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Molecular aspects of chronic radiation enteritis

Abstract

Purpose: Chronic radiation enteritis (CRE) is one of the most feared complications of abdominal or pelvic radiation therapy and the treatment of CRE is difficult and often controversial. Recent progress in molecular biology has shed some light on the pathogenesis of CRE, which is characterized by fibrosis. The purpose of this article is to summarize the current state of knowledge of molecular aspects of radiation induced intestinal fibrosis and to discuss potential therapeutic targets.

Methods: A review of the up-to-date published literature involving the possible molecular cascades in radiation-induced intestinal fibrosis and prospective targets for CRE were performed using the Pub-Med search engine.

Results: Fibrosis development is correlated with transforming growth factor $\beta 1$ (TGF- $\beta 1$) and its downstream effector Smad3, which stimulates fibrogenic downstream mediators, such as connective tissue growth factor (CTGF). Ras homologue (Rho) and Rho-associated kinase (ROCK) signaling pathway have been shown to play important roles in the development of CRE. The inhibition of these pathways ameliorated radiation-induced intestinal fibrosis *in vitro* and in animal studies; however, the relationship between the Smad3 and Rho signaling pathways has not been elucidated.

Conclusions: Rho/ROCK and TGF- $\beta 1$ /Smad3 signaling pathways have been shown to play a key role in intestinal fibrogenesis, which might provide with effective possibilities for clinical intervention. Understanding the cooperation between Smad3 and Rho, may therefore be critical to our overall understanding of fibrosis development and maintenance of CRE.

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Chronic radiation enteritis (CRE) is an increasing clinical problem in patients receiving radiation directed at the abdomen or pelvis. The incidence of CRE reported in medical literature is as high as 20% [1-3]. Progressive damage to intestine and some predisposing factors (including a low body mass index, previous abdominal surgery and the presence of comorbid conditions, as well as the concomitant use of chemotherapy) can lead to various gastrointestinal symptoms, which severely affect quality of life and are frequently poorly managed [4-6]. Despite improved techniques in radiation therapy, the percentage of patients exhibiting complications has not declined, likely because radiation therapy is increasingly being used following surgery or in association with chemotherapy. CRE is primarily characterized by intestinal fibrosis, and can be the underlying cause of stricture, obstruction, fistula and perforations of the intestine. The underlying mechanisms involved in development of intestinal fibrosis are still unclear; consequently, the medical and surgical treatment of CRE have often been disappointing [7-9]. In this review, we summarize the pathology, possible molecular cascades and future perspective treatment of CRE, and discuss the potential therapeutic targets.

Pathology of CRE

Excess radiation can damage normal cells, especially the rapidly growing mucosal epithelial cells of the gastrointestinal tract. The development of bowel toxicity is not entirely dose, volume and fractionation schedule related – it also depends on a complex interaction among physical, patient-related and genetic factors, such as body mass and previous abdominal surgery. It is now recognized that radiation-induced changes in cellular function, as well as secondary alterations, notably inflammatory changes, contribute substantially to the pathophysiologic manifestations of intestinal radiation toxicity. Oxidative damage, caused by the formation of free radicals, results in obliterative endarteritis leading to intestinal ischemia that may contribute to radiation injury. Ischemia can lead to diffuse fibrosis in the lamina propria and submucosa of the intestine. Subsequently, fibrosis, in turn, aggravates vascular damage and further worsens local ischemia. This persistent malignant cycle induces intestinal dysfunction and leads to severe gastrointestinal complications [10,11].

The cumulative effects of these pathological changes might gradually result in vascular degeneration, mucosal ulceration, intestinal wall necrosis and serosal adhesion formation. The vasculitis and fibrosis progress over time, resulting in narrowing of the intestinal lumen with dilation of the bowel proximal to the stricture. The affected segments of intestine and serosa be-

come thickened. The main feature of intestinal fibrosis is the accumulation of extracellular matrix that induces the loss of intestinal compliance, impairs function, and leads to severe complications. Ulceration, obstruction, necrosis, fistula, and occasional perforation of the intestine may occur. In a word, obliterative endarteritis, submucosal fibrosis, lymphatic dilatation and tissue necrosis are common features of CRE, while fibrosis is the most salient [1,12].

Current research on molecular pathogenesis of CRE

During the past few years, much progress has been made in elucidating the cellular mechanisms underlying CRE. The role of intestinal mesenchymal cells (myofibroblasts, fibroblasts and smooth muscle cells) in intestinal fibrosis is increasingly recognized. In normal bowel, these cells are involved in the maintenance of intestinal contraction and extracellular matrix homeostasis. Once activated via radiation, their role is enhanced as they are responsible for excessive deposition of extracellular matrix components and collagen, gradually leading to intestinal dysfunction. Compared with normal human smooth muscle cells, cells derived from radiation enteropathy displayed a specific radiation-induced fibrogenic differentiation with an altered cytoskeleton structure and prosecretory phenotype. In addition, similar features were observed in fibroblasts and myofibroblasts, which might reflect that common pathways maintain radiation-induced fibrogenic differentiation in intestinal mesenchymal cells. Chronic cellular activation within the mesenchymal compartment is one of the key mechanisms involved in radiation induced fibrosis (RIF) [13,14].

Research on the molecular actors involved in the radiation-induced fibrogenic differentiation has mostly focused on one significant fibrogenic growth factor, transforming growth factor $\beta 1$ (TGF- $\beta 1$) [15]. TGF- $\beta 1$ is a multifunctional cytokine that regulates homeostasis in various cell types, including epithelial and endothelial cells, and regulates other cell functions, such as growth, differentiation and apoptosis. It exerts its biological effects through a variety of signaling pathways of which receptor-regulated Smad signaling is considered to be the main mediator. Briefly, TGF- $\beta 1$ initiates its cellular response by binding to its specific receptor, TGF- β receptor II (T β R II). After ligand binding, T β R II activates T β R I kinase, which phosphorylates RSmads (Smad2 and Smad3). Activated Smads assemble with Smad4 and translocate into the nucleus where they bind to promoters and regulate the expression of various target genes involved in fibrosis. Connective tissue growth factor (CTGF) is the main downstream mediator of TGF- $\beta 1$ -induced activation of fibroblasts, and it is highly expressed in sub-acute and delayed radiation injury of the ileum

and colon. One hallmark of radiation-induced fibrogenic phenotype of intestinal smooth muscle cells is the high constitutive levels of CTGF [16-18]. It is believed that fibrosis is correlated with TGF- β 1 gene polymorphism and activation of the TGF- β 1/Smad pathway, which stimulates fibrogenic downstream mediators, such as CTGF, and deposition of extracellular matrix. Understanding the cooperation between TGF- β 1 and CTGF, is critical to our overall understanding of fibrosis development and maintenance [19,20].

Haydont and colleagues reported that nuclear accumulation of Smads in human smooth muscle cells, isolated from radiation enteritis (RE-SMC) treated with TGF- β 1, was lower than in TGF- β 1-treated human smooth muscle cells isolated from normal (N-SMC). Smad DNA-binding activity was also weaker in RE-SMC than N-SMC, suggesting the involvement of another signaling pathway in CTGF regulation. They found that nuclear accumulation of Rho, as well as their DNA-binding activity, was higher in RE-SMC than in N-SMC, suggesting a strong involvement of the Rho pathway in sustained fibrogenic differentiation [21].

Ras homologue (Rho) family small G proteins control many aspects of cell proliferation, including cell-cycle progression and cytokinesis, by acting as molecular switches, cycling between active (GTP-bound) and inactive (GDP-bound) states. Active GTPases interact with high affinity with one of several downstream effectors to modulate their activity and localization, including the Rho-associated kinase (ROCK). The small GTPases Rho and ROCK play important roles in graft fibrosis; it is now generally considered to be a major factor in the progression of chronic renal diseases, but their specific mechanisms are still unknown. Researches have demonstrated that Rho and ROCK play a key role in intestinal fibrogenesis, including formation of CTGF and type I collagen in smooth muscle cells derived from the intestine. Although the precise mechanism has yet to be determined, several lines of evidence point to its effect on cytoskeletal organization as well as its ability to modulate CTGF gene expression that suggest that the Rho signaling pathway is involved in the fibrogenic differentiation of intestinal mesenchymal cells. Considered together, Rho and ROCK display potent fibrogenic activity, and targeting the Rho-kinase signaling network may be a useful treatment for intestinal RIF [22,23].

Recently, more attention has been paid to whether there is a crosstalk between these two signaling pathways. Vardouli and colleagues demonstrated that Rho signaling regulated TGF- β -induced rapid actin reorganization in Swiss 3T3 fibroblasts. In mouse Swiss3T3 fibroblasts and human hepatoma HepG2 cells, they reported that TGF- β regulated long-term actin re-

modeling by increasing the steady-state mRNA levels of the RhoB gene. Adenovirus-mediated gene transfer of Smad2 and Smad3 in Swiss 3T3 fibroblasts induced transcription of the endogenous RhoB gene but not the RhoA gene. In addition, Smad2 and Smad3 triggered activation of RhoA and RhoB GTPases and long-term actin reorganization in Swiss 3T3 fibroblasts. They concluded that there was novel crosstalk between the classical TGF- β /Smad pathway and Rho GTPases, which might be involved in the fibroblast-myofibroblast differentiation [24]. The relationship between the Smad and Rho pathways has not yet been elucidated (Figure 1).

Future perspective treatments

CRE continues to be a hazard. Palliative surgical or medical interventions are the options of choice for the majority of CRE patients, but the results are disappointing [25]. In a prospective multicenter study, Larsen and colleagues demonstrated that at least 10% of patients with CRE die as a direct result of CRE and that most surviving patients suffer from chronic debilitat-

FIGURE 1. Fibrogenic molecular cascades in radiation induced fibrosis. The development of RIF requires the activation of a cytokines cascade controlled by fibrogenic growth factors including TGF- β 1 and Rho. These pathways control CTGF expression and extracellular matrix synthesis, and lead to the development and maintenance of fibrosis. Solid arrows indicate events that have been experimentally proven in previous studies. Dashed arrows indicate hypothetical events.

ing symptoms that negatively influence their quality of life for decades [26]. Radiation fibrosis was considered permanent and irreversible; however, this study of the cellular and molecular mechanisms involved in the persistence of human radiation enteropathy showed that severe fibrotic lesions are highly dynamic, and that biochemical maintenance of radiation fibrosis is a complex process that depends on continuous and integrated activation loops that involve cell differentiation and cross-talk between the various cellular signaling pathways, providing new opportunities for the development of antifibrotic therapies [1,13,24].

Due to its central role in the development of fibrosis, TGF- β 1 remains an attractive therapeutic target focusing on intestinal RIF. Soluble T β RRII receptor, neutralizing antibodies, sequestering agents, and antisense therapy have shown to prevent RIF development in various experimental models. Nevertheless, in neoplastic disease, TGF- β acts as a tumor suppressor in early phases, but later it acts as a pro-metastatic agent: tumor cells could escape selectively from the growth inhibitory effects of TGF- β , which then promotes metastasis. Thus, the effects of TGF- β inhibitors with radiotherapy under tumor prone conditions are disputable [13,17].

The availability of small molecules, such as statins (Rho isoprenylation inhibition) and Y-27632 (allosteric inhibitor of ROCK), acting as inhibitors of Rho/ROCK/CTGF pathway, has recently made possible a more targeted anti-fibrotic approach to CRE. The pleiotropic actions of statins are mediated by inhibition of the production of isoprenoid residues and sub-

sequent modulation of posttranslational protein prenylation, including that of Rho [27]. Preclinical studies suggest that pravastatin inhibited the Rho/ROCK/CTGF cascade in human samples *ex vivo* and showed decreased intestinal radiation-induced fibrosis *in vivo* [28,29]. Pravastatin protects normal intestine from radiation damage without interfering with the anticancer action of irradiation in experimental models, both *in vitro* and *in vivo*. Other studies using simvastatin confirmed these results [30,31]. Although additional mechanisms might be involved, such as endothelial barrier function, inflammation, platelet activation, thrombosis and oxidative stress, a highly significant observation using statins as Rho/ROCK inhibitors suggest possibilities for clinical intervention.

Researches have also reported that other molecules, including cyclooxygenase-2 inhibitor (rofecoxib), all-trans-retinoic acid, plasminogen activator inhibitor type-1 and γ -Tocotrienol, ameliorated irradiation-induced intestinal injury and fibrosis in animal models (Table 1) [32-35].

Radiation is genotoxic and induces DNA damage in human cells yet few researches have tried to identify regulators that influence the expression levels of radiation-responsive genes. The genetic analysis of human gene expression might expand our understanding of gene regulation and have clinical implications. Despite the progress acquired with *in vitro* and animal models, the potential contribution of research based on human tissue samples has never been greater [36-39].

Additionally, hyperbaric oxygen, possibly through stimulation of angiogenesis, appeared to be effective therapy for CRE.

TABLE 1. Current research on the amelioration of radiation enteropathy

Author	Object	Intervention	Results
Haydont <i>et al.</i> 2007 [28]	Human explants and Smooth muscle cells Rats	Pravastatin	Inhibition of Rho/ROCK/CTGF activity Inhibition of type I collagen and fibronectin Decreased deposition of CTGF and extracellular matrix, improved radiation enteropathy
Haydont <i>et al.</i> 2007 [29]	Rats; HT-29, HeLa, and PC-3 cells used for antitumor action	Pravastatin	Improved delayed radiation enteropathy Without interfering with the primary antitumor action of radiotherapy
Wang <i>et al.</i> 2007 [31]	Rats	Simvastatin	Ameliorated the intestinal radiation response Not related to protein C activation
Keskek <i>et al.</i> 2006 [32]	Rats	Rofecoxib	Alleviated intestinal radiation injury in rats after the acute phase
Okoshi <i>et al.</i> 2007 [33]	Mice	All-trans- retinoic acid	Ameliorated irradiation-induced intestinal fibrosis Inhibition expression of IL-6 and TGF β 1
Milliat <i>et al.</i> 2008 [34]	Mice	PAI-1	Increased survival and better intestinal function observed in PAI-1-/- mice
Berbee <i>et al.</i> 2009 [35]	Mice	γ -Tocotrienol	Ameliorated intestinal radiation injury and vascular injury by an HMG-CoA reductase-dependent mechanism

Rho: ras homologue; ROCK: Rho-associated kinase; CTGF: connective tissue growth factor; IL-6: interleukin 6; TGF β 1: transforming growth factor β 1; PAI-1: plasminogen activator inhibitor type-1

In a retrospective study, Marshall found that hyperbaric oxygen resulted in healing or clinically significant improvement in two thirds of patients with CRE [40]. Hyperbaric oxygen is apparently safe and effective in managing radiation-induced late side-effects, such as soft tissue necrosis, proctitis and fistulas [41]. Further multicenter investigation is warranted to confirm the efficacy of this treatment.

Conclusion

Current treatment of patients with CRE is only supportive and, not surprisingly, is ineffective, because it is not based on a thorough understanding of the pathogenesis of the disease at the molecular level. The identification of key molecular targets involved in radiation-induced fibrosis could, in turn, provide effective therapeutic targets.

List of Abbreviations

CRE	chronic radiation enteritis
RIF	radiation induced fibrosis
TGF- β 1	transforming growth factor β 1
CTGF	connective tissue growth factor
Rho	ras homologue
ROCK	Rho-associated kinase
RE-SMC	smooth muscle cells isolated from radiation enteritis
N-SMC	smooth muscle cells isolated from normal

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