is on the balance between pro-fibrotic factors and fibrosis endogenous inhibitors that renal fibrosis evolution and progression depend.

Finally, the fibrotic process also seems to be related to cell senescence and telomere shortening, as proved for example by Verzola et al. in a group of patients with Type 2 diabetic nephropathy [13]. The same link can probably be identified in chronic cyclosporine A (CsA) nephropathy, which shows some clinical features similar to those of renal organ aging, such as renal fibrosis and tubular atrophy. In fact, exposure of HK-2 cells (human proximal tubule cell line) and primary proximal tubular cells in vitro to CsA determines the inhibition of DNA synthesis and a reduction in telomere length [14].

**TGF-β1: signalling pathways and functions**

A key tissue fibrosis regulating factor is represented by TGF-β, which includes three highly homologous isoforms in mammals, TGF-β1, 2 and 3, which have similar fibrogenic effects in vitro. In vivo, the pro-fibrotic action of TGF-β1 has been widely studied, particularly at the renal level [15].

As we have previously described, both mesenchymal and epithelial cells play a role in ECM protein accumulation and in normal tissue architecture destruction: the former generally give the greatest contribution to the pathological accumulation of ECM and in the kidney, including the glomerular mesangial cells; the latter, at the renal level mostly represented by the tubular epithelial cells, can trans-differentiate in mesenchymal cells through the EMT process, in which TGF-β1 plays a fundamental role [16].

This mediator acts principally through the Smad signalling activation and constitutes the target of different kind of stimuli, such as hypoxia, which up-regulates the transcriptional activity of TGF-β1 promoter in various types of cultured cells, so acting as a potent physiological stimulus for collagen synthesis: the reduction in oxygen partial pressure, in fact, represents a typical aspect of the precocious phases of wound-healing mechanisms [17]. Another stimulus that leads to an increase in TGF-β1 expression is represented by Ang II [18], which also induces collagen

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Fig. 1. Events occurring during tubulo-interstitial fibrosis.
I and fibronectin synthesis, interacting with angiotensin II receptor, Type 1 (AT₁R) [19].

TGF-β1 release by renal cells or damaged kidney infiltrating cells moderates inflammatory reaction and contributes to tissue repair. TGF-β1, in fact, has many functions, sometimes anti-thetical. It has pro-inflammatory properties, since it has a chemiotactic action towards leucocytes [20] and induces cyclooxygenase-2 synthesis at the level of mesangial cells [21].

On the other hand, it also exerts an anti-inflammatory action, antagonizing pro-inflammatory cytokines interleukin-2 and tumour necrosis factor-α in glomerular diseases; furthermore, it is an important deactivator of macrophages in the course of renal insult [22].

In addition to its anti-inflammatory effects, TGF-β1 also plays a pro-fibrotic action, causing an increase in the matrix protein synthesis and in the expression of proteinase inhibitors, such as PAI-1, and a decrease in the synthesis of degrading ECM proteins, such as collagenase [23]. Consequently, the abnormal and sustained TGF-β1 expression results in the pathological accumulation of ECM in both glomerular and interstitial compartments [24].

Thanks to this pro-fibrotic activity, TGF-β1 acts as a potent EMT inducer, which determines the conversion of renal epithelial cells into myofibroblasts in the context of the damaged parenchyma.

For example, it seems to play a central role in the development of the nephropathy induced by CsA. Ling et al. studied the effects of 1D11, TGF-β1 neutralizing murine monoclonal antibody, in a mouse model of chronic CsA nephropathy. In these animals, CsA caused very extensive renal histopathological lesions, including: tubular injury, nephropathy. In these animals, CsA caused very extensive renal histopathological lesions, including: tubular injury, nephropathy. In these animals, CsA caused very extensive renal histopathological lesions, including: tubular injury, nephropathy. In these animals, CsA caused very extensive renal histopathological lesions, including: tubular injury, nephropathy.

In addition, the high levels of encoding TGF-β1 and 2 messenger RNA were significantly reduced. 1D11 also protected tubular epithelial cells from apoptosis to 48%. The effects of 1D11 on CsA-induced morphological alterations were then followed by a reduction in serum creatinine after 8 weeks of treatment [25]. The effects of 1D11 have also been studied in association with treatment involving enalapril in rats, in which a unilateral ureteral obstruction, a condition characterized by increased fibrosis and renal cell apoptosis, had been created. In such a context, both TGF-β1 and Ang II have been implicated and the latter effects seem to be mediated by TGF-β1 [26].

**Possible role of NGAL in renal fibrosis**

Neutrophil gelatinase-associated lipocalin (NGAL), also known as lipocalin-2, siderocalin, uterocalin and 24p3, is a small 25-kD peptide belonging to the lipocalin superfamily. Like the other members of this family, NGAL has a three-dimensional structure with an eight-stranded anti-parallel b-barrel surrounding a central pocket; throughout this central calix, it interacts with many low-molecular weight ligands [27]. The main NGAL ligand is represented by siderophores [28], small iron-carrying molecules which are necessary for bacterial and eukaryotic cells for their survival.

Initially, this protein was detected in activated neutrophils, where it plays the role of innate anti-bacterial factor. However, due to more and more evidence, NGAL is emerging in experimental and clinical nephrology as one of the most promising tubular biomarkers in the diagnostics of acute and CKDs.

In particular, in chronic nephropathies, NGAL levels closely reflect the entity of renal impairment and it also represents a strong and independent risk marker for the progression of kidney disease [29]. It seems that NGAL is not only a simple marker of renal injury but also an active player in disease progression. Viau et al. have recently demonstrated that the epidermal growth factor receptor, which is critical in the evolution of CKD, when activated, induces an increase in NGAL expression in mice and humans, and this could downregulate its mitogenic effect on tubular cells during renal deterioration [30]. Moreover, NGAL levels showed the highest correlation coefficient with tubular atrophy in patients affected by drug-induced tubulo-interstitial nephritis [31].

**Fibrosis and tumours**

According to Desmoulie`re et al [32], the formation of a fibrotic ECM disrupts the cells’ polarity and stimulates their proliferation, creating the conditions for cancer onset and development.

There is ample evidence in the literature in favour of the role played by fibroblast/myofibroblast transition in the interaction between epithelial cells and stroma that besides fibrosis, also underline neoplastic transformation and progression processes. Not surprisingly then, the presence of fibrotic lesions increases the risk of cancer in various tissues, including lung [33, 34], liver [35] and breast [36].

Another aspect which fibrosis and cancer have in common is the EMT process, which determines meaningful changes in epithelial cell morphology and behaviour, transforming them into myofibroblasts and intervenes not only physiologically during embryonic development but also in pathological processes like tumour progression and fibrosis [37]: in particular, snail genes, down-regulated during the mesenchymal–epithelial transition which occurs throughout renal development, are able to induce the whole inverse process, that of EMT, both in vitro and in vivo. The expression of these genes can remain silent in the mature kidney; their aberrant activation in the adult kidney is enough to induce a tubular EMT and the development of fibrosis in transgenic mice, and a pathological expression of snail genes can be observed in the fibrotic areas of human kidneys [38].

A lot of experimental evidence is also in favour of a possible role of TGF-β1 in the development and, above all, in the metastatic progression of neoplastic diseases.

In patients with breast cancer, TGF-β1 expression is related to that of breast cancer 2 and human epidermal growth factor receptor-2 (HER2) and these three factors act in synergy in promoting the disease [39]. TGF-β1 has also been shown to increase the levels of active collagenase matrix MMP-2 through different signalling pathways. This is related to human pancreatic carcinoma invasion and
metastasis processes [40]. Sub-cellular proteomics studies have highlighted the TGF-β1 production by human lung adenocarcinoma cells [41]. Furthermore, with regard to gastric adenocarcinoma, Guo et al. have recently observed that there is a variable association between the susceptibility to developing this form of cancer and the different TGF-β1 polymorphisms: those which constitute a greater risk are C-509T and T869C [42]. Finally, antagonists of TGF-β (1D11, already described, and LY2109761, a chemical inhibitor of the TGF-β receptors I and II kinases), used in a model of human metastatic breast cancer, have been demonstrated to possess a possible clinical utility, supporting the hypothesis that TGF-β plays an important role in both bone and lung metastases of basal-like breast cancer [43].

Do links between fibrosis, regeneration and cancer exist?

The fibrotic process constitutes the final common pathway of different types of renal insult. In this, different mediators play a role and among them, the most studied is TGF-β1.

We are not yet able to control and modulate this phenomenon, which seems to be the inevitable consequence of inflammatory processes which do not result in restitutio ad integrum. Therefore, it is necessary to develop experimental models which allow a better understanding of the molecular mechanisms that modulate fibrogenic events, to detect possible therapeutic strategies which interfere with fibrosis, and then stop the progression of renal disease [44].

The line of research called ‘regenerative medicine’ works toward this and aims to re-build a damaged organ through the use of stem cells [45–48] or the stimulation of angiogenetic processes [49, 50].

The literature is also full of studies showing the existence of a correlation between fibrosis and cancer. In fact, it appears that fibrosis in some way predisposes to neoplastic transformation of involved parenchyma. The need to further investigate the mechanisms underlying the fibrotic processes therefore raises its roots also in an attempt to identify possible mediators and pathways likely to be blocked to stop the probable transformation of a fibrotic tissue in a neoplastic one.

However, there is another plausible hypothesis (Figure 2): could fibrosis provide a sort of ‘protection’ from the development of cancer as a consequence of the intense proliferation that characterizes any inflammatory process?

**Fibrosis and regeneration through evolution: a new hypothesis**

Phlogosis is essentially a protective process aimed at eliminating the source of the damage and its consequences. The repair can occur by the regeneration of damaged tissue with cells of the same type or by the formation of a scar through replacement of parenchymal cells with connective tissue (fibrosis).

Hence, fibrosis enables the repair of damaged parenchyma, resulting, however, in a loss of organ function [51].

In superior organisms, and also in humans, we can consider fibrosis as an alternative to the regeneration that is observed in simpler organisms following an insult [52] and that in the human species this appears to be limited to few examples, including angiogenesis [53, 54].

Hydra, one of the simplest diploblasts, is able to regenerate its head after amputation because it has many undifferentiated cells that are constantly in mitosis [55]. Planaria, triploblasts, have the ability to regenerate missing parts of their body due to the presence of a population of undifferentiated cells (neoblasts) that actively proliferate [56]. Salamanders, more complex triploblasts, do not have undifferentiated cells in normal conditions: after a limb amputation, the stump cells undergo a process of dedifferentiation and proliferate, making a ‘regeneration blastema’, which forms the missing limb again with the same characteristics and the same size as the amputated one, as if there was a ‘memory’ that is stored and then used in the process of regeneration [57].

Through these examples, from simpler to more complex organisms, we can see how the regenerative process undergoes restrictions, being subject to more and more ‘control points’ that limit its potential. Why does this happen? Why has a seemingly beneficial process such as regeneration been negatively selected during evolution, to the point that it has almost disappeared in humans? In response to this question, several hypotheses have been proposed:

1. it is possible that a ‘decoupling’ has occurred between the molecular cascades involved in creation (embryogenesis) and those involved in the repair and maintenance of the adult structure (regeneration). Probable factors behind this alleged decoupling could be the size of the adult structure to be regenerated and/or longevity of a given species. In this case, it would not have been a negative selection on the restriction of the regenerative capacity of more complex organisms [58].

2. the loss of regenerative capacity with its replacement by healing in humans has its utility. This hypothesis has been put forward by Stéphane Roy in studies on the axolotl, an amphibian of the urodeles order which lives
in the lakes of Mexico, and is able to regenerate all
body tissues, including the eyes and central nervous
system [59, 60]. Roy noted that, if we cut an axolotl’s
leg, it can continue to swim and eat normally throughout
the regeneration period, which may require more
than a month. For terrestrial animals, however, it is not
favourable to stay with an open wound, because of the
possible risk of infection; scarring therefore, which is
faster than regeneration, can ensure their survival.

(3) the ability to self-repair is vital for organisms, such as
sessile hydra, starfish and plants, because they have
little means of avoiding being injured; on the contrary,
insects and vertebrates can escape from aggressors and
disguise themselves: so the lack of regenerative ca-
pacity is not a disadvantage. Thus, many regenerative
mechanisms have been progressively lost in more ad-
vanced animals, vertebrates and invertebrates [61].

(4) the negative selection of regeneration during evolution
may be related to the fact that maintaining or producing
pluripotent cells required to form a ‘blastema’ is
presumably incompatible with the long survival of a
species. In fact, uncontrolled production and prolifera-
tion of a pool of pluripotent cells can result in tumour
formation and possibly in the death of the organism [58].

The last assumption is certainly the most interesting and
significant from a biological point of view. In fact, the
active proliferation of undifferentiated cells can easily go
beyond the favourable aim of the regeneration of a dam-
gaged organ and result in a neoplastic process, to the detri-
ment of the individual and the survival of the human
species. In this perspective, fibrosis would play a protective
role, putting a brake on proliferative mechanisms which,
involving undifferentiated cells, could represent a greater
danger for the individual than the loss of function of an
organ affected by a chronic inflammatory process. If this is
true, then it is possible to assume the existence of molecular
mediators that, as a switch, would head towards one way or
the other.

Hence, fibrosis may represent an alternative way to can-
cer. A demonstration of this is the recent observation of a
possible increase in cancer risk in hypertensive patients
treated with angiotensin II AT1 receptor antagonists. These
drugs, known to be effective in controlling blood pressure,
also showed the ability to slow the progression of renal
fibrosis [62, 63]. Is it perhaps because of this anti-fibrotic
action that they are associated with an increased incidence
of cancer?

It can be argued that fibrosis and cancer represent two
alternate routes from one another, starting with evidence in
the literature concerning caveolin-1, a 22-kDa membrane
protein expressed by mesenchymal cells. It acts as an in-
hibitor of tissue fibrosis [64]; it is also commonly up-
regulated in malignant tumours [65, 66] and its expression
correlates in a statistically significant manner with drug
resistance and poor prognosis in patients affected by ad-
vanced non-small cell lung cancer patients treated with
gemcitabine [67].

Lastly, Oviedo et al. observed that regeneration, in an
apparently contradictory way, can probably both contribute
to the determination of abnormal cellular proliferation
and provide a means to prevent and correct cellular
growth abnormalities. With regard to the first hypothesis,
development of malignant tumours derives from a reduced
or incomplete regenerative process; in mammals, in the
epithelia exposed to chronic damage or to hypoxia and
inflammation conditions, tissue repair can result in growth
aberrations during the regenerative response. Conversely,
the regenerative process can keep the malignant cells’
autonomous growth under control: in fact, if the cellular
proliferation induced during regeneration is followed by
morphogenetic processes, regeneration has the ability to
prevent an abnormal growth and, more surprisingly, to
reverse the malignant process (this phenomenon is espe-
cially observed in species with very high regenerative
potential) [68].

**Conclusion**

The links existing between fibrosis, regeneration and carci-
nogenesis are complex and sometimes contradictory. The
most plausible and most biologically significant hypothesis
is that in superior organisms, regeneration may have been

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<td>Blockage of renin–angiotensin–aldosterone system</td>
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<td>Inhibition of pro-fibrotic cytokines</td>
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<td>Administration of recombinant HGF</td>
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selected negatively and replaced by fibrosis in the course of evolution, to warrant species survival: in fact, unchecked pluripotent cell production and proliferation can lead to tumour development and the potential death of a single individual.

Tumours could represent the outcome of the failure of fibrotic processes, most likely due to some mediators predominating over others. So, it is necessary to develop valid experimental models that allow the study of molecular factors involved in these phenomena; in this way, it is possible to understand the complex interactions that exist between fibrosis and tumours and to evaluate the real advantage of therapies, in many cases still in an experimental phase, that aim to inhibit the fibrotic process at the renal or other organs level. In fact, the ideal approach would be to limit fibrosis and then organ function loss but without exposing the patient to risks of developing a tumour, starting from as early as the drugs prescribed (Table 1).

Conflict of interest statement. None declared.

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