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World J Gastroenterol 2007 May 21; 13(19): 2675-2683
World Journal of Gastroenterology ISSN 1007-9327

TOPIC HIGHLIGHT

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Maintenance of radiation-induced intestinal fibrosis: Cellular and molecular features

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Received: 2006-12-28 Accepted: 2007-02-25

Abstract

Recent advances in cell and molecular radiobiology clearly showed that tissue response to radiation injury cannot be restricted to a simple cell-killing process, but depends upon continuous and integrated pathogenic processes, involving cell differentiation and crosstalk between the various cellular components of the tissue within the extracellular matrix. Thus, the prior concept of primary cell target in which a single-cell type (whatever it's epithelial or endothelial cells) dictates the whole tissue response to radiation injury has to be replaced by the occurrence of coordinated multicellular response that may either lead to tissue recovery or to sequel development. In this context, the present review will focus on the maintenance of the radiation-induced wound healing and fibrogenic signals triggered by and through the microenvironment toward the mesenchymal cell compartment, and will highlight how sequential and sustained modifications in cell phenotypes will in cascade modify cell-to-cell interactions and tissue composition.

Key words: Radiotherapy; Fibrosis; Fibrogenic differentiation; TGF-β1; CCN2; Smad; Rho; ROCK; Actin cytoskeleton

Haydont V, Vozenin-Brotons MC. Maintenance of radiation-induced intestinal fibrosis: Cellular and molecular features. *World J Gastroenterol* 2007; 13(19): 2675-2683

http://www.wjgnet.com/1007-9327/13/2675.asp

INTRODUCTION

Treatment of tumor by radiation therapy faces a crucial dilemma that is delivering sufficient radiation rate for tumor cure, while limiting, as far as possible, normal tissue exposure and injury. Despite the recent sophisticated irradiation modalities development, as 3D-conformal or intensity-modulated radiation therapy, increased radiation ballistic performance in pelvic and abdominal cancer treatment[1], the intestine remains a major dose-limiting organ. Indeed, chronic gastro-intestinal side effects (diarrhea, fecal urgency, proctitis, bleeding, fistula, *etc.*) affect the daily quality of life of 6% to 78% patients[2]. Moreover, 5% to 10% of patients will develop severe intestinal toxicity mainly characterized by intestinal narrowing and transmural fibrosis leading to obstruction[3]. Excessive deposition of collagens and extracellular matrix components in the *submucosa* induces the loss of compliance of the *mucosa* over the *muscularis propria* required for aboral propulsion. In addition, thickening of the intestinal wall contributes to stricture formation. Globally, this loss of compliance and the stricture formation lead to intestinal obstruction[4]. Although antioxidant-based anti-fibrotic treatments have been proposed to patients, including the combination of pentoxifylline and tocopherol[5,6], their efficacy in delayed radiation-induced intestinal toxicity is disputed[7] and surgical resection remains today the only therapeutic option for patients with delayed radiation enteropathy. These inconsistent clinical reports add confusion to the old, yet unresolved controversy about the reversibility of radiation fibrosis[8]. Thus, one challenge for translational research in radiopathology/radiotherapy is to characterize the specific molecular mechanisms and cellular contributions involved in the maintenance of fibrosis to define efficient curative strategies. We will see in the present review that contrary to the conventional wisdom, severe fibrotic lesions observed in human radiation enteropathy are highly dynamic[9,10], thus opening real perspective for therapeutic interventions. These curative strategies are particularly relevant in oncology as they won't interfere with anti-cancer treatments and would be applicable to treat established radiation injury in case of radiation accidents or acts of terrorism[11].

RADIATION-INDUCED INTESTINAL FIBROSIS: THE PATHOLOGIST DEFINITION

The main pathological feature of delayed radiation toxicity is the transmural fibrosis consisting of severe deposition of extracellular matrix component within the *mucosa*, *submucosa*, *muscularis propria* and *subserosa* (Figure 1). The number of crypts is reduced and a collagenous infiltration in the *lamina propria* is observed. Around the microvessels, accumulation of inflammatory cells suggesting an increased vascular permeability likely caused by endothelial cell damages. The *muscularis mucosa* is thickened with zones of complete disruption with infiltration of muscular-like structures within the *submucosa*. Submucosal layers always exhibit an altered but heterogeneous morphology. Some zones are composed of dense cords of collagen fibers with few fibroblasts whereas others are edematous or contained fibrosis-related fibroblasts and inflammatory cells located around hyalinized vessels with interlaced fibers. The *muscularis propria* is thickened and dystrophic with infiltration of connective septa. The Auerbach plexus, located between the circular and longitudinal muscular layers, are mostly hypertrophied. The *subserosa* also revealed a severe heterogeneous fibrosis containing newly formed microvessels, myofibroblasts, inflammatory cells, and paucicellular zones composed of stromal accumulation.

More than 30 years ago the World Health Organization proposed to define fibrosis "as the presence of excess collagen due to new fiber formation"[12]. The same is true for radiation fibrosis that was classically considered as a chronic and progressive process in which normal tissue is replaced by fixed and irreversible fibrotic tissue. This view has however been challenged and fibrosis has been recently redefined as a dynamic process resembling chronic wound healing[9,10,13].

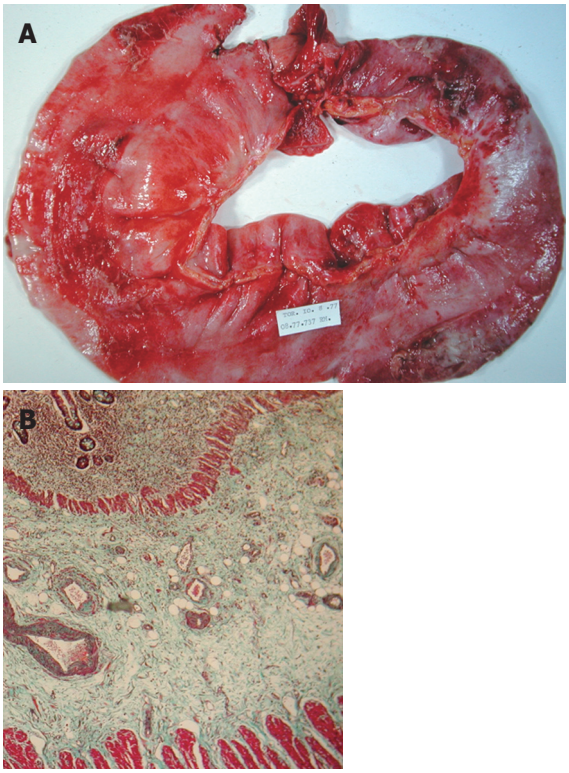

Figure 1 **A**: Resection of human intestine with radiation-induced fibrosis; **B**: Bright field photomicrograph showing transmural collagen accumulation as green stain in human radiation enteropathy after Masson's trichrome staining.

THE INITIATION OF THE FIBROGENIC PROCESS: WHO IS GUILTY? OR THROUGH A MULTICOMPONENT AND INTEGRATED VISION OF THE PATHOGENESIS

The pathophysiological mechanisms of acute intestinal lesions after irradiation have been well investigated but the mechanisms underlying delayed radiation-induced intestinal complications and the precise sequence of cellular and molecular events that initiates fibrogenesis are still discussed. Classical radiobiological views presents radiation-induced tissue injury as the direct consequence of DNA damages and cell death induction in target cells, meaning that the severity of tissue damages would be directly related to cell depletion during the acute phase.

In murine models of acute gastrointestinal syndromes, the injury has been mainly attributed to apoptosis and depletion of both microvascular endothelial cells[14] and epithelial stem cells[15]. The primary role of the vascular compartment in triggering radiation-induced normal tissue damages was introduced more than 40 years ago by Rubin and Casarett[16]. Endothelial cell dysfunctions precede other cell-type response as well as fibrin deposition. In addition, peri-vascular edema always precedes collagen accumulation[17]. This vascular hypothesis represents a matter of debate because the endothelial compartment was seen as a single entity. The detractors of the vascular hypothesis argued that if endothelial cells were at the initiation of late damages the relationship between radiation dose and tissue lesion should be similar in all organs[18]. Today, the progress made in cell biology clearly demonstrate that endothelial cell phenotype depends upon the vessel type (artery, veins, micro-vessels) and the tissue[19]. These observations suggest that the biological effects of irradiation on endothelial cells might be tissue specific. Although specific responses of endothelial cells isolated from various tissues to ionizing radiation remains to be studied, their role in normal tissue response to radiation-injury is today undisputable.

In rodents, acute mucosal damage is required for the development of delayed intestinal complications[20,21]. These observations suggest that acute mucosal lesions contributed to late toxicity[22] and lead to the idea that increasing the pool of epithelial cells before irradiation would improve acute damages and inhibit the development of late injury[22]. This hypothesis has been fully validated in experimental models using mucosal trophic growth factors like KGF[23,24] and GLP-2[25]. Yet, the use of trophic factors faced a crucial problem in cancer patient related to their stimulatory action on tumor growth[26,27]. The functional consequences of radiation-induced epithelial depletion are probably far beyond the barrier function. One indirect consequence of the epithelial rupture is the exposure of the intestinal stroma to luminal flora, involved

in specific lymphocyte T helper (TH) polarization. The role of TH orientation and the local production of specific cytokines associated with this polarization have been well investigated. On the one hand TH1 orientation, notably characterized by the secretion of interferon γ, is associated with resolution of the wound healing process. On the other hand TH2 orientation, characterized by the secretion of IL-4, IL-13 and TGF-β1, triggers tissue response toward fibrosis probably mediated by the pro-fibrotic growth factor: TGF-β1[28-30]. Exposure of intestinal stroma to bacteria is known to induce a TH1 polarization[29], but in the lung persistent exposure to bacterial antigens reorients TH1 polarization toward a TH2 profile suggesting that chronic epithelial depletion is fibrosis-prone[30]. In addition, seven days after γ-irradiation (10 Gy) a TH2 orientation has been described in rats[31], suggesting that a fibrosis-prone polarization occurs that remains to be fully characterized. The importance of the immune compartment to intestinal response to ionizing radiation.

The extrapolation from observations obtained in rodents to patients probably has to be moderated. Indeed, whether the consequential component occurs in radiotherapy patients is more controversial. Whereas, fractionation protocols significantly minimizes mucosal damages[32], late toxicity does occur[33]. In addition, several clinical reports showed the absence of correlation between the severity of early lesions and the probability of late effect development[34-37]. These data suggest that delayed tissue response to radiation injury depends upon continuous and integrated pathogenic processes, involving cell differentiation and crosstalk between the various cellular components of the tissue, within the extracellular matrix[38]. In this context, the role of the mesenchymal compartment (*i.e.* in the gut, smooth muscle cells, *submucosa* fibroblasts, subepithelial myofibroblasts and the extracellular matrix) is probably the most indubitable for the development and the maintenance of fibrosis as these cells are responsible of the pathological extracellular matrix accumulation observed in fibrosis.

WHAT DIFFERENTIATION STATUS FOR THE MESENCHYMAL CELLS IN RADIATION-INDUCED INTESTINAL FIBROSIS?

Radiation-induced intestinal fibrosis is characterized by the accumulation of extracellular matrix due to a global deregulation of the synthesis/degradation balance[9,10]. Whether this dynamic remodeling process is a cause or a consequence of the phenotypic alteration of the resident mesenchymal cells is not known[33]. However, this pathological differentiation contributes to the intestinal loss of function and obstruction. After injury, tissue regeneration relies on the differentiation capacity of its resident cells. Thus, understanding the mechanisms involved in the differentiation of intestinal mesenchymal cells would provide new insight to design new therapeutic strategies.

Normal wound healing and fibrosis: the skin as "model system"

After injury, the loss of dermal homeostasis induces mechanical tension in the clot. This contractile stress added to the secretion of cytokines, such as PDGF, triggers fibroblast recruitment and their morphological change[39] into proto-myofibroblasts[40]. Then, the mechanical tension associated with ED-A fibronectin and TGF-β1 deposition induce the differentiation of proto-myofibroblasts into myofibroblasts. The latters are mainly characterized by altered cytoskeleton with prominent stress fibers (composed of α-Sm actin, myosin, tropomyosin, α-actinin and filamin), anchored at the cytoplasmic membrane by molecular complex named the focal adhesion point *in-vitro* and the fibronexus *in-vivo*[41]. These focal adhesion points connect the actin cytoskeleton to the extracellular matrix *via* integrin receptors and control the mechanical exchanges between the myofibroblats and the extracellular matrix. Subsequently, contraction of the granulation tissue occurs and leads to wound healing closure[42,43]. In addition, myofibroblasts are connected directly to each other through gap junctions, composed of several hemichannels containing distinct but functionally related proteins called connexins[44]. Thus, myofibroblasts might form a syncytial structure composed of multicellular contractile units. In summary, the myofibroblastic differentiation is an intermediate differentiation between fibroblast and smooth muscle cells and ensured the contraction of the granulation tissue and the neo-synthesis of the extracellular matrix.

What is known about the mesenchymal cell differentiation in the intestine?

In the intestine, the mesenchymal compartment is composed of 3 cell types: the sub-epithelial myofibroblasts, the submucosal fibroblasts and the smooth muscle cells of *muscularis mucosa* and *muscularis propria*. The respective contribution of these 3 cell types to fibrosis is not clearly defined, but the pathological collagen deposition seemed mainly achieved by smooth muscle cells[45], and their differentiation profile seemed comparable at the molecular level[46].

After injury, the differentiation of smooth muscle cells is characterized by a phenotypic switch from a contractile function to a secretory activity[47]. This switch is associated with cytoskeleton modifications defined using the VDA classification proposed by Gabbiani : Vimentin-Desmin-α-sm Actin[48]. In human intestinal radiation-induced fibrosis, an overall increase in the relative number of cells defined as fibroblasts/myofibroblasts were detected in the *mucosa* ($V^+/D^-/A^+$) and *submucosa* ($V^+/D^-/A^{-/+}$), differentiated smooth muscle cells are also found in the hyalinized vessel wall ($V^+/D^-/A^+$) and in the dystrophic *muscularis propria* ($V/D^+/A^+$)[33]. The strong plasticity of smooth muscle cells allowed profound alterations in their phenotype in response to changes in local environment[47,49] and in return these differentiated smooth muscle cells controlled tissue response.

Terminal differentiation versus immature phenotype

For a long time, the conventional wisdom presented mesenchymal cell differentiation in radiation-induced fibrosis as terminal and thus irreversible[50,51]. This hypothesis was based on phenotypical characterizations performed on fibroblasts irradiated *in vitro*, which exhibit a premature senescent and pro-secretory phenotype (extracellular matrix secretion). Thus, necrosis or apoptosis

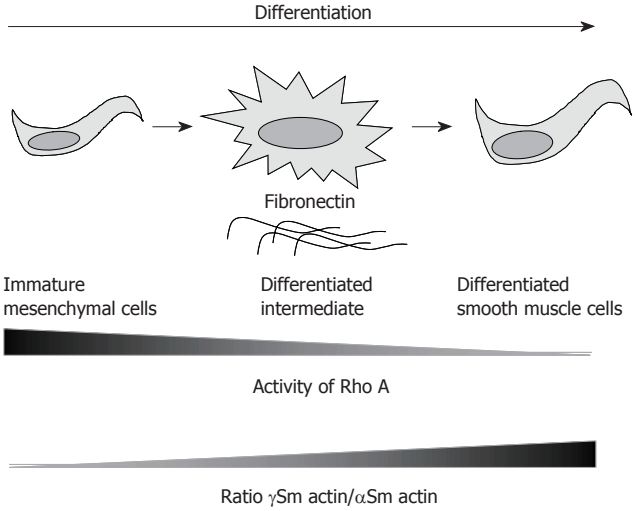

Figure 2 Differentiation of the smooth muscle cells during myogenesis is correlated with increased γSm actin/αSm actin ratio in the intestine[54] and with decreased Rho activity in the lung. In addition in the lung Beqaj *et al*[55] isolated a cell exhibiting an intermediate differentiation status after spreading of precursors cells onto fibronectin coating.

of these fibrosis-activated mesenchymal cells were the unique solution conceivable to cure fibrosis[13,52].

Further investigation on the characterization of the fibrogenic differentiation of intestinal smooth muscle cells isolated from radiation enteropathy suggested another hypothesis, in which fibrosis-derived intestinal smooth muscle cells (RE-SMC) seemed more immature than their normal counterpart (N-SMC). The first evidence was given by cytoskeleton analysis since an alteration of the γ-Sm actin/α-Sm actin ratio was found. Indeed, RE-SMCs exhibit higher expression level of the α-Sm actin than their normal counterparts whereas the level γ-Sm actin remained stable[53]. Because this ratio is an indicator of intestinal smooth muscle cell differentiation[54] *i.e.* increased ratio indicates a differentiated phenotype whereas decreased ratio reveals immaturity, the profile found in RE-SMC suggests the maintenance of an immature phenotype (Figure 2). This immaturity is further supported by the comparison study by Beqaj *et al*[55], who demonstrated an inverse correlation between smooth muscle cell differentiation during bronchial myogenesis and Rho activity: *i.e.* Rho activity decreased when cells became mature. In RE-SMC, a global profiling approach performed by cDNA array revealed a deregulation of the genes coding for Rho pathway as compared to N-SMC[53]. Furthermore the Rho pathway is preferentially activated upon TGF-β1 stimulation in RE-SMC[56]. The last evidence is related to the extracellular matrix composition and in particular to the secretion of fibronectin. Fibronectin is known to control the differentiation of smooth muscle cells in the lung. Indeed, Bequaj *et al*[55] have been able to isolate a specific cell-type that spreads on fibronectin and exhibits an intermediate differentiation status between mesenchyme precursors and bronchial smooth muscle cells. These intermediate cells are larger and more spread than the differentiated lung smooth muscle cells (Figure 2). Similarly RE-SMC are more spread and larger than their normal counterparts (Figure 3A) and secrete high level

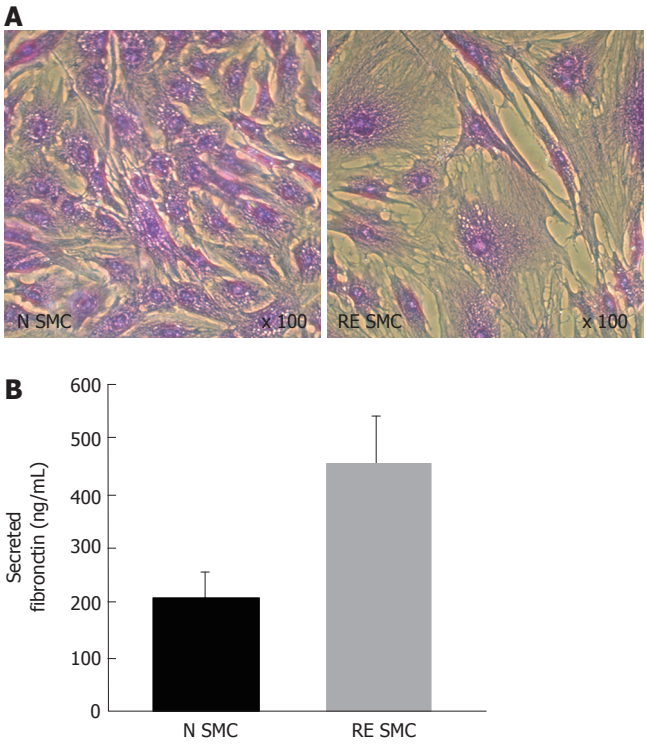

Figure 3 **A**: Bright field photomicrograph of fibrosis-derived intestinal smooth muscle cells (RE SMC) and normal cells (N SMC) observed after crystal violet staining; **B**: Fibronectin secretion level in RE SMC and N SMC assessed by ELISA (Chemicon).

of fibronectin (Figure 3B). This immaturity concept has important clinical implications as it suggests that fibrotic tissue has high regenerative potential and imply that fibrosis might be reversible.

MEDIATORS TRIGGERING THE MAINTENANCE OF MESENCHYMAL DIFFERENTIATION IN FIBROSIS

If defining the differentiation status of fibrosis-related cells is essential to investigate the regenerative potential of irradiated tissue, another important issue is to characterize the mediators involved in the maintenance of this pathological phenotype. Numerous actors are involved, yet we choose to focus on important mediators involved in the criss-cross relationship between mesenchymal cells and their micro-environment leading to the establishment of sequential and chronic activation loops.

TGF-β1 is a pleïotropic cytokine involved in the regulation of various biological processes including maturation of the immune cells, proliferation, differentiation, apoptosis as well as normal and pathological wound healing response[57]. In the context of the maintenance of fibrosis, one relevant function of TGF-β1 is related to its self-induction ability[58-62]. This auto-induction, mainly triggered by the transcription factor AP-1, probably constitutes one of the first fibrogenic activation loops contributing to fibrosis maintenance by persistent extracellular matrix production and continuous induction of the fibrogenic differentiation of mesenchymal

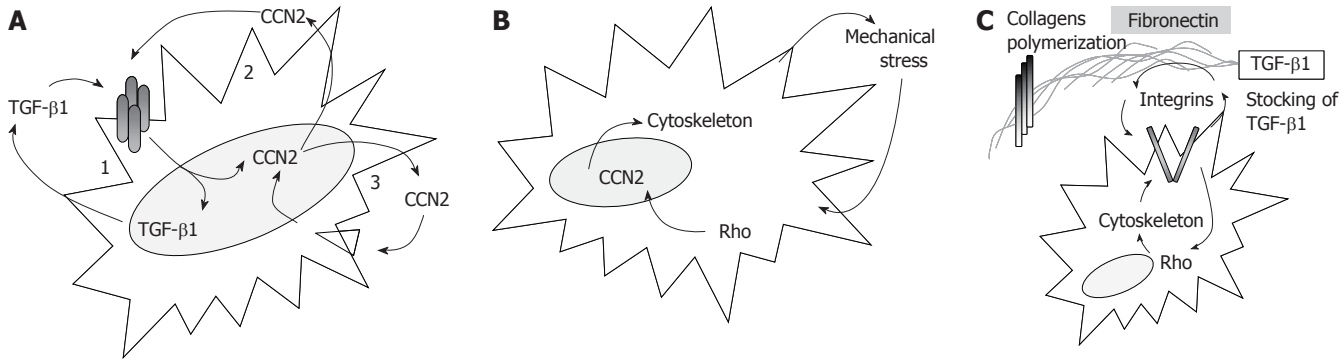

Figure 4 Chronic molecular activation loops, established between mesenchymal cells and its microenvironment, involved in the maintenance of fibrosis. **A**: 1-TGF-β1 auto-induction; 3-CCN2 auto-induction; 2-cooperation between CCN2 and TGF-β1; **B**: Mechanical stress induces Rho pathway activation[73] and CCN2 expression[72]. Rho activation induced modulation of cytoskeleton polymerization and, as a consequence, generated new mechanical stress in the environment; **C**: Fibronectin is involved in the extra-cellular matrix sequestration of TGF-β1[76], in the polymerization of collagen fibers[77,78] and in the activation of the Rho pathway[79]. The activation of the Rho pathway through integrins, modulated the polymerization of cytoskeleton. This structural cell change produced integrin's movement at the cell surface and thus modulated the polymerization of fibronectin.

cells (Figure 4A).

CCN2 (also called CTGF) is another relevant growth factor involved in the maintenance of the fibrogenic differentiation and in the control of the extracellular matrix remodeling. In normal tissue, CCN2 is absent or expressed at extremely low concentration, whereas it is highly and specifically expressed in established fibrotic tissue[63] including Crohn disease[64] and intestinal radiation-induced fibrosis[33]. As TGF-β1, CCN2 exhibits auto-induction properties constituting another chronic activation loop particularly relevant for the maintenance of fibrosis as it seems restricted to fibrosis-derived cells[65] (Figure 4A). The cooperation between CCN2 and TGF-β1 constitutes an additional chronic activation loop: TGF-β1 is one of the primary inductor of CCN2[66,67] which in return enhances TGF-β1 binding to the TGF-β-type Ⅱ receptor and increases Smad pathway activation[68] (Figure 4A). Yet, the mechanisms involved in the sustained and constitutive expression of CCN2 found in long-term established fibrosis is rather obscure. Thereby, in delayed radiation enteropathy[33] and scleroderma[69,70], a paradoxal situation occurs as *in-situ* TGF-β1 deposition is low, whereas CCN2 is highly expressed and correlates with the severity of the pathology. To explain this paradox, Grotendorst *et al*[71] proposed that transient TGF-β1 induction might trigger long-term mesenchymal cell differentiation and CCN2 expression, that perpetuates in time without TGF-β1, but additional cooperative signals between the cells and their microenvironment might be involved.

First, the mechanical stress produced by wound contraction triggered the chronic production of CCN2 by direct transactivation of the CCN2 gene expression[72] *via* the stretch-responsive element located in its promoter. This mechanical stress-induced CCN2 activation occurs through Rho/Rho kinase (ROCK) pathway activation and stress fibers polymerization[73] thus generating novel mechanical stress and subsequent CCN2 activation (Figure 4B). Second, CCN2 triggers fibronectin over-secretion by fibrosis-derived cells[74]. In return, fibronectin has a crucial role in the maintenance of fibrosis, acting in combination with CCN2 to sustain CCN2 own expression[75], controlling the extracellular matrix sequestration of TGF-β1 through its latent complex LTBP-1/TGF-β1[76] and playing an essential structural role in collagen network formation (*i.e.* polymerization of the fibrillar collagens type I and Ⅲ).[77,78] Beyond these direct fibrogenic actions, fibronectin binding to the α5β1 and αvβ3 integrins activates the Rho pathway[79], thus increasing the proliferation and differentiation of the smooth muscle cells[80] and regulating the cytoskeleton polymerization (Figure 4C). These structural actions of fibronectin rely on its integrity, that might be damaged by the reactive oxygen species produced upon irradiation (H_2O_2) and might thus triggered the fibrogenic differentiation of human lung fibroblasts[81,82].

Interestingly these various chronic activation loops involved in the sustained expression of CCN2, depend upon the Rho pathway. Furthermore, despite the high constitutive expression of CCN2 in fibrosis-derived cells[46,53,56], the canonical TGF-β/Smad3/4 pathway is only poorly activated after stimulation with TGF-β1 in dermal myofibroblasts[83] and intestinal smooth muscle cells isolated from radiation fibrosis[56]. Indeed the Smad3/4 pathway is activated in cells derived from normal tissue, whereas in the fibrosis-derived cells the TGF-β1 signal is mainly transduced by the Rho/ROCK pathway[56]. The Rho proteins are small GTPases (from "Ras homologous") acting as molecular switches to control a wide range of cellular functions like cell adhesion, formation of stress fibers, and cellular contractility through the reorganization of actin-based cytoskeletal structures. These functions are accomplished specifically *via* their effectors, the ROCKs[84-87], after Rho anchorage to the cell membrane by prenylation[88]. The anchorage allows cycling between the inactivated GDP-bound form to the activated GTP-bound, also controlled by specific activators/inhibitors: the GEFs (Guanosine nucleotide exchange factor), the GDI (Guanine dissociation inhibitors) and the GAP (GTPase Activating Protein) (for review[84,89,90]). Thus, Rho activity might be controlled by inhibitors of HMG-CoA reductase including the statins, that might provide safe and efficient tools for the development of anti-fibrotic strategies.

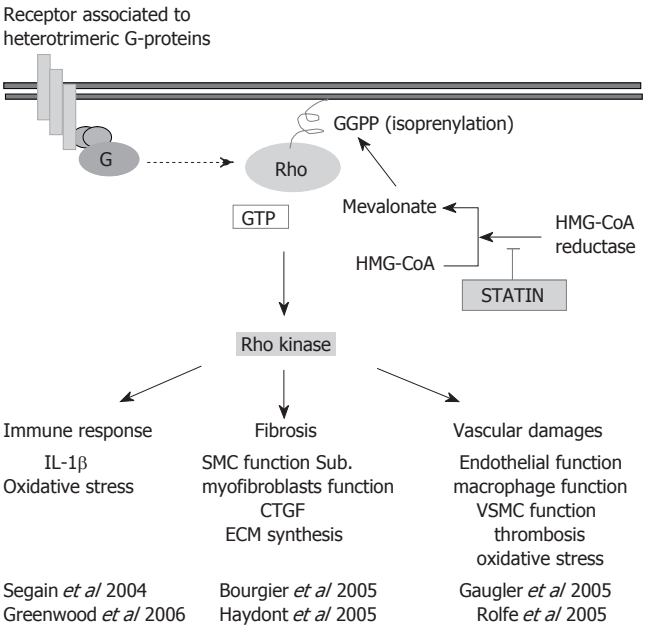

Figure 5 Targeting the Rho/ROCK pathway for normal tissue protection. Molecular binding on receptor associated to heterotrimeric G-proteins activated Rho protein by a shift between Rho-guanosin diphosphate (Rho-GDP) to Rho-guanosin triphosphate (Rho-GTP). This activation of Rho induced in cascade Rho kinase activation. As a consequence immune response, fibrosis and vascular damage were modulated. Rho protein anchorage to cytoplasmic membrane depends on the isoprenylation. Geranylgeranyl pyrophosphate (GGPP) required for this post-translational modification come from cholesterol metabolism, and may be inhibited by statins.

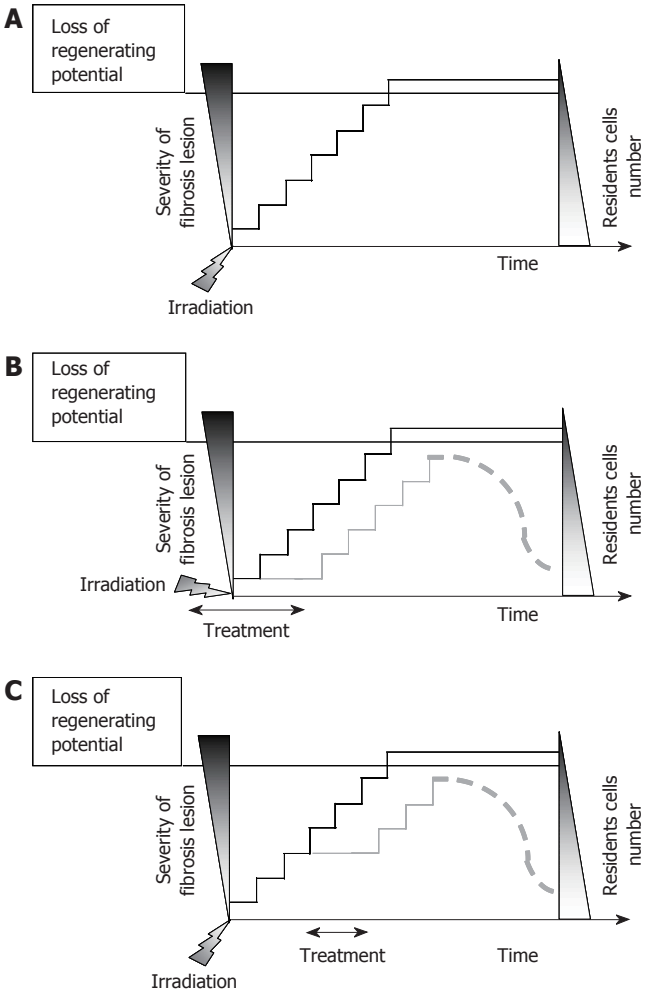

Figure 6 **A**: Fibrosis is a progressive pathology that involves sequential activation of chronic molecular loops. Gradation of fibrosis severity correlates with a decreased number of resident cells and decreased the potential of recovery; **B**: Impact of prophylactic treatment on the development of fibrosis: early inhibition of one or few molecular loops would help to preserve the regenerative potential of the tissue; **C**: Impact of curative treatment on fibrosis maintenance: inhibition of one or few chronic loop would help to preserve the regenerative potential of the tissue thus leading to tissue recovery.

THERAPEUTIC PERSPECTIVES

The development of curative anti-fibrotic strategy is nowadays highly expected by both patients and physicians[8]. Indeed, the high efficacy of the current anti-cancer treatments increases patient's overall survival, but also increases late complications occurrence especially in the gut[2]. The development of high-throughput biological approaches highlighted by the recent concept of cellular plasticity helps answering this difficult question by the identification of biologically-based therapeutic targets. Thus, targeting one central pathway involved in vascular, immune, and stromal pathogenic response would provide an efficient anti-fibrotic strategy and thus we propose that targeting the Rho/ROCK pathway may help to achieve this aim (Figure 5)[53,56,91,94].

The Rho pathway is known to control vascular functions[94] mediating endothelial barrier functions, inflammation and transendothelial leukocyte migration, platelet activation, thrombosis, and oxidative stress, as well as the homeostasis of vascular smooth muscle cells[93,95-97]. It also controls immune functions[92] as pharmacological inhibitors of Rho including the statins modulate the TH1/TH2 balance thus interfering with chronic inflammation[92,98]. Consistently, statins (lovastatin) displays an anti-fibrotic efficacy in a mice model of radiation-induced pulmonary fibrosis[99]. In addition, reversion of the fibrogenic phenotype of intestinal smooth muscle cells isolated from human radiation enteropathy was shown using Rho (pravastatin)[56] and ROCK (Y-27632) inhibitors[53]. These observations open new perspective for anti-fibrotic therapies by specific inhibition of the Rho/ROCK pathway.

CONCLUSION

In light of several investigations on the differentiation status of mesenchymal cells during fibrosis, it appeared that the secretory phenotype of pathological cells would be associated to a less maturation compared to such in normal cells. Thus, the biochemical maintenance of radiation fibrosis is a complex process that depends upon continuous and integrated activation loops involving cell differentiation, and crosstalk between the various cellular components of the tissue within the matrix[38]. However, the time and kinetic notion has never been evoked to explain the maintenance of fibrosis. Yet, fibrotic pathogenesis is progressive and results from successive and

sequential induction of chronic molecular activation loops. The evolution of the pathology during time correlates with a decrease in the cell number present within the tissue[5] and with a progressive loss of regenerating potential. In this context, when a new molecular loop is activated, the severity of fibrosis increases and correlates to decreased number in the resident cell number (Figure 6A). As a consequence, both preventive (Figure 6B) and curative (Figure 6C) therapeutic strategies might be efficient since inhibition of one or several steps would inhibit fibrosis evolution and preserve tissue-regenerating potential.

ACKNOWLEDGMENTS

VH is fellow of IRSN/region Ile de France. MCVB group is supported by "Association pour la Recherche sur le cancer" grant n°3881.

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