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Responses of Normal Cells to Ionizing Radiation

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Radiation-induced alterations in cellular tissue homeostasis triggered by various molecular responses at the level of inter- and intracellular signaling processes cause both acute and late effects in normal tissue after radiation therapy. Some of the underlying molecular and cellular response pathways leading to radiation-induced tissue remodeling will be discussed, with special emphasis on vascular and parenchymal tissues.
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Treatment of cancer patients with radiation can be significantly compromised by the development of severe acute and late damage to normal tissue, which occurs within days to weeks or months to years after exposure.[1,2] Normal tissue complications induced by ionizing radiation differ depending on the target organ and cell types. Acute or early reactions are primarily characterized by rapidly occurring changes within hours, such as increased endothelial cell swelling, vascular permeability, and edema as well as lymphocyte adhesion and infiltration. Apoptosis of endothelial cells is an important feature in the concert of radiation-induced acute alterations in the vascular system of irradiated organs. Thus, response of the vascular system is reflected by the rates of radiation-induced cell death and regeneration by surviving stem cells.

Late reactions occurring months to years after radiation exposure are primarily the result of radiation-dependent depletion of tissue-specific stem cells or progenitor cells leading to fibrosis, organ dysfunction, and necrosis. Although the early inciting molecular steps (i.e., induction of specific molecular pathways of inter- and intracellular signaling) resulting in the manifestation of late reactions certainly occur shortly after radiation exposure, the cellular events and tissue remodeling processes triggered by these stimulated mechanisms evolve slowly over time. This indicates that acute processes, such as radiation-induced cell death and apoptosis, are not the major or critical events underlying late tissue reactions. At least for the development of radiation-induced fibrosis in skin and lung, accelerated terminal differentiation of fibroblast progenitor cells seems to be a major driving cellular process. Yet, independent of whether acute or late reactions of normal tissues to radiation therapy occur, the molecular and cellular processes responsible for tissue remodeling are triggered by a number of inter- and intracellular signaling cascades regulating cell cycle progression as well as cell survival. This review will thus focus on the molecular and cellular processes regulating the signaling pathways primarily involved in acute and late reactions in vascular and parenchymal tissues.

Vascular Tissue

The radiation response of the vascular tissue actually occurs in 2 waves. The acute vascular changes within 24 hours are dominated by the radiation-induced apoptotic cell death of endothelial cells.[3] Late vascular effects occur within months after irradiation and include capillary collapse, thickening of basement membrane, scarring of the surrounding tissue as well as telangiectasias, and a loss of clonogenic capacity.[4] Radiation-induced late vascular damage may thus contribute to late radiation responses of various normal tissues.

Capillaries are the most radiosensitive component of the vasculature; thus, capillary vascular injury may be at the crux of tissue radiosensitivity.[5,6] For example, capillary endothelium responds to radiation by leukocyte attachment, endothelial cell swelling, and increased capillary permeability.[7] Characteristic changes in capillary histology after irradiation include detachment of endothelial cells from the basal lamina, cell pyknosis, thrombosis, and loss of entire capillary segments, resulting in tissue ischemia or regrowth of lost vessels in some organs.[8] In response to ionizing radiation, capillaries show considerably more morphological changes than larger vessels and a marked shift in the size distribution of the microvasculature to larger diameters, apparently from capillary obliteration with dilation of the remaining vessels.[9]

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Responses of Endothelium

The cellular and molecular responses of endothelial cells to ionizing radiation have been well characterized by in vitro studies.[10] Within hours of irradiation changes in specific endothelial cells, structural proteins occur, which affect the physiological appearance of these cells. Among these structural changes, alterations in the distribution of endothelial cell F-actin, cell retraction, and a dose-dependent increase in transendothelial flux of low–molecular-weight solutes are prominent.[11,12] Irradiated endothelial cells, furthermore, exhibit alterations in the synthesis and secretion of a variety of biomolecules, such as growth factors, chemoattractants, and specific injury markers, such as thrombomodulin, von Willebrand factor, heparinase, and leukocyte endothelial-cell adhesion molecule 1.[13,14] Endothelial cells that have survived radiation exposure display compensatory cytoplasmic hypertrophy,[15] leading to the formation of giant cells permanently arrested in G1-phase or G0-phase.

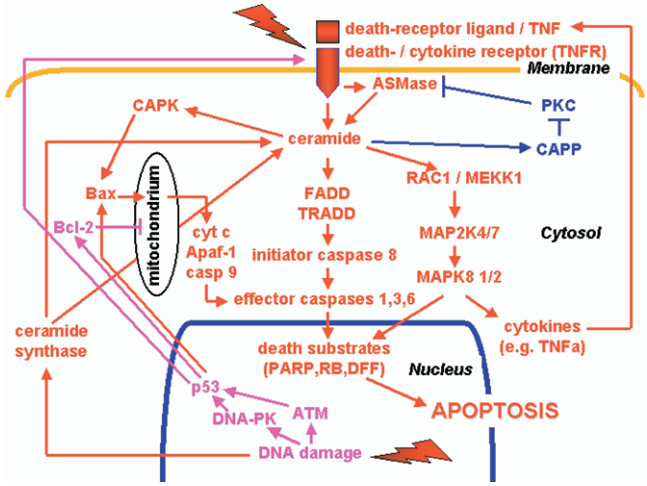

Figure 1 DNA damage-independent and -dependent pathways of endothelial cell apoptosis.

Pathways of Endothelial Cell Death

Because radiation-induced death of endothelial cells seems to be the major cause of the high radiation sensitivity of the vascular system, studies have focused on the specific pathways of these cell death processes. Special attention was drawn to the role of radiation-induced apoptotic processes, which are primarily DNA damage independent but membrane damage dependent. These processes are mediated through radiation-induced activation of acid sphingomyelinases (ASMases) and the generation of ceramide.[16] Yet, in most of these studies, rather high single doses of ionizing radiation were applied (between 10 and 20 Gy). Thus, to what extent these clearly defined processes contribute to radiation-induced death of endothelial cells under more clinically relevant doses cannot sufficiently be answered at present.

The sphingomyeline pathway is a ubiquitous, highly evolutionarily conserved signaling system, analogous to conventional systems such as the cyclic adenosine monophosphate and phosphoinositol pathways. Ceramide is generated from sphingomyeline by the action of a neutral or ASMase or by de novo synthesis coordinated through the enzyme ceramide synthase. Once generated, ceramide may serve as a second-messenger molecule in signaling responses to physiologic or environmental stimuli, or it may be converted to a variety of structural or effector molecules. For endothelial cells, radiation-induced elevation of intracellular levels of ceramide has been attributed to a direct or indirect activation of ASMase by membrane damage.[17] As outlined in Figure 1, ceramide released from membrane-bound sphingosine, either by activation of ASMase, binding of death receptor ligands or by radiation damage to the membrane, mediates the activation of 3 major cascades: the MAPK8 pathway, the mitochondrial pathway, and the death receptor pathway.

Ceramide specifically targets and activates the ceramide-activated protein kinase (CAPK) and the ceramide-activated phosphatase.[18,19] CAPK can directly activate RAC1, a component of the stress pathway, and can also stimulate RAF1, a component of the protein kinase C (PKC)/RAF1/MAPK cytoprotective pathway.[20] Ceramide-activated phosphatase, through its phosphatase function, can directly inactivate protein kinase C, an enzyme involved in antiapoptotic signaling.[21] For maintaining ceramide's key role in triggering apoptosis, inactivation of the antiapoptotic PKC seems essential because this kinase can block the hydrolysis of sphingomyelin and thus prevent the release of ceramide from cellular membranes.[22,23] With respect to apoptosis, however, the most important target of ceramide is the RAC1/MEKK1 pathway, which directly leads to activation of MAPK8. This protein kinase has been implicated in apoptosis induced by tumor necrosis factor (TNF), the cytokine activating the TNF-receptor and other environmental stresses.[24] Activation of the MAPK8 pathway results in apoptosis through the activation of effector caspases, namely caspases 1, 3, and 6 as well as the autocrine stimulation of the death receptor pathway.

Ceramide may also initiate proteins in the mitochondria that facilitate apoptosis through an alternative activation pathway of caspases, mainly via caspase 9. This pathway is initiated by ceramide-dependent stimulation of CAPK and promotes apoptosis through processes involving the pro-apoptotic proteins the bcl-2-associated protein X (BAX) and the bcl-2 antagonist of cell death protein (BAD).[25] BAD exerts its function by binding to the antiapoptotic proteins BCL2 and BCL2L1 and prevents inhibition of cell death mediated by BAX. The antiapoptotic action of BCL2 and BCL2L1 is based on their ability to maintain mitochondrial function (i.e., stabilizing mitochondrial membrane integrity and preventing release of cytochrome C into the cytoplasm).[26,27] Release of cytochrome C would result in the activation of the so-called apoptosome complex composed of APA1, cytochrome C, and desoxy-ATP, which activates caspase 9. This enzyme will then cleave and activate downstream effector caspases, such as caspase 3. Activated caspase 3 degrades a variety of cell death substrates, such as poly(adenosine-5′-diphosphate-ribose)

polymerase, the retinoblastoma protein, or the DNA fragmentation factors A or B and thereby initiates apoptosis.[28]

Ceramide released via the action of death or TNF receptor can trigger a direct apoptotic pathway through various adapter protein complexes, such as Fas-associated death domain and TNFαR-associated death domain).[29] These complexes initiate the activation of cytoplasmic promoters of apoptosis, notably the procaspase 8. This enzyme, in turn, activates caspases 1, 3, and 6. As outlined earlier, activated caspases 1, 3, and 6 initiate apoptosis through degradation of cell death substrates.

In 1994, it was first described by Haimovitz-Friedman and coworkers[22,23] that ionizing radiation acts on cellular membranes of endothelial cells to generate ceramide and initiate apoptosis. This study provided conclusive evidence that apoptotic signals can also generate DNA damage independently as a result of radiation-induced membrane damage. However, a second source of ceramide resulting from ceramide synthase activation may be directly related to DNA-damage induced by ionizing radiation because DNA double-strand breaks can also trigger the activation of ceramide synthase[30] and thus the production of ceramide, which will subsequently induce apoptotic cell death. Yet, this ceramide synthase pathway will require de novo protein synthesis; consequently, this proapoptotic mechanism displays a slower kinetics and may thus be responsible for more sustained effects as compared with those resulting from immediate release of ceramide after ASMase activation.

It is standard knowledge that radiation-induced DNA-damage can lead to apoptosis through p53-dependent processes. DNA damage, mainly DNA double-strand breaks, is able to activate the protein kinases ATM and DNA-PK, both located in the nucleus (Fig 1). ATM and DNA-PK will then phosphorylate p53 at specific serine residues, which results in its stabilization and activation. Once activated, p53 will exert different functions with respect to regulation of the cell-cycle progression and transactivation of genes involved in pro- and antiapoptotic cascades. The direct p53-dependent regulation of BAX and BCL2 proteins leads to the activation or blockage of the mitochondrial pathway, which will result in the activation of the apoptosome and induction of effector caspases, as addressed earlier, through the release of cytochrome C from mitochondria.[31-33] Moreover, evidence has accumulated over recent years that p53, when activated, is also able to upregulate the death receptor-ligand system and thereby stimulates apoptosis, as described earlier and outlined in Figure 1.[34,35]

Yet, ionizing radiation must not necessarily lead to apoptosis of endothelial cells. This may be dependent on the radiation dose applied. It seems clear that irradiation with high single doses (>5-10 Gy), as mostly used in these investigations, is a potent inducer of apoptosis via activation of ASMase and generation of ceramide. However, more recently, Tan and coworkers[36] reported that endothelial cell survival after radiation with a single dose of 3 Gy is dependent on the activation of protein kinase B/AKT (PKB/AKT) signaling. Within minutes of irradiation, phosphorylation of the serine/threonin protein kinase PKB/AKT at serine-residue 473 appeared. This activation of PKB/AKT contributes to inhibition of glycogen synthase kinase-3beta (GSK3beta), which has a clear inhibitory role in endothelial cell survival. As a consequence, in comparison with high-dose irradiation (single doses of 10 Gy or more), low-dose radiation (i.e., single dose of 3 Gy) can mediate AKT-dependent inhibition of GSK-3beta and thus promote endothelial cell survival and capillary formation. As reported by Toulany and coworkers,[37] radiation-induced PKB/AKT signaling contributes to cell survival after ionizing radiation by stimulation of DNA double-strand break repair via activation of DNA-PK, a major enzyme of the nonhomologous end-joining repair mechanism. Taken together, endothelial cell apoptosis both in vitro and in vivo depends very much on the dose of radiation applied and, moreover, on the balance between the activities of pro- and antiapoptotic signaling cascades.

PKC can block ASMase activity and thus prevent the release of ceramide from cellular membranes.[22,23] Because basic fibroblast growth factor is able to stimulate PKC activity, the protective effect of basic fibroblast growth factor in irradiated endothelial cells has been attributed to the PKC-dependent downregulation of ASMase activity and inhibition of ceramide-dependent apoptosis.[4,38-40] In addition, Zundel and Giaccia[41] have shown that stress-induced apoptosis is associated with ceramide-mediated downregulation of phosphatidyl-inositol-3′-kinase (PI3K) and subsequent inhibition of the kinase AKT. The inhibition of this pathway resulted in decreased phosphorylation of the BAD protein, which is essential for the mitochondrial pathway of apoptosis. Hence, radiation-induced apoptotic signaling through ceramide appears to involve multiple, coordinated pathways, and the apoptotic outcome, in vitro as well as in vivo, depends on the balance between the activities of pro- and antiapoptotic signaling systems.[4]

Parenchymal and Connective Tissues

The pathological progression of radiation toxicity in many normal tissues seems to be a consequence of an early inflammatory phase followed by late stromal alterations. In the lung, for example, radiation-induced pneumonitis is followed by fibrosis. Likewise, in the kidney, radiation nephritis is succeeded by renal sclerosis.[6] Because blood vessels in irradiated normal tissue often undergo adverse changes as discussed earlier, these changes often precede the development of radiation fibrosis. Especially in the lung, injury of capillary endothelial cells as well as type II pneumocytes, which have the highest mitotic rate and are the cells most susceptible to radiation, has long been thought to initiate alveolar edema, exudation, and vascular congestion.[42,43]

Cellular and Molecular Components of Fibrotic Tissue Response to Radiation

Like any other fibrotic tissue response, radiation-induced connective tissue remodeling is a multicellular process driven by intercellular communications via cytokines and growth

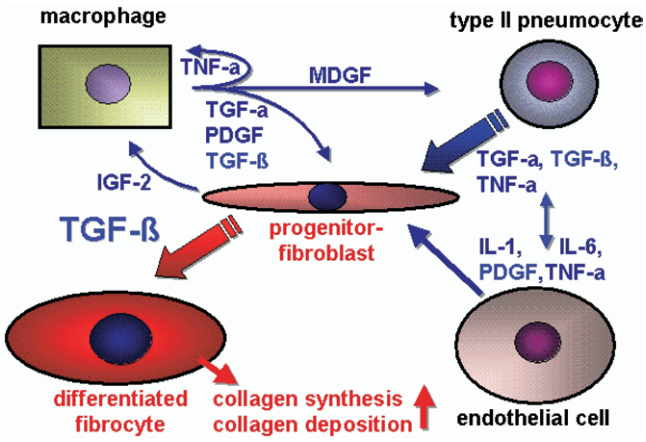

Figure 2 Cytokine-mediated multicellular interactions in connective tissue remodeling.

factors, which are induced during the radiation response of each participating cell type (Fig 2). The fibrosis-specific biochemical changes such as accumulation of collagen and other extracellular matrix proteins are predominantly based on the reactivity of the fibroblast cell system.[43-45] It has been shown that ionizing radiation induces premature terminal differentiation of potentially mitotic progenitor fibroblasts to irreversible postmitotic fibrocytes.[46-52] Terminally differentiated fibrocytes are the biochemically functioning and active cells of the fibroblast system responsible for the production of tissue-specific collagen and matrix molecules, as well as growth factors and cytokines.[44,47,52-54] Based on these functional properties of the different cell types of the fibroblast/fibrocyte cell system, it can be concluded that the radiation-induced accumulation of postmitotic fibrocytes results in the pronounced elevation of synthesis and extracellular deposition of collagen, characteristic of fibrotic tissue alteration.

In the light of the recent developments in cellular and molecular biology, the concept of a single target cell, which can explain the dynamic sequence of events occurring after radiation exposure, is supplanted by that of multiple cell systems interacting. Thus, as already addressed (Fig 2), radiation-induced fibrotic remodeling of connective tissues represents a multicellular process with initiation and sustaining of the fibrogenic process through an intercommunication between different cell types, with the activated fibroblasts functioning as the biochemically responsible cell. Within hours after radiation exposure, an increased expression of genes coding for growth factors, like PDGF, interleukin-1, TNF-α, and especially transforming growth factor β (TGF-β)1 occurs.[44,55,56] This early modulation of gene expression has profound effects on the pathophysiology of the late radiation effect. Altered expression of growth factors and cytokines may thus result in a modulation of the interactions between cell types involved in the fibrotic reaction. For example, TGF-β1 stimulates proliferation of early progenitor fibroblasts and myofibroblasts, which may be one initial step in the onset of fibrosis.[44,47,57] Radiation-exposure induces TGF-β1 production by fibroblasts, which most likely triggers the accelerated terminal differentiation process of progenitor fibroblasts into postmitotic functioning fibrocytes known to produce and deposit collagen at elevated levels.[47] Although all of the molecular actions of TGF-β1 in the process of radiation-induced fibroblast differentiation/activation and connective tissue remodeling are not completely understood at present, it can be stated that this factor plays an important role in radiation-induced tissue reactions.

The regulatory role of TGF-β1 in cell growth and differentiation is well documented for many cell types.[57,58] TGF-β is synthesized by several cell types including platelets, macrophages, lymphocytes, epithelial and endothelial cells, and fibroblasts.[58] Three isoforms of TGF-β exist, TGF-β1, 2, and 3, with specific as well as overlapping activities in various cellular processes.[57,58] TGF-β1, a 25-kDa protein, is the most prominent isoform of TGF-β. It is synthesized as an inactive, latent form, which associates to the latency-associated pep-

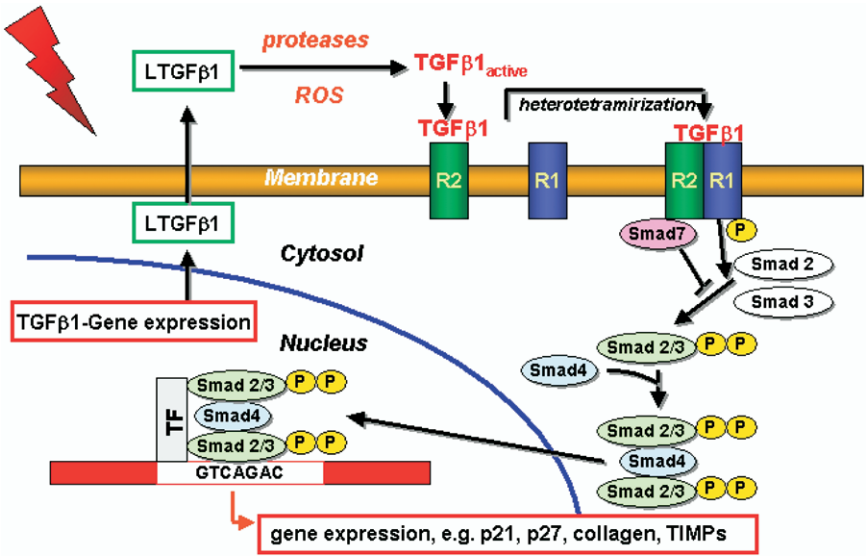

Figure 3 TGF-β1-dependent signal transduction and gene expression.

tide before secretion into the extracellular space (Fig 3)[59-61] and can bind to the latent TGF-β–binding protein, which is linked to the extracellular matrix through disulphide bonds. Latent and inactive TGF-β (LTGF-β) is activated by the function of specific extracellular proteases.[60-62] Activation of extracellular matrix-bound latent TGF-β1 can also be a consequence of radiation exposure (i.e., ionizing radiation) as was shown in the stroma of mammary glands.[62,63] This mechanism involves the activation of specific proteolytic enzymes from inactive proenzymes and is most likely dependent on the presence of reactive oxygen species (ROS). Scavengers of radiation-induced free radicals or ROS, such as amifostine, can inhibit ROS-induced activation of LTGFβ as well as diminish the plasma level of TGF-β1. As a consequence, manifestation of radiation-induced pneumonitis and radiation-induced collagen deposition, as a biochemical marker of fibrotic tissue remodeling in rat lung, is markedly reduced.[64] Likewise, intratracheal injection of plasmids carrying the gene for Mn-dependent superoxide dismutase into mice prevents impairment of lung function and prolongs survival of mice irradiated with a single dose of 20 Gy to the total lung volume.[65] As shown in this study, at the molecular level, Mn-dependent superoxide dismutase, which is an important enzyme for detoxification of the superoxide radicals, inhibits significantly radiation-induced gene expression of TGF-β1. Thus, downregulation of TGF-β1 gene expression diminishes the level of extracellular LTGF-β, which could be activated to its functional form in response to irradiation.

Once activated, TGF-β1 can bind to specific TGF-β1-receptors, the TGF-β1-receptor types I and II, which are located on the cell membrane (Fig 3). Both receptors exhibit serine/threonine kinase activities. The TGF-β1 receptor II first binds active TGF-β1 and presents it to the receptor type I to form the heteroterameric receptor complex, which is the active form of the TGF-β1-receptor. Activation of the receptor kinase activity generates the first step of the TGF-β1–signaling pathway, which is the phosphorylation of specific Smad-proteins, namely Smad2 and/or Smad3. On phosphorylation activation, these 2 proteins can form a complex with another Smad-protein, Smad4. This protein complex is able to translocate from the cytoplasm to the nucleus. Within the nucleus, the activated Smad-complex acts together with other transcription factors as a transactivator complex for specific target genes to be activated in response to the TGF-β1 stimulus.[66]

Depending on the cell type, TGF-β1 promotes and regulates a number of rapid responses in cells, including alterations in proliferation, differentiation, and apoptosis. The most pronounced effect of TGF-β1, which is observable in epithelial, endothelial, and hematopoietic cells as well as fibroblasts, is the inhibition of cell growth by inducing both a reversible and/or irreversible G1 cell-cycle arrest. In this context, TGF-β1 induces, through TGF-β1 receptor and Smad signaling, the transactivation of inhibitor proteins of the cyclin-dependent kinases, such as p15, p21, and p27.[67,68] Although the main effect of TGF-β1 with respect to cell-cycle control is inhibition of cell proliferation, TGF-β1 can, at least to a certain degree, promote proliferation of mesenchymal cells, such as early progenitor fibroblasts.[47] This growth stimulatory effect of TGF-β1 has also been shown in highly proliferative populations of human embryonic progenitor fibroblasts and mouse fibroblasts, which present an infinite growth in vitro.[69,70] In these cells, TGF-β1 downregulates the cyclin-dependent kinase inhibitors p21 and p27. Thus concerning cell proliferation, TGF-β1 can be a bifunctional cytokine at least for certain cell types.

With respect to tissue remodeling, the most important function of TGF-β1 is to control homeostasis of the extracellular matrix.[57] Remodeling of the extracellular matrix is caused through the ability of TGF-β1 to simultaneously stimulate the synthesis of most matrix proteins, decrease the production of matrix degrading enzymes, increase the production of inhibitors of these enzymes, and modulate the expression of integrins.[57,71] Thus, through these multiple functions, TGF-β1 has a central role in development and normal wound healing, as well as in the development of fibrotic tissue alterations.

Like fibrosis in irradiated skin or lung tissue, delayed radiation enteritis is a relatively frequent side effect of abdominal and pelvic radiation therapy. Thus, pathological tissue response to radiotherapy can lead to progressive development of fibrosis and consequently to intestinal obstruction, which can significantly impair the quality of life of cancer survivors. After radiation-induced tissue injury, intestinal mesenchymal cells, mainly represented by smooth muscle cells and subepithelial myofibroblasts, are released from their quiescent state to take part in the wound-healing process.[72] Sometimes, however, excessive wound healing occurs resulting in transmural accumulation of extracellular matrix components and chronic fibrosis.[73] The connective tissue growth factor (CTGF/CON2) has been identified to be a driving force and an essential mediator of delayed radiation enteritis.[74] CTGF expression can be induced by various fibrogenic stimuli, such as TGF-β, thrombin, or even mechanical stretch.[75] With respect to TGF-β, this cytokine not only promotes CTGF expression but also physically interacts with existing CTGF. This complex enhances binding of TGF-β to its receptor and stimulates TGF-β signaling to further promote CTGF expression.[75] In normal intestinal smooth muscle cells, TGF-β–dependent CTGF expression depends exclusively on the regular TGF-β–Smad signaling pathway, whereas in intestinal smooth muscle cells isolated from patients with delayed radiation enteritis, CTGF expression depends mainly on the Rho/ROCK pathway stimulated primarily by TGF-β. These are the first results indicating the important role of CTGF-induction by TGF-β in the maintenance of intestinal fibrosis.

The function of TGF-β1 in the regulation of cell growth and differentiation as well as homeostasis of extracellular matrix proteins have initiated clinical investigations into the role of this growth factor during the induction, progression, and manifestation of radiation-induced late tissue complications, like fibrosis. Alterations in the plasma levels of active TGF-β1 could be correlated to the susceptibility of radiotherapy patients to the development of pneumonitis, which eventually leads to lung fibrosis.[76-78] Likewise, clinical evidence

for the role of TGF-β1 in the development of radiogenic lung fibrosis has been reported by Bentzen and coworkers.[79] Breast cancer patients undergoing radiation therapy and treated with the antiestrogen tamoxifen presented a higher risk for radiation fibrosis than patients who did not receive the drug; it is suggested that this result is most likely because of the known induction of TGF-β1 expression by tamoxifen.

Consequently, on the basis of variety of experimental and clinical datasets, the following scenario for the cellular and molecular processes underlying the late fibrotic connective tissue remodeling process in patients after radiation therapy can be established. In response to the radiation insult, cells of the connective tissue present a stimulated production and secretion of TGF-β1. With respect to the fibroblast cell system, in a paracrine and autocrine manner, this cytokine is then responsible for a short wave of proliferation of early progenitor fibroblasts and myofibroblasts.[44,57] Depending on the tissue type, one main function of TGF-β1, however, is the immediate transition of already existing late progenitor fibroblasts into terminally differentiated fibrocytes.[44,48-50,52] Thus, these proliferation and differentiation events result in the accumulation of postmitotic biochemically activated fibrocytes producing and secreting collagens and other extracellular matrix proteins at high level.[47,48] These radiation-induced and TGF-β1–mediated alterations in cellular homeostasis of the fibroblast system represent the basis for the biochemical changes of connective tissue remodeling (i.e., enhanced production and extracellular deposition of interstitial collagen molecules by activated terminally differentiated fibrocytes). In intestinal tissue with the prevailing cell type of smooth muscle cells, radiation-induced TGF-β mainly triggers the induction of CTGF, which then leads to both the induction and maintenance of the fibrotic phenotype.

Radiation-Mediated Inhibition of Extracellular Matrix Protein Degradation

Although the precise cell types, mediators, and pathogenic mechanisms involved in tissue fibrosis after radiation exposure exhibit some organ specificity, a common feature is excessive accumulation of extracellular matrix proteins (ECMs), mainly collagen. The latter results not only from increased synthesis, primarily by postmitotic fibroblasts and myofibroblasts, but also from decreased degradation. With respect to collagen, it has clearly been shown that TGF-β1–mediated gene expression and production of tissue inhibitors of matrix metalloproteinases is responsible for decreased degradation of newly synthesized and deposited extracellular collagen.[57] The plasmin activator (PA) system is another key regulator of fibrinolysis and ECM degradation. Urokinase PA and tissue-type PA are arginine-specific proteinases that convert the inactive zymogen plasminogen to the active broad-spectrum serine protease plasmin.[80] Plasmin can then degrade ECM, both directly by its own proteolytic activity and by activation of latent matrix metalloproteinases. The activity of the PA/plasmin system is regulated by a family of PA inhibitors. The most important inhibitor is the plasmin activator inhibitor PAI-1.[81] Secretion of PAI-1 can efficiently be stimulated by IL-1, TNF-α, and TGF-β1. Yet, especially after radiation exposure, radiation-induced redox status and activation of TGF-β1, presumably via nonoverlapping signaling events, seem to be the prime stimuli to induce PAI-1.[82,83]

Another interesting aspect in the context of radiation-induced tissue remodeling has been reported by Strup-Perrot and coworkers[84] in 2006. The authors investigated the contribution of MMPs in the ECM remodeling involved in the 2 phases of colonic response to ionizing radiation (ie, epithelial denudation and subsequent restitution). This study indicates that in the denudation phase, radiation-induced alteration in the mucosal structure is concomitant with local increased expression and activation of MMP subtypes involved in basement membrane degradation, such as MMP-2, -3, and -9. However, during the restitution phase, a pronounced induction of MMP inhibitors such as tissue inhibitors of matrix metalloproteinases 1 and PAI-1 was apparent.

Conclusion

The understanding of normal tissue responses to radiation therapy at the mechanistic level has been markedly improved by the successful implementation of modern technologies of molecular biology into radiobiological and experimental radiation oncology research. The combination of these "new" results with "old" histopathological datasets on normal tissue alterations as a consequence of radiation therapy will thus foster the interpretation and understanding of the interactions of complex multiple-cell systems. This knowledge will then allow the development of preventive/interfering strategies to improve the quality of life of radiotherapy patients.

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