



Understanding the mechanism of radiation induced fibrosis and therapy options



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ABSTRACT

Radiation therapy has been increasingly employed as a tool to cure and palliate majority of solid tumors. Although radiotherapy has shown promising results in preserving structure and function of organs, it is associated with late side effects mainly manifested in the form of tissue fibrosis. Recent advances in molecular biology techniques has helped better understand the molecular mechanisms involved in radiation induced fibrosis. Currently, very few treatment modalities are available to treat the condition with moderate success rate. Stem cell therapies and particularly adipose tissue and adipose derived stem cells therapies have shown promising results in clinical applications. Identification of the key factors involved in the mitigation process will help to enhance the beneficial effects and develop new therapy approaches.

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1. Introduction

Radiotherapy is a fundamental component of modern cancer management regime, and > 50% of the newly diagnosed cancer patients undergo radiation therapy either alone or as an adjuvant to chemotherapy, surgery, or both (Govindan & DeVita, 2009). An estimated half million cancer patients undergo radiotherapy in the United States alone each year (Citrin, 2017; Panel, 2016). Radiotherapy, when compared to

both chemotherapy and surgery, carries benefit of being cost effective, targeted, non-invasive, and preserve the organ loss (Bentzen, 2006). Use of high energy packets consisting of photons beams and particles of electrons, protons, and neutrons having sub-millimeter precision and better penetration capacity is the method of choice in conducting modern external beam radiotherapy (De Ruyscher et al., 2019). Ionizing radiation induced skin changes have been known since 1902 and, despite the advancement in the field of radiotherapy, its application is associated with acute (occurring during or within weeks) and chronic side effects (occurrence range from 6 months to years) post therapy (Barnett et al., 2009; Ryan, 2012). Acute effects include skin rashes, erythema, mucositis, nausea, diarrhea, and alopecia, while chronic effects range from radiation induced fibrosis, atrophy, neural damage, vascular damage, endocrine defects, and cardiac toxicity to infertility depending on the anatomical region exposed to radiations

Abbreviations: RIF, Radiation Induced Fibrosis; HGF, Hepatocyte Growth Factor; TGF, Transforming Growth Factor; ECM, Extracellular Matrix; ROS, Reactive Oxygen Species; CTGF, Connecting Tissue Growth Factor; IL, Interleukins; miRNA, Micro RNA; ASCs, Adipose Derived Stem Cells.

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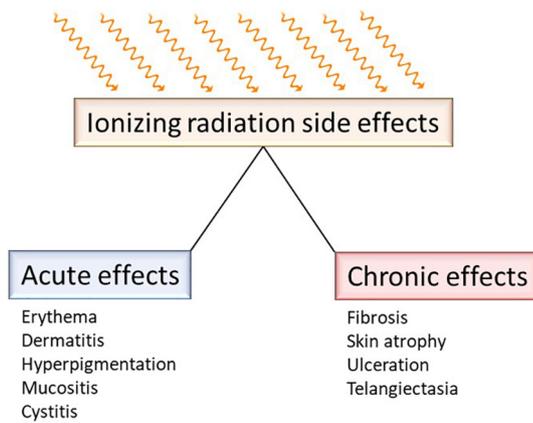


Fig. 1. Acute and chronic toxic effects of radiation. Acute or short-term side effects of radiation exposure occur in skin shortly after the radiations and resolve in a relatively short duration. Late or chronic side effects appear relatively slowly, ranging from months to years post radiation exposure, and are long lasting and difficult to treat, thus exerting a treatment dose limitation effect.

(Fig. 1) (Brush et al., 2007; Ryan, 2012). Late side effects can result in decreased quality of life, with the severity of the side effects corresponding to the radiation dose and manifesting in organs exposed to the radiation field. Side effects from radiation injuries also exert a dose limiting effect which influence the efficiency of the treatment.

Injuries from radiations have gained attention and international efforts have been boosted to develop medical countermeasures during the last decades due to an increasing risk of nuclear attacks. Likelihood of radiation toxicities are directly related to the dose, irradiated body proportion, tissue volume, and time of exposure (Table 1) (Barnett et al., 2009; Ryan, 2012). By virtue of improvement in therapeutic and surgical techniques used for cancer management, the survival rate has significantly increased, meaning that many of these survivors will be living with the late side effects having a notable impact on the social and economic system (Bryant, Banegas, Martinez, Mell, & Murphy, 2017; DeSantis et al., 2014; Hawkins, 2004). Radiation induced skin/muscle fibrosis is among the most prominent late side effects of radiation exposure, characterized as an uncontrolled wound healing response resulting in excessive extracellular matrix deposition at the radiation injury site (J. H. Kim, Kolozsvary, Jenrow, & Brown, 2013; Yarnold & Brotons, 2010). Recent advances in molecular radiobiology has improved our understanding of the complex and persistent mechanisms involved in radiation induced skin injuries and has open new directions for the development of therapeutic strategies to mitigate tissue damage.

2. Clinical manifestations

Radiation exposure can cause fibrosis in almost any organ involved, but skin is the most prevalent site affected by radiation therapy. Almost 95% of the patients receiving radiation therapy develop acute or late skin reactions (Bray, Simmons, Wolfson, & Nouri, 2016; Salvo et al.,

Table 1
Effect of radiation dose on skin reaction.

Radiation dose (Gy)	Skin Reaction	Time onset post radiation
Acute effect		
2	Erythema	Within hours
6–10	Epilation	7–10 days
12–20	Hyperpigmentation	2–3 weeks
20–25	Dry desquamation	3–4 weeks
30–40	Moist desquamation	≥ 4 weeks
≥40	Ulceration	≥ 6 weeks
Late effect		
≥45	Fibrosis	6 Month to ≥1 year
≥45	Telangiectasia	6 Month to ≥1 year

2010). Skin of the head, neck, and trunk is more prone to fibrosis, and patients having breast, head and neck, or lung cancer mostly develop skin fibrosis attributed to heavy dose radiation treatment (Archambeau, Pezner, & Wasserman, 1995; McQuestion, 2011; O'Donovan, Coleman, Harris, & Herst, 2015; Rigotti et al., 2007; Salvo et al., 2010). Survivors of accidental exposure and patients previously treated with radiation for skin conditions are also at risk for developing skin fibrosis (Gottlöber et al., 2001; Hymes, Strom, & Fife, 2006).

Radiation induced fibrosis (RIF) develops late after the therapy and can take from 6 months to several years to develop, reflected by signs and symptoms like pain, induration, ulceration, alopecia, and ulceration (Sylvie Delanian, Balla-Mekias, & Lefaix, 1999). Late fibrosis is a persistent condition characterized by atrophy, hypo- or hyper pigmentation, and cutaneous malignancies which normally do not resolve on its own. Development of late fibrosis after radiation therapy of head and neck region can result in trismus, a condition which affects the mastication muscle, resulting in loss of ability to open the mouth (Sciubba & Goldenberg, 2006). Severe late cutaneous injuries may reflect as xerotic, scaly and dyspigmented skin and can result in loss of hair follicles, nails, skin appendages, and sebaceous glands (Bray et al., 2016; Hymes et al., 2006). As the fibrosis of skin and subcutaneous tissue develop, the range of motion decreases with contractures, pain, skin necrosis, ulceration and lymphedema (Harper, Franklin, Jenrette, & Aguero, 2004; Mendelsohn, Divino, Reis, & Kerstein, 2002).

Although the clinical presentation of acute and late response to radiation injury is well documented, the investigation of normal tissue response is complicated and depends on the anatomical site exposed to radiations, requiring a well-defined tissue specific end point and severity of the effect (Barnett et al., 2009). Use of National Cancer Institute (NCI) Common Terminology Criteria for Adverse Effects version 3.0 (CTCAEv3.0) has become the first multimodality grading system for recording acute and late effects, replacing the previously used guidelines: the LENT-SOMA (Late Effects Normal Tissues: Subjective, Objective, Management and Analytic) system and RTOG (Radiation Therapy Oncology Group)- EORTC late effect scale (Denis et al., 2003; Hoeller et al., 2003). Radiation therapy effects are graded based on severity, but timescale is an important factor to be considered as the late effects can manifest years after the therapy; however, confirmation of the fibrosis should be made based on the tissue biopsy analyses (Bray et al., 2016; Johansson, Svensson, & Denekamp, 2000). In general, grade 0 reflects no symptoms, grade 1 for mild symptoms, grade 3 reflects medically significant but not life threatening, grade 4 refers to life-threatening, and grade 5 is death due to radiotherapy toxicity (De Ruyscher et al., 2019).

3. Risk factors for developing radiation fibrosis

Several extrinsic and intrinsic factors have been identified which contribute to the development and severity of RIF. The dose of applied radiations is the most crucial extrinsic factor which contributes in fibrosis development. The other influential extrinsic factors include tissue volume, radio-sensitivity of exposed tissue, and time gap between fractionated delivery of radiations (Stubblefield, 2011; Zackrisson, Mercke, Strander, Wennerberg, & Cavallin-Staahl, 2003). Fractionation of total dose into smaller units exert fewer toxic side effects and enables application of higher total dose; thus, hyper fractionated treatment design increase the radiation tolerance of skin (Olschewski, Bajor, Lang, Lang, & Seegenschmiedt, 2006). Excellent cosmesis was achieved by fractionating a dose of 57 Gy into 5 × 3 Gy/week dose with no tumor recurrence in basal cell carcinoma patients (Olschewski, et al., 2006). Furthermore, the quality of the radiation beam, the particle type, and the chemotherapy drug combination also influence the outcomes of treatment.

In addition to extrinsic factors, there are several intrinsic factors that determine the risk of developing late side effects of radiotherapy. These intrinsic factors may include patient age, health condition, skin

condition, nutritional status, comorbid diseases, ethnicity, living habits, and genetic disposition (Morgan, 2014; Zackrisson, et al., 2003). Patients with smoking habits, implants in case of breast tissue, obesity, or HIV infection have a greater predisposing to develop skin fibrosis reactions (Housri, Yarchoan, & Kaushal, 2010; Vandeweyer & Deraemaeker, 2000). Genetics conditions such as a mutational defect in DNA repair gene ATM, Nijmegen breaking syndrome, Fanconi's anemia, Bloom syndrome and xeroderma pigmentosum predispose patients to developing skin reactions (Bray et al., 2016; Iannuzzi, Atencio, Green, Stock, & Rosenstein, 2002).

4. Mechanisms of radiation induced fibrosis

Radiation induced fibrosis is a complex process which involves several cellular and non-cellular factors which mediate a healing process after radiation injury. A normal wound healing process can be subdivided into three phases: an immediate phase of inflammation in which epithelial and endothelial cells play major role, a phase of cell proliferation and differentiation characterized by high density of myofibroblasts in an unorganized matrix and poorly cellularized fibrotic areas of senescent fibrocytes, and the remodeling phase named late fibro atrophic phase (Sylvie Delanian & Lefaix, 2007; Gurtner, Werner, Barrandon, & Longaker, 2008; Vallée, Lecarpentier, Guillevin, & Vallée, 2017). Fine tuning of the cellular and molecular activities during these three phases result in a normal healed wound, but during the RIF development this healing process is dysregulated which results in an excessive production and deposition of extracellular matrix at the radiation injury site (Jones, Foster, Hu, & Longaker, 2019). Regulation of different phases during normal wound healing and RIF are illustrated in (Fig. 2).

Development of radiation induced fibrosis involves cellular and molecular mechanisms that are common to other fibrotic diseases.

4.1. Cellular mechanisms involved in RIF

The pathological mechanism of RIF is complex and involves many cell types. The development of fibrosis in irradiated tissue challenged the target-cell hypothesis, the idea that the main effects of radiation are due to loss of the target cells population (Rodemann & Bamberg, 1995). Mechanistic studies conducted to understand the process of RIF showed the importance of cell-cell communication and bystander cellular toxicity. Radiation exposure results in an initial damage to epithelial and endothelial cells lining the vasculature, with the predominantly affected cell type depending upon the tissue type (Toshkov et al., 2017; Venkatesulu et al., 2018). Injury to epithelial lining can cause skin damage and breakdown of mucosal membranes. Radiation damage disrupts the self-renewal capability of the epidermis due to loss of stem cells, resulting in dermatitis. In addition, radiations also damage keratinocytes and melanocytes in the skin (Hymes et al., 2006). The release of inflammatory mediators from these damaged cells activate platelets which in turns activate blood-clot formation and a provisional ECM deposition (Ahamed & Laurence, 2017). In addition, activated platelets degranulate and promote vasodilation to facilitate the influx of inflammatory cells to the site of injury (Ahamed & Laurence, 2017; Wynn, 2008). Cytokines, chemokines, and damage-associated molecular patterns (DAMPs) produced by damaged cells further promote the infiltration of leukocytes, predominately macrophages and neutrophils, which eliminate dead cells, debris, and invading pathogens (Denham & Hauer-Jensen, 2002; Meziari, Deutsch, & Mondini, 2018; Travis, 2001). Radiation-induced cell death and DAMPs are mainly responsible for a

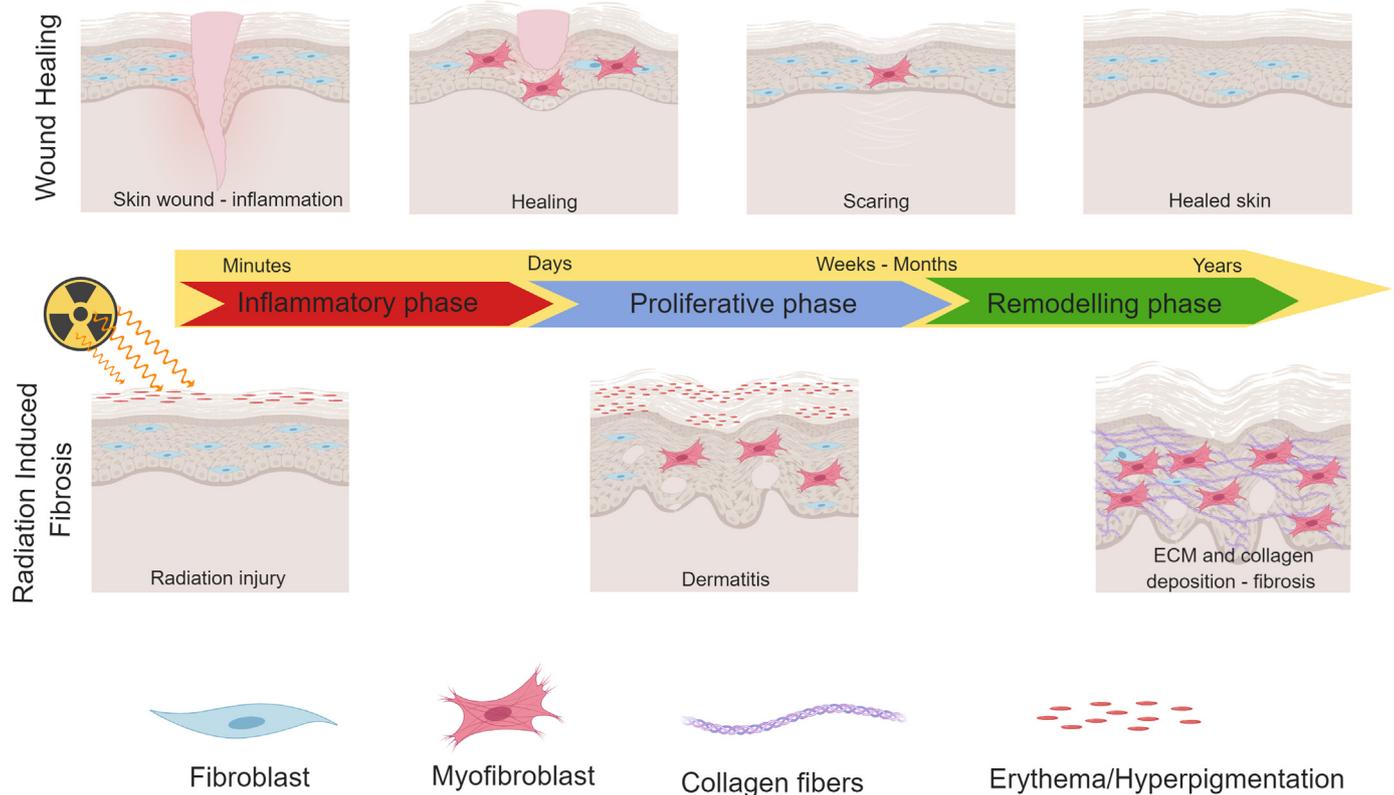


Fig. 2. Normal wound healing vs radiation delayed wound healing. Normal wound healing is characterized by three distinct phases: inflammatory phase, proliferative phase, and remodeling phase resulting in restoration of normal skin structure. Inflammatory phase is characterized by an immediate platelet response, release of chemokines, cytokines, and influx of inflammatory immune cells, while the proliferative phase comprises of angiogenesis and transformation of fibroblasts to myofibroblasts, leading to scar formation by extracellular matrix and collagen deposition. The remodeling phase comprises of degradation of extra collagen and extracellular matrix, resulting in restoration of normal skin architecture. In the case of radiation injury induced fibrosis development, there is a prolonged phase of proliferation and a defective phase of remodeling, resulting in extensive deposition of extracellular matrix and collagen.

dose dependent activation of the inflammasome pathway in macrophages, NK cells, dendritic cells, B cells and T cells which produce acute inflammation response (Stoecklein et al., 2015). Moreover, the inflammatory signals produced by damaged cells and macrophages after irradiation result in generation of an absopal inflammatory response leading to a perpetual cycle of inflammatory cytokines release (Mukherjee, Coates, Lorimore, & Wright, 2014). IL17 $\gamma\delta$ T cells plays an important role in development of radiation skin fibrosis (Liao, Hei, & Cheng, 2017).

4.1.1. Role of Myofibroblasts in RIF

Myofibroblasts are the major player in the process of fibrogenesis. Myofibroblasts are derived from tissue resident or migratory fibroblasts and are associated with tissue repair and fibrosis (Abraham, Eckes, Rajkumar, & Krieg, 2007; V. Kumar, 2005; Quan, Cowper, & Bucala, 2006). In addition, myofibroblasts are derived from epithelial or endothelial cells through a process called epithelial or endothelial to mesenchymal transition (Jung et al., 2007; Pardali, Sanchez-Duffhues, Gomez-Puerto, & ten Dijke, 2017; Yi et al., 2018). Chemokine signaling via CCL2-CCL5 ligand /CCR4 receptor contributes in epithelial to mesenchymal transition (Zhong et al., 2019). Myofibroblast differentiation is associated with upregulation of α smooth muscle actin (α SMA) fibers which are responsible for retractile role of myofibroblasts and these differentiated myofibroblast produce collagens, fibronectins and other matrix molecules (Jun Wei, Bhattacharyya, Jain, & Varga, 2012; Yarnold & Brotons, 2010). Association of transforming growth factor- β 1 (TGF- β 1) with extra domain A (EDA) along with tissue stiffness and other chemical factors is required for differentiation of fibroblasts into myofibroblasts (Goffin et al., 2006; Serini et al., 1998; Tomasek, Gabbiani, Hinz, Chaponnier, & Brown, 2002). In addition, α SMA synthesis is also regulated by TGF- β 1 (Hinz, Celetta, Tomasek, Gabbiani, & Chaponnier, 2001). Under normal homeostatic conditions, fibroblasts are responsible for maintaining a fine network of β -actin fibers and contact with extracellular matrix (ECM). After normal wound healing, myofibroblasts undergo apoptosis, but upon radiation injury, perpetual cycle of inflammatory signals drives the differentiation of fibroblast to

myofibroblasts which are then responsible for excessive deposition of the ECM (Bentzen, 2006; Hinz, 2010; Hinz et al., 2007). Other sources of myofibroblast origin include vascular smooth muscle cells and bone marrow origin fibrocytes that express CD34, CD45, and type I collagen (Fig. 3) (Brittan et al., 2002; Forbes et al., 2004; Willis, dubois, & Borok, 2006). A recent study using genetic lineage tracing and transplantation assays has identified single somatic-derived fibroblast lineage dermal cells expressing CD26/dipeptidyl peptidase-4 (DPP4) which are responsible for the bulk of connective tissue deposition upon RIF (Rinkevich et al., 2015).

4.1.2. Role of immune cells in RIF

Skin provides both a physical and immunological barrier to environmental insults (Gerber et al., 2015). Langerhans cells are the key immunological cells surveilling the skin and, along with the dermal dendritic cells, perform the essential antigen presentation role (Otsuka, Egawa, & Kabashima, 2018). In addition, dermis harbor mast cells and T cells play an important role in radiation-induced immune responses (Komi, Khomtchouk, & Santa Maria, 2019; MacLeod et al., 2014; Müller & Meineke, 2011; Nguyen & Soulika, 2019). Radiation-induced damage to keratinocytes, fibroblasts, and endothelial cells leads to cell damage and apoptosis, which results in a breach of the skin barrier. Skin resident Langerhans and dendritic cells are responsible for the uptake of invading antigens and presentation of antigens to T cells (Carvalho & Villar, 2018; Deckers, Hammad, & Hoste, 2018; Müller & Meineke, 2007). These interactions result in influx of more immune cells at the irradiated site and the subsequent release of cytokines and chemokines. Acute radiation damage to keratinocytes and ROS results in the release of various cytokines and chemokine including IL-1 α , IL-1 β , IL-6, IL-8, CCL4, CXCL10 and CCL2 (Ryan, 2012).

4.1.3. Role of bone marrow derived cells in RIF

Hallmark histological features of radiation damage are reduction in parenchymal cells and damage to endothelial cells which culminates in development of tissue hypoxia. Radiation induced acute vascular injuries are one of the main causes of late manifestations of RIF (J. H.

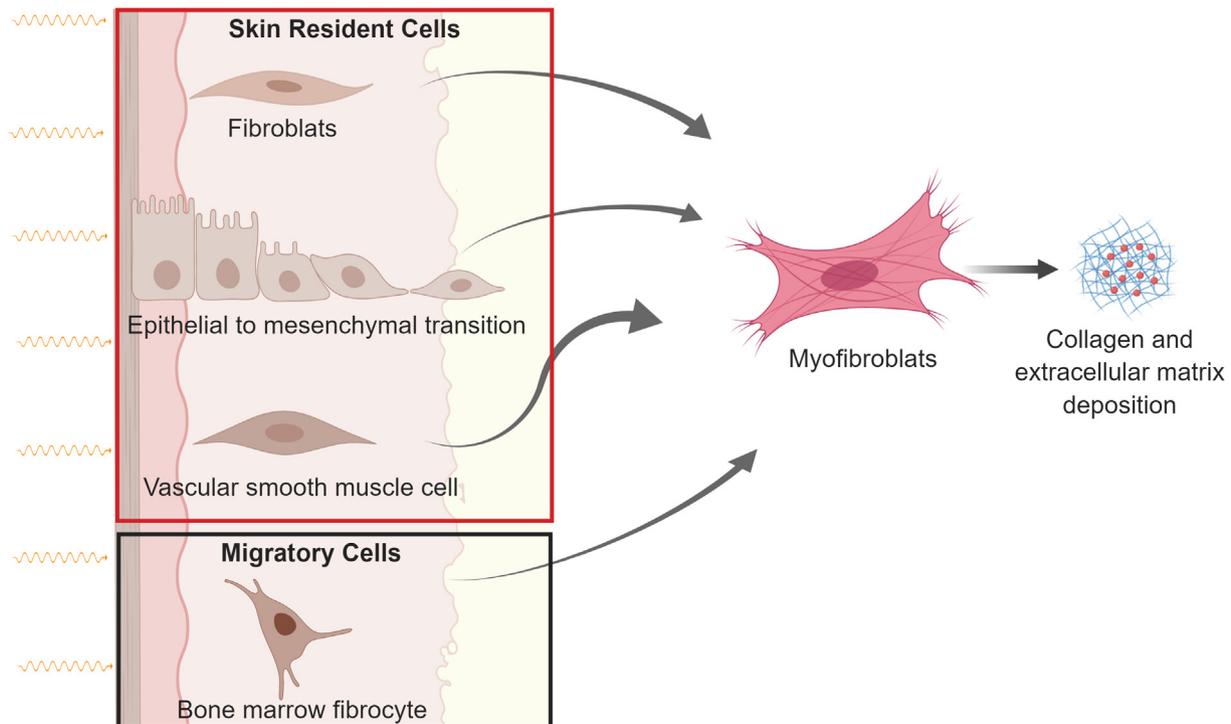


Fig. 3. Myofibroblasts play an important role in fibrogenesis process by active synthesis and deposition of excessive collagen and extracellular matrix. Myofibroblasts can originate from fibroblasts, vascular smooth muscle cell, bone marrow-derived fibrocytes, and endothelial or epithelial cells via a process of epithelial-mesenchymal transition (EMT).

Kim et al., 2013). Angiogenesis and vasculogenesis are the two mechanisms responsible for the repair of vascular damage (Patan, 2004). Cytokines and growth factors like CXCR4 and SDF-1 mediate the migration and proliferation of endothelial cells to accomplish angiogenesis and vasculogenesis which is further supported by influx of bone marrow origin cells to the radiation injury site (Bastianutto et al., 2007). Mesenchymal stem cells, endothelial progenitor cells, and myelomonocytic cells predominantly migrate from bone marrow to the irradiated site of injury. CD11b expressing myelomonocytic cells are the most abundant cell population of bone marrow origin which localize to irradiated skin, lungs, brain, and bone marrow and stimulate vessels repair releasing angiogenic factors. (Bastianutto et al., 2007; J. H. Kim et al., 2013). Thus, bone marrow derived cells migrate to the irradiated tissue and participate in the reconstruction process.

4.2. Molecular mechanisms involved in RIF

The process of RIF involves several interactive molecular mechanisms which eventually result in the activation of cellular responses involved in establishment of fibrosis. Various cytokines such as TGF- β 1, connective tissue growth factor (CTGF), IL-6 and chemokines like CCL3 (macrophage inflammatory protein 1 α) and CCL2 (monocyte chemoattractant protein-1) induce the fibrosis process (A. Ejaz, Epperly, Hou, Greenberger, & Rubin, 2019; Weigel, Schmezer, Plass, & Popanda, 2015). In addition, epigenetic modifications, hypoxia, DNA damage, and ROS production drive the fibrogenic process.

4.2.1. TGF- β 1 role in radiation fibrosis

Transforming growth factor β superfamily consists of three isoforms TGF- β 1, β 2, and β 3, which regulate physiological processes like embryonic development, cell cycle, and wound healing (Derynck & Budi, 2019; Ma et al., 2017; Massague, 1990; Massagué, 1998). TGF- β 1 isoforms have shown a key role in fibroproliferative diseases. It is a 25 kDa homodimer that is secreted in an inactive form complexed through disulfide linkages with latent TGF- β 1 binding protein, which inhibits the latent form from binding to its receptors (Liénart et al., 2018; Nunes et al., 1998; Saharinen, Taipale, & Keski-Oja, 1996; Sheppard, 2015). Transforming growth factor is secreted by the cells and a relatively large pool of latent TGF- β 1 is always present in the extracellular space associated with ECM proteins (D. A. Lawrence, 2001; Werb, 1997). Triggering events like wound healing or ionizing radiations exposure induce activation of TGF- β 1 within hours (Ehrhart, Segarini, Tsang, Carroll, & Barcellos-Hoff, 1997; Leask & Abraham, 2004). Upregulation of plasmin proteases MMP-2, MMP-9, and $\alpha_v\beta_6$ integrin in epithelial cells during wound healing is also associated with activation of TGF- β 1 (Jenkins, 2008; Yu & Stamenkovic, 2000). In addition, thrombospondin-1 (TSP-1), a protein associated with angiogenesis, cell adhesion, and matrix reconstruction also promotes the activation of TGF- β 1 (Murphy-Ullrich & Suto, 2018).

The active TGF- β 1 binds to a two subunit receptor complex with serine/threonine kinase activity; engagement of both TGF- β type 1 and TGF- β type 2 receptors is essential for the downstream signal transduction (Derynck & Budi, 2019). The downstream intracellular signaling effectors are mainly the Smad proteins, although non-Smad pathways, like mitogen activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K)-AKT-mammalian target of rapamycin (mTOR) pathways, are also activated by TGF- β (Derynck & Budi, 2019). Smad protein family consists of eight members of which two Smads, Smad 6 and 7, antagonize TGF- β signaling (Hata & Chen, 2016; Hill, 2016; Katsuno et al., 2018). Smad are the major TGF- β family transducers, and activation of the TGF- β receptors in turn activate Smads through C-terminal serine phosphorylation. These activated Smads translocate to the nucleus and regulate gene expression via interactions with high-affinity DNA binding transcription factors (Massagué, 2012). Smad proteins can regulate large number of target genes, with the activation or repression outcome of a gene depending on the interaction of Smad proteins

with transcription factors and co-factors as well as the physiological state of the cell.

Although TGF- β 1 has a role in inhibition of cell growth and immunosuppression, its role in regulation of extracellular matrix depositions designate it as a key player in fibrogenesis (Meng, Nikolic-Paterson, & Lan, 2016). TGF- β 1 regulates extracellular matrix remodeling by balancing its synthesis and degradation. The synthesis and deposition of ECM post irradiation injury is facilitated by a simultaneous upregulation of the genes responsible for ECM synthesis and down-regulation of the matrix degrading proteases (Blobe, Schiemann, & Lodish, 2000; Dutta et al., 2015; Schultze-Mosgau et al., 2004). The profibrotic role of TGF- β 1 is supported by the observation that both exogenous injection of TGF- β 1 or genetic manipulation to overexpress TGF- β 1 in mice result in establishment of fibrosis (Michèle Martin, Lefaix, & Delanian, 2000). Upon a single dose exposure of 35 Gy TGF- β 1, gene expression was upregulated in the skin by 24 h and the expression is further increased to several hundred folds by day 14 post exposure. Expression of other profibrotic genes like connective tissue growth factor (CTGF) and collagens type 1–6 coincided with TGF- β 1 expression (A. Ejaz et al., 2019; Randall & Coggle, 1995). Keratinocytes and fibroblasts showed an increased activity of transcription factor Ap-1 upon radiation exposure which in turns upregulate TGF- β 1 transcription by binding to the TGF- β 1 promoter (M Martin et al., 1997). Smad signaling downstream of TGF- β 1 plays the key role in ECM synthesis and fibrosis, as mice lacking intact Smad signaling cascade were resistant to fibrosis development as a result of irradiation (A. Ejaz et al., 2019; Flanders, Sato, Ooshima, Russo, & Roberts, 2004; Roberts, Russo, Felici, & Flanders, 2003). An overview of TGF- β signaling in radiation fibrogenesis is shown in Fig. 4.

4.2.2. CTGF role in radiation fibrosis

CTGF is a matricellular protein belonging to a small family of proteins having a highly conserved disulphide bonding pattern and modulates the interaction of cells with matrix to modify the cellular phenotype (Lipson, Wong, Teng, & Spong, 2012). CTGF binds directly to TGF- β 1, enhance its binding to the receptor and regulating its expression in a positive feedback loop manner (Abreu, Ketpura, Reversade, & De Robertis, 2002). CTGF expression is upregulated upon irradiation, and it facilitates transdifferentiation of other cell types into myofibroblasts and stimulates the ECM deposition by myofibroblasts (A. Ejaz et al., 2019; Gore-Hyer et al., 2002; Lipson et al., 2012; Tsai, Wu, Kau, & Wei, 2018). CTGF promotes TGF- β 1 mediated ECM deposition and is an essential facilitator for TGF- β 1 mediated sustained fibrotic response, as exogenous application of either TGF- β 1 or CTGF fail to generate a sustained response (Bickelhaupt et al., 2017; Mori et al., 1999).

4.2.3. Chemokines and interleukins role in radiation fibrosis

Irradiated damaged cells and migratory leukocyte secrete chemoattractant that augment profibrotic cytokines in development of fibrosis by recruiting myofibroblasts and more immune cells to the radiation injury site. CCL3 (macrophage inflammatory protein 1 α) and CCL2 (monocyte chemoattractant protein-1) are the prominent mediators of fibrosis (Groves, Johnston, Williams, & Finkelstein, 2018; Wynn, 2008; Yang et al., 2011). Macrophages and epithelial cells are the main source of CCL3, and blockade of CCL3 or its receptors, CCR1 or CCR5, reduce fibrosis development (Yang et al., 2011).

In addition to TGF- β 1, interleukin 1 (IL-1) and Interleukin 6 (IL-6) play an important role in the pathogenesis of radiation induced fibrosis. IL-1 contributes to acute inflammatory response post irradiation and activates T and B cells; it also facilitates fibroblast proliferation and collagen synthesis during the late fibrotic (Müller & Meineke, 2007; Zhang, Jiang, & Ren, 2019). IL-6, a pro-inflammatory cytokine produced by fibroblasts, endothelial cells, immune cells, and bone marrow cells is upregulated during the acute phase of radiation injury (Di Maggio et al., 2015; A. Ejaz et al., 2019). In vitro studies have demonstrated

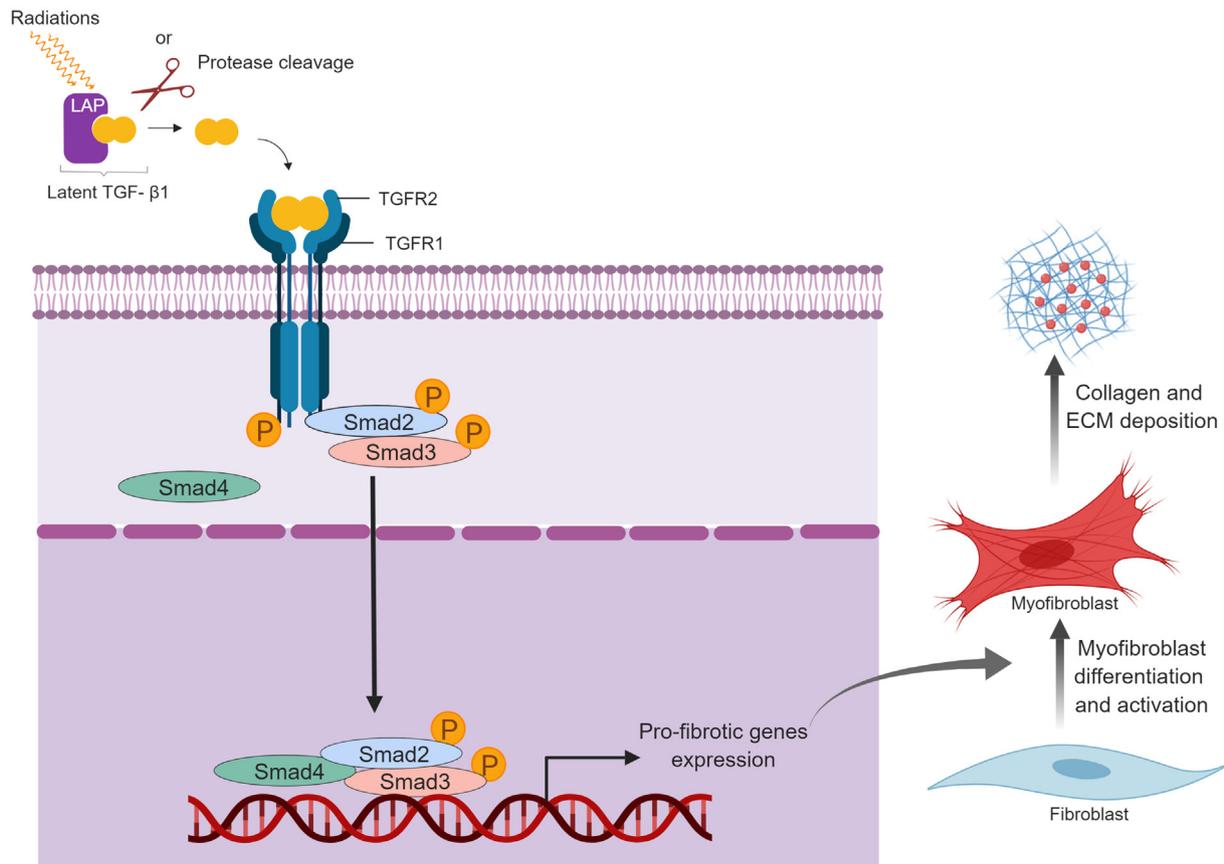


Fig. 4. TGF- β signaling in radiation fibrogenesis. Ionizing radiations and/or protease activity lead to cleavage of latent associated peptide (LAP) rendering TGF- β free to bind to its receptor TGFR2 which then recruits and activates TGFR1. Activated TGF- β receptor complex phosphorylate Smad 2 and Smad 3, which further complex with Smad 4 and translocate into the nucleus where they activate the expression of pro-fibrotic genes. The products of pro-fibrotic genes result in differentiation of fibroblasts into myofibroblasts and deposition of collagen and extracellular matrix.

production of IL-6 by fibroblasts, keratinocytes, and endothelial cells upon irradiation (Beetz et al., 1997; A. Ejaz et al., 2019; Petit-Frère et al., 2000). Increase in IL-6 levels post irradiation is linked to worst therapeutic outcome or toxicity (Marconi et al., 2019), as well as the development of fibrosis in the late phase (Saito-Fujita et al., 2011).

4.2.4. Reactive oxygen species in radiation fibrosis

Generation of reactive oxygen species (ROS) after irradiation is one of the driving mechanisms involved in RIF. ROS promote differentiation and persistence of myofibroblast cells by inducing epigenetic changes (Shrishrimal, Kosmacek, & Oberley-Deegan, 2019). Ionizing radiations result in generation of hydroxyl radicals ($\cdot\text{OH}$), hydroperoxyl radicals ($\text{HO}\cdot$), hydrogen peroxide (H_2O_2), and superoxide ($\text{O}_2\cdot^-$) (Azzam, Jay-Gerin, & Pain, 2012). ROS generation results in metabolic and epigenetic changes responsible for establishment of RIF. Mitochondria produce basal levels of superoxide and epigenetic metabolites such as nicotinamide adenine dinucleotide (NAD), α -ketoglutarate (α -KG), S-adenosyl methionine (SAM), and acetyl-co which contribute to epigenetic modifications like methylation, acetylation, and ribosylation. Ionizing radiations increase mitochondria ROS production and alter epigenetic metabolite concentrations, leading to modifications of the cells' epigenome (Pannkuk, Laiakis, Fornace Jr, Fatanmi, & Singh, 2018; Yamamori et al., 2017). The decrease in the concentration of NAD^+ immediately after radiation exposure reduces the ability of cells to counter oxidative stress and repair damaged DNA. In addition to the altered metabolites, ROS play an important role in regulating TGF- β signaling (Richter & Kietzmann, 2016; Richter, Konzack, Pihlajaniemi, Heljasvaara, & Kietzmann, 2015). ROS activate TGF- β signaling by mediating the release of TGF- β from its complex with latency associated peptide (LAP). Activated TGF- β activates the Smad signaling cascade,

which in turns positively regulates ROS production via transcriptional upregulation of NADPH oxidase 4 (NOX4), thus establishing a positive feedback mechanism between TGF- β and ROS production, with both positively regulating the other (Cheng et al., 2019; R.-M. Liu & Desai, 2015).

4.2.5. Epigenetics in radiation fibrosis

DNA methylation is a well characterized epigenetic modification which controls gene expression by the addition of methyl groups to DNA using DNA methyltransferases (DNMTs) (Laisné, Gupta, Kirsh, Pradhan, & Defossez, 2018). Activation of the TGF- β pathway increases the protein expression of DNMT1 and DNMT3a via PI3K-AKT-mTOR pathway in fibroblasts (Koh, Scruggs, & Huang, 2016). Studies in fibrotic tissue have shown the role of DNA methylation in pro-survival signals in the activated myofibroblasts. Even so, no appreciable direct effect on DNA cytosine methylation patterns in human cells exposed to acute γ -radiation was observed (Lahtz et al., 2012). Post translational modifications of histone proteins regulate chromatin architecture and gene expression. Histone acetylation and deacetylation is generally associated with an increase or decrease in transcription, respectively (M. Lawrence, Daujat, & Schneider, 2016). Exposure of skin to radiation results in TGF- β dependent upregulation of acetyltransferase enzymes P300 and CBP in myofibroblasts cells as a contributing mechanism to RIF (Shrishrimal et al., 2019).

Non-coding micro-RNAs (miRNAs) play an important role in regulating epigenetic modifications involved in RIF. Studies have shown deregulation of several fibrosis related miRNAs upon irradiation exposure fibrosis (Simone et al., 2009). The miRNAs which were upregulated include let-7d, let-7 g, let-7i, miR-26b, miR-663, let-7e, miR-15b, miR-21, miR-768-3p, and miR-768-5p; the prominent miRNAs

down-regulated upon irradiation include: miR-24, let-7a, miR100, miR125b, miR222, let-7b, and miR-638 (Simone et al., 2009). These miRNAs regulate many key pathway molecules involved in fibrosis (Weigel et al., 2015).

5. Treatment strategies for radiation induced fibrosis

Currently there are very few therapeutical option for RIF having moderate clinical success. Recent work has improved our understanding regarding the pathological mechanisms involved in RIF. Current therapy candidates in clinics and in pipeline are based on modulating these pathophysiological mechanisms. RIF is a multiphase phenomenon governed by different molecular players, thus making it difficult to design drugs which can work for all stages of RIF (i.e. from acute to established RIF). Current treatment strategies are mainly focused to limit aggravating influences. A two-prong therapy approach based on: (1) preventing the progression of the condition and (2) reconstruction of damaged tissue can be a potential way forward. Based on the progression of the condition, therapeutical strategies can be characterized as acute and chronic phase therapies.

5.1. Topical emulsions

Topical emulsions like Triethanolamine have been used in the clinic for a long time to treat radiation injuries. It promotes healing by recruiting macrophages and enhancing the growth of granulation tissue (Del Rosso & Bikowski, 2008). A meta-analysis of seven studies concluded that trolamine cannot be considered a standardized product to prevent or treat radiation dermatitis in patients with breast, head, or neck cancers (Meneses, Reis, Guerra, Canto, & Ferreira, 2018). Biafine is another emulsion formulation containing triethanolamine which is approved for wound healing. Application of biafine significantly reduce skin toxicity in women receiving radiation therapy of the chest wall (Szumacher et al., 2001).

5.2. Antioxidant therapies for RIF

Superoxide dismutases (SODs) catalyze the conversion of molecular oxygen to hydrogen peroxide and oxygen. Application of liposomal form of SOD (LipSOD) has shown reversal of established RIF in patients and pigs (S Delanian et al., 1994; Lefaix et al., 1996). Mechanistically, LipSOD results in reduction in transforming growth factor beta 1 expression. Development of low cost synthetic SOD has opened new avenues for treatment of RIF (Wei et al., 2019). Use of SOD in different formulations has shown radio-protective effects in lungs, esophagus, and skin injury models (Campana et al., 2004; J. H. Kim et al., 2013; Rabbani et al., 2005). Application of synthetic SOD EUK-207 resulted in reduced wet desquamation, inflammation, and enhanced wound contracture in rat model of skin radiation injury (A Rosenthal et al., 2011).

Alpha-tocopherol (Vitamin E), is an antioxidant which protects membrane phospholipids from oxidative damage by scavenging the reactive oxygen species that are generated during oxidative stress (Patel & McGurk, 2017). It also inhibits protein kinase C activity, platelet aggregation, nitric oxide, and superoxide production in endothelial cells and macrophages, respectively (Azzi, Ricciarelli, & Zingg, 2002). Use of vitamin E has shown clinical benefits in clinics with a mean regression of 40% after 6 months of treatment (Sylvie Delanian, Porcher, Balla-Mekias, & Lefaix, 2003). In addition, vitamin E rinses have demonstrated radioprotection in head and neck cancer patients (Ferreira et al., 2004). The clinical benefits could only be achieved at high doses of vitamin E, which has reflected side effects in terms of increase in all-cause total mortality (E. R. Miller et al., 2005). A combined use of vitamin E and pentoxifylline, a methylxanthine derivative, has shown promising results (Delanian et al., 1999; Haddad, Kalaghchi, & Amouzegar-Hashemi, 2005). This combination therapy works by blocking the

TGF- β signaling in irradiated tissues (Hung, Huang, Chen, Lee, & Tsai, 2003; Lin et al., 2005).

5.3. Hyperbaric oxygen therapy for RIF

Hyperbaric oxygen therapy is a technique applied to treat hypoxic radiation wound by increasing the partial pressure of oxygen in blood. Although the mechanism of mitigation of hyperbaric oxygen in irradiated wounds is not clearly understood, is postulated that mitigation is due to the increase in local oxygen tension resulting in enhanced angiogenesis (Borab, Mirmanesh, Gantz, Cusano, & Pu, 2017). Patients are usually treated by application of 2–2.5 ATA pressure for 60–120 min (Bennett, Feldmeier, Hampson, Smees, & Milross, 2016). A systemic review of eight different studies concluded hyperbaric oxygen treatment to be a safe and effective treatment option for RIF (Borab et al., 2017). Another meta-analysis concluded efficacy of hyperbaric oxygen therapy for osteoradionecrosis in the head and neck and for radiation proctitis (Kalman, Zhao, Anscher, & Urdaneta, 2017).

5.4. Adipose tissue-based therapies for RIF

Autologous fat transfer (AFT) is a minimally invasive surgical procedure in the USA and is an emerging modality able to reverse and rejuvenate the fibrotic changes in skin (Borrelli et al., 2019; M. H. Lee et al., 2009). Adipose tissue is harvested with low morbidity from subcutaneous regions of the abdomen, flank, or hips using a hollow bore suction cannula (Strong, Cederna, Rubin, Coleman, & Levi, 2015) The harvest yields particles of adipose tissue, approximately 3–5 mm, consisting of adipocytes, vessel fragments, connective tissue, and ASCs. The harvested adipose tissue is injected into local tissues with specialized cannulas that can infiltrate scarred and fibrosed planes. First clinical evidence showing improvement in fibrotic skin wound and tissue function was provided by Rigotti et al. in 2007. Treating a group of twenty patients, they showed that injection of lipoaspirate enriched with stromal vascular fraction improved LENT-SOMA score, enhanced neovessel formation, and improved hydration of the irradiated skin (Rigotti et al., 2007). Succeeding studies confirmed the initial observation and showed improvement in clinical symptoms and scores (Lindgren et al., 2019; Mohan & Singh, 2017; Panettiere, Marchetti, & Accorsi, 2009; Phulpin et al., 2009; Serra-Renom, Muñoz-Olmo, & Serra-Mestre, 2010). While there is some experimental evidence that physical (Carlson, Longaker, & Thompson, 2003; Fluck et al., 1998; Grinnell, Zhu, Carlson, & Abrams, 1999) or dilutional (Verhoekx, Mudera, Walbeehm, & Hovius, 2013) effects of fat grafting may affect fibroblast survival or activity, most evidence suggests that adipose-derived stem cells (ASCs) are responsible for the beneficial effects of lipoaspirate injection in tissue with chronic radiation sequelae (Borovikova et al., 2018; Borrelli et al., 2019). ASCs, found in high concentrations in the perivascular region and throughout the adipose stroma, contribute to graft survival and vascularization by releasing angiogenic growth factors. Enzymatic digestion of adipose tissue with collagenase produce two main fractions: the adipocyte fraction and the stromal vascular fraction (SVF) (Berry et al., 2014). The SVF comprises of ASCs, lymphocytes, endothelial cells, pericytes, and fibroblasts (Berry & Rodeheffer, 2013). Immunophenotypically, ASCs are defined as CD34⁺CD90⁺CD29⁺CD45⁻CD31⁻ cells of mesenchymal origin, exhibiting tri-lineage differentiation capabilities into bone, cartilage, or fat (Asim Ejaz, Mattesich, & Zwerschke, 2017; Ejaz et al., 2016; Minteer, Marra, & Rubin, 2013; Varma et al., 2007; Zimmerlin, Donnenberg, Rubin, & Donnenberg, 2013). In white adipose tissues, these cells mainly reside in the vascular stroma around small blood vessels and can proliferate and differentiate into adipocytes (Horl et al., 2017; Tang et al., 2008; Zwierzina et al., 2015). ASCs exhibit similarities to bone marrow-derived mesenchymal stem cells (De Ugarte et al., 2003; Kern, Eichler, Stoeve, Kluter, & Bieback, 2006), but have multiple advantages including sample abundance in most patients, relative ease of harvest, ability to culture and amplify, and higher stem

cell yield in adipose tissue compared to bone marrow (De Ugarte et al., 2003; Guilak et al., 2006; Sterodimas, de Faria, Nicaretta, & Pitanguy, 2010).

5.4.1. Role of adipose-derived stem cells in mitigation of radiation induced fibrosis

Several properties of ASCs mainly attributed to a plethora of growth factors and cytokines released by them are suggested to contribute to the mitigation of radiation injury (Fig. 5):

- I. *Pro-angiogenic*. ASCs can differentiate into vascular endothelium and secrete growth factors such as vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF) that stimulate neoangiogenesis (Cao et al., 2005; Gehmert et al., 2011; Hausman & Richardson, 2004; Kilroy et al., 2007; Kinnaird et al., 2004; S. Liu et al., 2011; Matsuda et al., 2013; Philips et al., 2013; Philips, Marra, & Rubin, 2012; Rehman et al., 2004; H. Suga, Glotzbach, Sorkin, Longaker, & Gurtner, 2014). In addition, injection of ASCs facilitates the influx of bone marrow stem cells to fibrotic regions (Butala et al., 2010; A. Ejaz et al., 2019). Fat grafting enhances vascularization in fibrotic skin (Borovikova et al., 2018).
- II. *Antioxidant*. Decrease in vascularization of fibrotic tissue result in hypoxia and increased ROS production. In addition, influx of macrophages at the site of radiation injury further enhances ROS production and accumulation. Increase in vascularity upon ASCs treatment exert antioxidation effects. Several secreted factors such as insulin-like growth factor (IGF), HGF, pigment epithelium-derived factor (PEDF) and PDGF are purported to confer antioxidant properties of ASCs (W. S. Kim et al., 2008; W. S. Kim, Park, & Sung, 2009; Pinheiro et al., 2012). In addition, ASCs confer a direct anti-oxidant effect by upregulation of anti-oxidant markers like glutathione peroxidase (GPx), glutathione reductase, heme oxygenase, NAD(P)H quinone oxidoreductase, and endothelial nitric oxide (NO) synthase (Borovikova et al., 2018).

- III. *Immunomodulatory*. The role of ASCs in immunomodulation is complex; however, suppression of immune activity and anti-inflammatory effects have been observed (Gonzalez, Gonzalez-Rey, Rico, Buscher, & Delgado, 2009; Keyser, Beagles, & Kiem, 2007; Pinheiro et al., 2012; Puissant et al., 2005). ASCs exert immunosuppressive effect via release of factors like prostaglandin E2 and indoleamine 2,3- dioxygenase (DelaRosa et al., 2009; McIntosh et al., 2006a). In addition, ASCs have potent anti-inflammatory properties, interacting with innate and adaptive immune responses and capacity to down regulate T cell proliferation (McIntosh et al., 2006b; Plock et al., 2015; Plock et al., 2017; Waldner et al., 2018). ASCs mediate a phenotypic switch from pro-inflammatory switch of M1 macrophages to anti-inflammatory M2 type (Shang et al., 2015). Whole genome analysis of patients treated with AFT for fibrosis revealed modulation of inflammatory pathways (Lindegren et al., 2019).
- IV. *Regenerative*. Since the discovery of their pluripotency (Zuk et al., 2002), ASCs have been shown to differentiate into adipogenic, osteogenic, chondrogenic, myogenic, cardiomyogenic, and neurogenic-type cells (Philips et al., 2012; Strem et al., 2005; Tsuji, Rubin, & Marra, 2014). Regenerative ability of ASCs is in part attributed to their ability to withstand tissue hypoxia. Most of the mature adipocytes resorb after AFT but ASCs survive the hypoxic environment, proliferate, and differentiate into mature adipocytes (Mizuno et al., 2008).
- V. *Antiapoptotic*. Though the signaling mechanisms are unclear, treatment with ASCs has been shown to reduce apoptosis rates in endothelial cells and fibroblasts (W. S. Kim et al., 2008; Rehman et al., 2004).
- VI. *Antifibrotic*. Studies have shown a decrease in TGF- β gene expression in fibrotic tissue upon ASCs/lipoaspirate administration. Injection of ASCs result in a decrease in scar size, TGF- β expression, and scar remodeling due to increased MMP expression in minipigs (Yun et al., 2012). Using a rabbit model of radiation induced muscular fibrosis, Sun et al. has shown a decrease in collagenous fibrosis area and TGF- β 1 expression upon ASCs

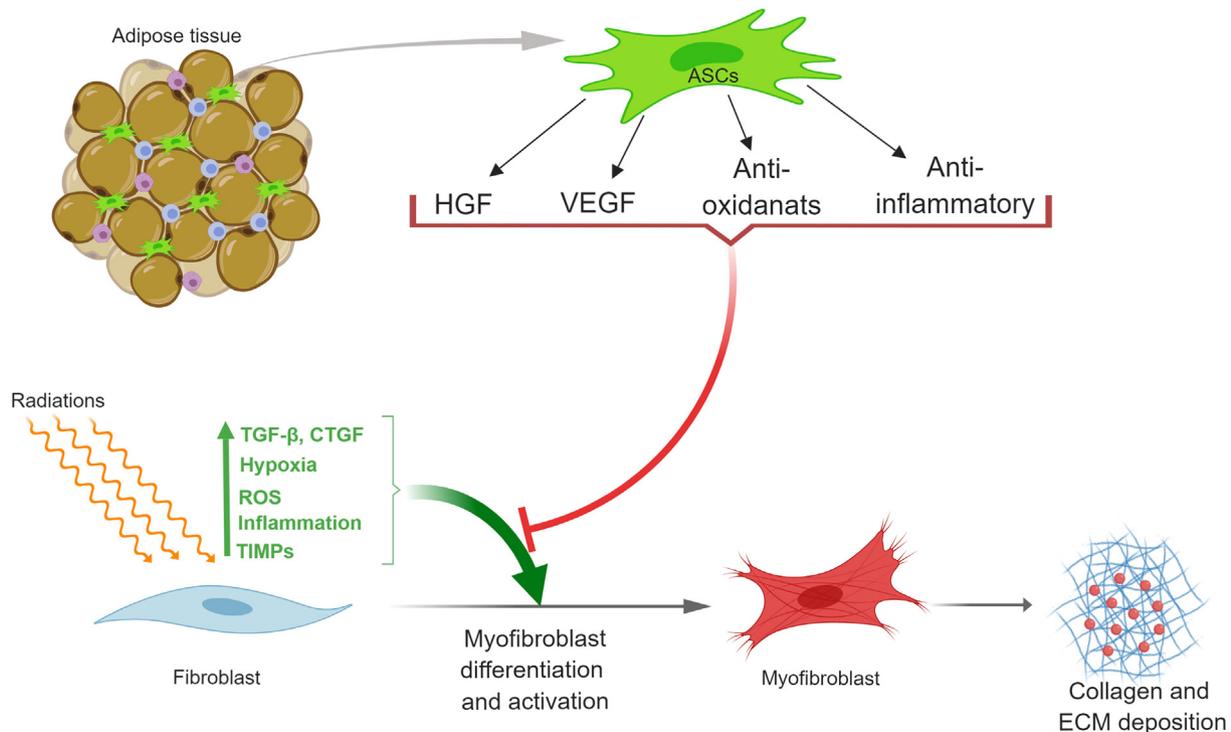


Fig. 5. Adipose derived stem cells regulate TGF- β signaling, inflammation, hypoxia, reactive oxygen species (ROS) levels, and tissue inhibitors of metalloproteinases (TIMPs) to attenuate radiation induced skin fibrosis.

therapy (Sun et al., 2016). The key adipogenesis transcription factor, peroxisome proliferator-activated receptor gamma, mediates antifibrotic effects via adiponectin and blocks the TGF- β -Smad signaling cascade (Fang et al., 2012). Advocating for a possible paracrine mechanism, ASC's conditioned medium reduced the expression of collagens in TGF- β treated human dermal fibroblasts (Spiekman et al., 2014). ASCs release basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and hepatocyte growth factor (HGF) in response to mechanical stress (Banyard et al., 2016). bFGF promotes HGF release by ASCs via the c-jun N-terminal kinase (JNK) pathway (Suga et al., 2009). bFGF mediates anti-fibrotic effects of ASC based therapies through induction of myofibroblasts apoptosis (Funato, Moriyama, Shimokawa, & Kuroda, 1997), collagen remodeling, MMP-1 upregulation, TIMP-1 downregulation and inhibition of TGF- β 1 signaling (Shi et al., 2013).

5.4.2. Hepatocyte growth factor is the key weapon for ASCs against fibrosis

HGF is a single inactive polypeptide secreted by mesenchymal cells and cleaved into its active form in the extracellular space by serine proteases. Like TGF- β , HGF is widely distributed in the extracellular matrix of most tissues. Its receptor, HGFR (Hepatocyte Growth Factor Receptor), is expressed by mesenchymal, endothelial, and epithelial cell lines. The receptor has a complex structure and triggers many intracellular pathways. Antifibrotic effects of HGF have been demonstrated in skin, myocardial, pulmonary, hepatic, and renal fibrosis (Cahill, Kennelly, Carty, Mahon, & English, 2016; Chen et al., 2016; Ejaz et al., 2019; Iekushi et al., 2010; Ogaly, Eltablawy, El-Beairy, El-Hindi, & Abd-Elsalam, 2015).

A direct role of HGF in blocking the nuclear translocation of TGF- β 1 has been depicted (Youhua Liu, 2004). HGF exerts anti-inflammatory effects in fibrotic tissue environment by reducing the

expression of TNF- α , INF- γ , MCP-1, and IL-12 through reduction in NF- κ B (Youhua Liu, 2004; Y Liu & Yang, 2006). Employing a transwell co-culture system, we observed that ASCs downregulate expression of fibrosis related genes TGF- β , CTGF, IL1, NF- κ B, TNF, and Collagen1-6 in co-cultured irradiated human foreskin fibroblasts. We observed HGF secreted by ASCs as the paracrine factor responsible for directly shutting down fibrosis related genes in damaged cells and recruiting bone marrow cells to irradiated tissue for damage repair (A. Ejaz et al., 2019) (Fig. 6). Although there is evidence of antifibrotic properties of ASCs (Yun et al., 2012), paradoxically they can also secrete profibrotic factors and stimulate the proliferation and migration of fibroblasts, but the overall outcome of ASCs treatment is remodeling of the fibrous tissue (W. S. Kim et al., 2007; R. Kumar, Griffin, Adigbli, Kalavrezos, & Butler, 2016; S. H. Lee, Jin, Song, Seo, & Cho, 2012).

5.5. Other therapeutic strategies

Statins play an important role in regulating cholesterol biosynthesis. Statins have shown a mitigation effect against RIF, mainly by downregulating inflammatory pathways mediated by TGF- β , TNF α , and Rho kinases (Fritz, Henninger, & Huelsenbeck, 2011; Kalman et al., 2017; Monceau et al., 2010; Ostrau et al., 2009). A phase II clinical study has demonstrated beneficial effects of statin use in reversing the RIF in head and neck squamous cell carcinomas patient treated with RT (Bourgier et al., 2019).

Direct inhibitors of inflammatory mediators of radiation fibrosis, TGF- β and TNF- α , have been investigated as a potential therapeutic approach. Halofuginone, a plant alkaloid which inhibits TGF- β , has shown to be effective in mitigating radiation-induced fibrosis in mice (Xavier et al., 2004). Inhibition of TGF- β also mitigated fibrosis in lungs (Dadrich et al., 2016). Similarly, blocking TNF- α mitigated radiation injury of the skin in mice (J. H. Kim, Jenrow, & Brown, 2014).

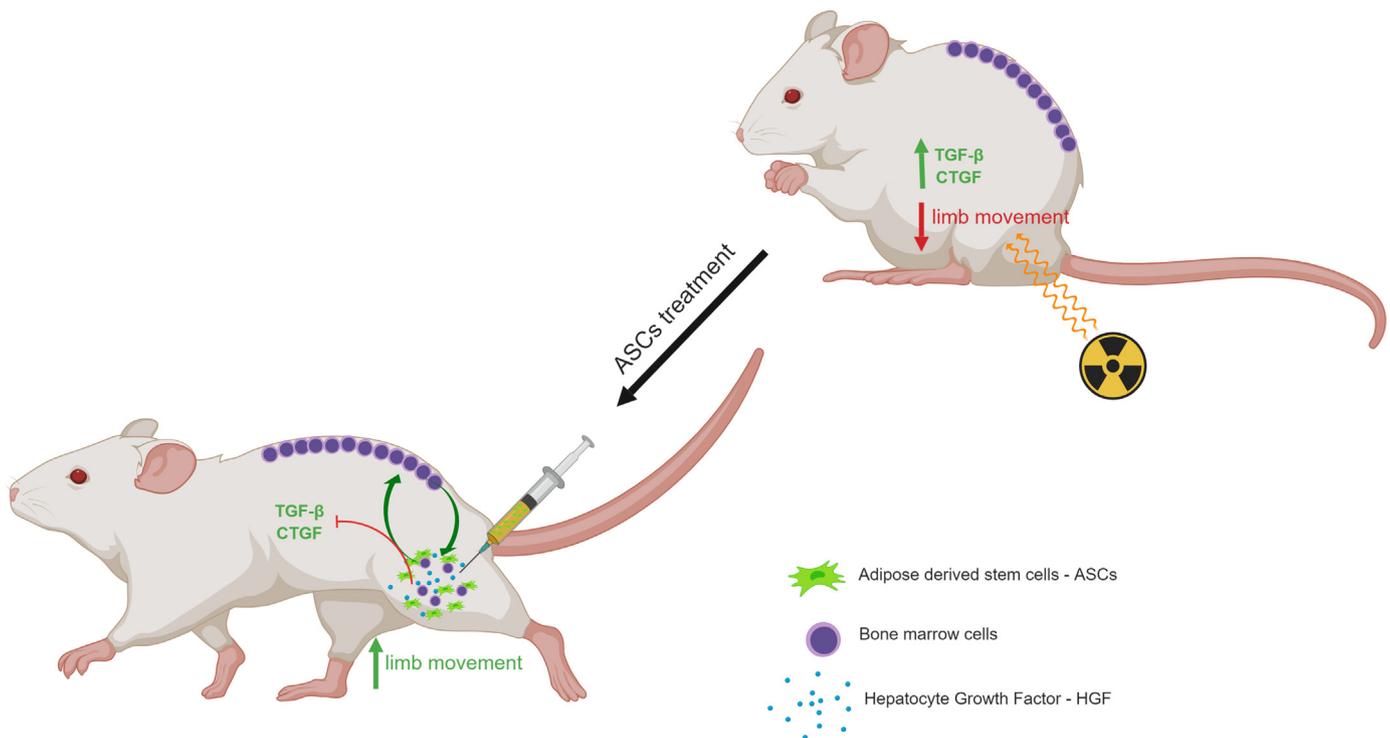


Fig. 6. Role of hepatocyte growth factor in mitigation of radiation induced fibrosis. Focus radiation dose of 35Gy to mouse hind limb leads to an upregulation of pro-fibrotic gene expression and loss of limb movement. Adipose derived stem cells release hepatocyte growth factor upon exposure to irradiated environment which mitigate fibrosis by: (1) down regulating the expression of pro-fibrotic genes TGF- β and CTGF, thus halting the progression of fibrosis and (2) promoting migration of bone marrow cells to irradiated tissue to participate in tissue regeneration.

6. Conclusion

Radiation therapy is the mainstay of modern cancer management. The field of radiation therapy has undergone enormous technological advancement enabling radiation oncologists to escalate therapy doses with minimal side effects. Despite the progress, radiation therapy exerts acute and late side effects on the surrounding tissues, thus limiting the intensity of radiation dose required for a precise loco-regional tumor control. Radiation induced fibrosis is one of the major late side effects which could have life-threatening consequences. The advancement in molecular biology techniques have enabled researchers to better understand the molecular mechanisms involved in RIF development. Currently there are very few therapy options with low efficacy. Stem cells therapies, particularly those using adipose tissue derived stem cells, have shown clinical promise in treating radiation fibrosis. Future research to elucidate the cellular and molecular mechanisms involved in development of RIF will help to improve current approaches and develop new therapeutical strategies.

Disclosure of potential conflicts of interest

The authors indicated no potential conflicts of interest.

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References

- Abraham, D. J., Eckes, B., Rajkumar, V., & Krieg, T. (2007). New developments in fibroblast and myofibroblast biology: Implications for fibrosis and scleroderma. *Current Rheumatology Reports* 9, 136–143.
- Abreu, J. G., Ketpura, N. L., Reversade, B., & De Robertis, E. (2002). Connective-tissue growth factor (CTGF) modulates cell signalling by BMP and TGF- β . *Nature Cell Biology* 4, 599.
- Ahamed, J., & Laurence, J. (2017). Role of platelet-derived transforming growth factor- β 1 and reactive oxygen species in radiation-induced organ fibrosis. *Antioxidants & Redox Signaling* 27, 977–988.
- Archambeau, J. O., Pezner, R., & Wasserman, T. (1995). Pathophysiology of irradiated skin and breast. *International Journal of Radiation Oncology* Biology* Physics* 31, 1171–1185.
- Azzam, E. I., Jay-Gerin, J.-P., & Pain, D. (2012). Ionizing radiation-induced metabolic oxidative stress and prolonged cell injury. *Cancer Letters* 327, 48–60.
- Azzi, A., Ricciarelli, R., & Zingg, J.-M. (2002). Non-antioxidant molecular functions of α -tocopherol (vitamin E). *FEBS Letters* 519, 8–10.
- Banyard, D. A., Sarantopoulos, C. N., Borovikova, A. A., Qiu, X., Wirth, G. A., Paydar, K. Z., ... Widgerow, A. D. (2016). Phenotypic analysis of stromal vascular fraction after mechanical shear reveals stress-induced progenitor populations. *Plastic and Reconstructive Surgery* 138, 237e–247e.
- Barnett, G. C., West, C. M., Dunning, A. M., Elliott, R. M., Coles, C. E., Pharoah, P. D., & Burnet, N. G. (2009). Normal tissue reactions to radiotherapy: Towards tailoring treatment dose by genotype. *Nature Reviews Cancer* 9, 134.
- Bastianutto, C., Mian, A., Symes, J., Mocanu, J., Alajez, N., Sleep, G., ... Gospodarowicz, M. (2007). Local radiotherapy induces homing of hematopoietic stem cells to the irradiated bone marrow. *Cancer Research* 67, 10112–10116.
- Beetz, A., Messer, G., Oppel, T., van Beuningen, D., Peter, R. U., & Kind, P. (1997). Induction of interleukin 6 by ionizing radiation in a human epithelial cell line: Control by corticosteroids. *International Journal of Radiation Biology* 72, 33–43.
- Bennett, M. H., Feldmeier, J., Hampson, N. B., Smees, R., & Milross, C. (2016). Hyperbaric oxygen therapy for late radiation tissue injury. *Cochrane Database of Systematic Reviews* 4 (CD005005).
- Bentzen, S. M. (2006). Preventing or reducing late side effects of radiation therapy: Radio-biology meets molecular pathology. *Nature Reviews Cancer* 6, 702.
- Berry, R., Church, C. D., Gericke, M. T., Jeffery, E., Colman, L., & Rodeheffer, M. S. (2014). Imaging of adipose tissue. *Methods in Enzymology* 537, 47–73.
- Berry, R., & Rodeheffer, M. S. (2013). Characterization of the adipocyte cellular lineage in vivo. *Nature Cell Biology* 15, 302–308.
- Bickelhaupt, S., Erbel, C., Timke, C., Wirkner, U., Dadrich, M., Flechsig, P., ... Peschke, P. (2017). Effects of CTGF blockade on attenuation and reversal of radiation-induced pulmonary fibrosis. *JNCI: Journal of the National Cancer Institute* 109, djw339.
- Blobe, G. C., Schiemann, W. P., & Lodish, H. F. (2000). Role of transforming growth factor β in human disease. *New England Journal of Medicine* 342, 1350–1358.
- Borab, Z., Mirmanesh, M. D., Gantz, M., Cusano, A., & Pu, L. L. (2017). Systematic review of hyperbaric oxygen therapy for the treatment of radiation-induced skin necrosis. *Journal of Plastic, Reconstructive & Aesthetic Surgery* 70, 529–538.
- Borovikova, A. A., Ziegler, M. E., Banyard, D. A., Wirth, G. A., Paydar, K. Z., Evans, G. R., & Widgerow, A. D. (2018). Adipose-derived tissue in the treatment of dermal fibrosis: Antifibrotic effects of adipose-derived stem cells. *Annals of Plastic Surgery* 80, 297–307.
- Borrelli, M. R., Patel, R. A., Sokol, J., Nguyen, D., Momeni, A., Longaker, M. T., & Wan, D. C. (2019). Fat chance: The rejuvenation of irradiated skin. *Plastic and Reconstructive Surgery Global Open* 7.
- Bourgier, C., Auperin, A., Rivera, S., Boisselier, P., Petit, B., Lang, P., ... Azria, D. (2019). Pravastatin reverses established radiation-induced cutaneous and subcutaneous fibrosis in patients with head and neck cancer: Results of the biology-driven phase II clinical trial pravacur. *International Journal of Radiation Oncology* Biology* Physics* 104(2), 365–373.
- Bray, F. N., Simmons, B. J., Wolfson, A. H., & Nouri, K. (2016). Acute and chronic cutaneous reactions to ionizing radiation therapy. *Dermatology and Therapy* 6, 185–206.
- Brittan, M., Hunt, T., Jeffery, R., Poulson, R., Forbes, S., Hodiola-Dilke, K., ... Wright, N. (2002). Bone marrow derivation of pericycral myofibroblasts in the mouse and human small intestine and colon. *Gut* 50, 752–757.
- Brush, J., Lipnick, S. L., Phillips, T., Sitko, J., McDonald, J. T., & McBride, W. H. (2007). Molecular mechanisms of late normal tissue injury. *Seminars in Radiation Oncology*. Vol. 17. (pp. 121–130). Elsevier.
- Bryant, A. K., Banegas, M. P., Martinez, M. E., Mell, L. K., & Murphy, J. D. (2017). Trends in radiation therapy among cancer survivors in the United States, 2000–2030. *Cancer Epidemiology and Prevention Biomarkers* 26, 963–970.
- Butala, P., Sultan, S., Davidson, E., Crawford, J., Szpalski, C., Knobel, D., ... Coleman, S. (2010). 6: Augmentation of fat graft survival with progenitor cell mobilization. *Plastic and Reconstructive Surgery* 125, 12.
- Cahill, E. F., Kenedy, H., Carty, F., Mahon, B. P., & English, K. (2016). Hepatocyte growth factor is required for mesenchymal stromal cell protection against bleomycin-induced pulmonary fibrosis. *Stem Cells Translational Medicine* 5, 1307–1318.
- Campana, F., Zervoudis, S., Perdereau, B., Gez, E., Fourquet, A., Badiu, C., ... Koukouloglou, S. (2004). Topical superoxide dismutase reduces post-irradiation breast cancer fibrosis. *Journal of Cellular and Molecular Medicine* 8, 109–116.
- Cao, Y., Sun, Z., Liao, L., Meng, Y., Han, Q., & Zhao, R. C. (2005). Human adipose tissue-derived stem cells differentiate into endothelial cells in vitro and improve postnatal neovascularization in vivo. *Biochemical and Biophysical Research Communications* 332, 370–379.
- Carlson, M. A., Longaker, M. T., & Thompson, J. S. (2003). Wound splinting regulates granulation tissue survival. *Journal of Surgical Research* 110, 304–309.
- Carvalho, H. A., & Villar, R. C. (2018). Radiotherapy and immune response: The systemic effects of a local treatment. *Clinics (Sao Paulo)* 73, e557s.
- Chen, H., Xia, R., Li, Z., Zhang, L., Xia, C., Ai, H., ... Guo, Y. (2016). Mesenchymal stem cells combined with hepatocyte growth factor therapy for attenuating ischaemic myocardial fibrosis: Assessment using multimodal molecular imaging. *Scientific Reports* 6, 33700.
- Cheng, Q., Li, C., Yang, C.-F., Zhong, Y.-J., Wu, D., Shi, L., ... Li, L. (2019). Methyl ferulic acid attenuates liver fibrosis and hepatic stellate cell activation through the TGF- β 1/Smad and NOX4/ROS pathways. *Chemico-Biological Interactions* 299, 131–139.
- Citrin, D. E. (2017). Recent developments in radiotherapy. *New England Journal of Medicine* 377, 1065–1075.
- Dadrich, M., Nicolay, N. H., Flechsig, P., Bickelhaupt, S., Hoeltgen, L., Roeder, F., ... Lopez, R. (2016). Combined inhibition of TGF β and PDGF signaling attenuates radiation-induced pulmonary fibrosis. *Oncotarget* 7, e123366.
- De Ruyscher, D., Niedermann, G., Burnet, N. G., Siva, S., Lee, A. W., & Hegi-Johnson, F. (2019). Radiotherapy toxicity. *Nature Reviews Disease Primers* 5, 13.
- De Ugarte, D. A., Morizono, K., Elbarbary, A., Alfonso, Z., Zuk, P. A., Zhu, M., ... Hedrick, M. H. (2003). Comparison of multi-lineage cells from human adipose tissue and bone marrow. *Cells, Tissues, Organs* 174, 101–109.
- Deckers, J., Hammad, H., & Hoste, E. (2018). Langerhans cells: Sensing the environment in health and disease. *Frontiers in Immunology* 9, 93.
- Del Rosso, J. Q., & Bikowski, J. (2008). Trolamine-containing topical emulsion: Clinical applications in dermatology. *CUTIS-New York* 81, 209.
- Delanian, S., Baillet, F., Huart, J., Lefaix, J.-L., Maulard, C., & Housset, M. (1994). Successful treatment of radiation-induced fibrosis using liposomal Cu Zn superoxide dismutase: Clinical trial. *Radiotherapy and Oncology* 32, 12–20.
- Delanian, S., Balla-Mekias, S., & Lefaix, J.-L. (1999). Striking regression of chronic radiotherapy damage in a clinical trial of combined pentoxifylline and tocopherol. *Journal of Clinical Oncology* 17, 3283–3290.
- Delanian, S., & Lefaix, J.-L. (2007). Current management for late normal tissue injury: Radiation-induced fibrosis and necrosis. *Seminars in Radiation Oncology*. Vol. 17. (pp. 99–107). Elsevier.
- Delanian, S., Porcher, R., Balla-Mekias, S., & Lefaix, J.-L. (2003). Randomized, placebo-controlled trial of combined pentoxifylline and tocopherol for regression of superficial radiation-induced fibrosis. *Journal of Clinical Oncology* 21, 2545–2550.
- DelaRosa, O., Lombardo, E., Beraza, A., Mancheco-Corvo, P., Ramirez, C., Menta, R., ... Abad, J. L. (2009). Requirement of IFN- γ -mediated indoleamine 2, 3-dioxygenase expression in the modulation of lymphocyte proliferation by human adipose-derived stem cells. *Tissue Engineering Part A* 15, 2795–2806.
- Denham, J. W., & Hauer-Jensen, M. (2002). The radiotherapeutic injury—a complex ‘wound’. *Radiotherapy and Oncology* 63, 129–145.
- Denis, F., Garaud, P., Bardet, E., Alfonsi, M., Sire, C., Germain, T., ... Oudinot, P. (2003). Late toxicity results of the GORTEC 94-01 randomized trial comparing radiotherapy with concomitant radiochemotherapy for advanced-stage oropharynx carcinoma: Comparison of LENT/SOMA, RTOG/EORTC, and NCI-CTC scoring systems. *International Journal of Radiation Oncology* Biology* Physics* 55, 93–98.
- Derynck, R., & Budi, E. H. (2019). Specificity, versatility, and control of TGF- β family signaling. *Sci. Signal.* 12 eaav5183.

- DeSantis, C. E., Lin, C. C., Mariotto, A. B., Siegel, R. L., Stein, K. D., Kramer, J. L., ... Jemal, A. (2014). Cancer treatment and survivorship statistics, 2014. *CA: a Cancer Journal for Clinicians* 64, 252–271.
- Di Maggio, F. M., Minafra, L., Forte, G. I., Cammarata, F. P., Lio, D., Messa, C., ... Bravatà, V. (2015). Portrait of inflammatory response to ionizing radiation treatment. *Journal of Inflammation* 12, 14.
- Dutta, A., Li, J., Fedele, C., Sayeed, A., Singh, A., Violette, S. M., ... Languino, L. R. (2015). $\alpha\text{v}\beta 6$ integrin is required for TGF β 1-mediated matrix metalloproteinase2 expression. *Biochemical Journal* 466, 525–536.
- Ehrhart, E., Segarini, P., Tsang, M., Carroll, A. G., & Barcellos-Hoff, M. -H. (1997). Latent transforming growth factor beta1 activation in situ: Quantitative and functional evidence after low-dose gamma-irradiation. *The FASEB Journal* 11, 991–1002.
- Ejaz, A., Epperly, M. W., Hou, W., Greenberger, J. S., & Rubin, P. J. (2019). Adipose-derived stem cell therapy ameliorates ionizing irradiation fibrosis (RIF) via hepatocyte growth factor mediated TGF-beta down regulation and recruitment of bone marrow cells. *Stem Cells* 37(6), 791–802.
- Ejaz, A., Mattesich, M., & Zwerschke, W. (2017). Silencing of the small GTPase DIRAS3 induces cellular senescence in human white adipose stromal/progenitor cells. *Aging (Albany NY)* 9, 860.
- Ejaz, A., Mitterberger, M. C., Lu, Z., Mattesich, M., Zwierzina, M. E., Hörl, S., ... Meryk, A. (2016). Weight loss upregulates the small GTPase DIRAS3 in human white adipose progenitor cells, which negatively regulates adipogenesis and activates autophagy via Akt-mTOR inhibition. *EBioMedicine* 6, 149–161.
- Fang, F., Liu, L., Yang, Y., Tamaki, Z., Wei, J., Marangoni, R. G., ... Varga, J. (2012). The adipokine adiponectin has potent anti-fibrotic effects mediated via adenosine monophosphate-activated protein kinase: Novel target for fibrosis therapy. *Arthritis Research & Therapy* 14, R229.
- Ferreira, P. R., Fleck, J. F., Diehl, A., Barletta, D., Braga-Filho, A., Barletta, A., & Ilha, L. (2004). Protective effect of alpha-tocopherol in head and neck cancer radiation-induced mucositis: A double-blind randomized trial. *Head & Neck: Journal for the Sciences and Specialties of the Head and Neck* 26, 313–321.
- Flanders, K., Sato, M., Ooshima, A., Russo, A., & Roberts, A. (2004). Smad-3 as a mediator of the fibrotic response. *International Journal of Experimental Pathology* 85(2), 47–64.
- Fluck, J., Querfeld, C., Cremer, A., Niland, S., Krieg, T., & Sollberg, S. (1998). Normal human primary fibroblasts undergo apoptosis in three-dimensional contractile collagen gels. *Journal of Investigative Dermatology* 110, 153–157.
- Forbes, S. J., Russo, F. P., Rey, V., Burra, P., Rugege, M., Wright, N. A., & Alison, M. R. (2004). A significant proportion of myofibroblasts are of bone marrow origin in human liver fibrosis. *Gastroenterology* 126, 955–963.
- Fritz, G., Henninger, C., & Huelsenbeck, J. (2011). Potential use of HMG-CoA reductase inhibitors (statins) as radioprotective agents. *British Medical Bulletin* 97, 17–26.
- Funato, N., Moriyama, K., Shimokawa, H., & Kuroda, T. (1997). Basic fibroblast growth factor induces apoptosis in myofibroblastic cells isolated from rat palatal mucosa. *Biochemical and Biophysical Research Communications* 240, 21–26.
- Gehmert, S., Gehmert, S., Hidayat, M., Sultan, M., Berner, A., Klein, S., ... Prantl, L. (2011). Angiogenesis: The role of PDGF-BB on adipose-tissue derived stem cells (ASCs). *Clinical Hemorheology and Microcirculation* 48, 5–13.
- Gerber, S. A., Cummings, R. J., Judge, J. L., Barlow, M. L., Nanduri, J., Johnson, D. E. M., ... Ryan, J. L. (2015). Interleukin-12 preserves the cutaneous physical and immunological barrier after radiation exposure. *Radiation Research* 183, 72–81.
- Goffin, J. M., Pittet, P., Csucs, G., Lussi, J. W., Meister, J. -J., & Hinz, B. (2006). Focal adhesion size controls tension-dependent recruitment of α -smooth muscle actin to stress fibers. *The Journal of Cell Biology* 172, 259–268.
- Gonzalez, M. A., Gonzalez-Rey, E., Rico, L., Buscher, D., & Delgado, M. (2009). Adipose-derived mesenchymal stem cells alleviate experimental colitis by inhibiting inflammatory and autoimmune responses. *Gastroenterology* 136, 978–989.
- Gore-Hyer, E., Shegogue, D., Markiewicz, M., Lo, S., Hazen-Martin, D., Greene, E. L., ... Trojanowska, M. (2002). TGF- β and CTGF have overlapping and distinct fibrogenic effects on human renal cells. *American Journal of Physiology-Renal Physiology* 283, F707–F716.
- Gottlöber, P., Steinert, M., Weiss, M., Bebeskio, V., Belyi, D., Nadejina, N., ... Peter, R. U. (2001). The outcome of local radiation injuries: 14 years of follow-up after the Chernobyl accident. *Radiation Research* 155, 409–416.
- Govindan, R., & DeVita, V. T. (2009). *DeVita, Hellman, and Rosenberg's cancer: Principles & practice of oncology review*. Lippincott Williams & Wilkins.
- Grinnell, F., Zhu, M., Carlson, M. A., & Abrams, J. M. (1999). Release of mechanical tension triggers apoptosis of human fibroblasts in a model of regressing granulation tissue. *Experimental Cell Research* 248, 608–619.
- Groves, A. M., Johnston, C. J., Williams, J. P., & Finkelstein, J. N. (2018). Role of infiltrating monocytes in the development of radiation-induced pulmonary fibrosis. *Radiation Research* 189, 300–311.
- Guilak, F., Lott, K. E., Awad, H. A., Cao, Q., Hicok, K. C., Fermor, B., & Gimple, J. M. (2006). Clonal analysis of the differentiation potential of human adipose-derived adult stem cells. *Journal of Cellular Physiology* 206, 229–237.
- Gurtner, G. C., Werner, S., Barrandon, Y., & Longaker, M. T. (2008). Wound repair and regeneration. *Nature* 453, 314.
- Haddad, P., Kalaghchi, B., & Amouzegar-Hashemi, F. (2005). Pentoxifylline and vitamin E combination for superficial radiation-induced fibrosis: A phase II clinical trial. *Radiotherapy and Oncology* 77, 324–326.
- Harper, J. L., Franklin, L. E., Jenrette, J. M., & Aguero, E. G. (2004). Skin toxicity during breast irradiation: Pathophysiology and management. *Southern Medical Journal* 97, 989–994.
- Hata, A., & Chen, Y. -G. (2016). TGF- β signaling from receptors to Smads. *Cold Spring Harbor Perspectives in Biology* 8, a022061.
- Hausman, G. J., & Richardson, R. L. (2004). Adipose tissue angiogenesis. *Journal of Animal Science* 82, 925–934.
- Hawkins, M. M. (2004). Long-term survivors of childhood cancers: What knowledge have we gained? *Nature Reviews Clinical Oncology* 1, 26.
- Hill, C. S. (2016). Transcriptional control by the SMADs. *Cold Spring Harbor Perspectives in Biology* 8, a022079.
- Hinz, B. (2010). The myofibroblast: Paradigm for a mechanically active cell. *Journal of Biomechanics* 43, 146–155.
- Hinz, B., Celetta, G., Tomasek, J. J., Gabbiani, G., & Chaponnier, C. (2001). Alpha-smooth muscle actin expression upregulates fibroblast contractile activity. *Molecular Biology of the Cell* 12, 2730–2741.
- Hinz, B., Phan, S. H., Thannickal, V. J., Galli, A., Bochaton-Piallat, M. -L., & Gabbiani, G. (2007). The myofibroblast: One function, multiple origins. *The American Journal of Pathology* 170, 1807–1816.
- Hoeller, U., Tribius, S., Kuhlmei, A., Grader, K., Fehlaue, F., & Alberti, W. (2003). Increasing the rate of late toxicity by changing the score? A comparison of RTOG/EORTC and LENT/SOMA scores. *International Journal of Radiation Oncology* Biology* Physics* 55, 1013–1018.
- Horl, S., Ejaz, A., Ernst, S., Mattesich, M., Kaiser, A., Jenewein, B., ... Zwerschke, W. (2017). CD146 (MCAM) in human cs-DLK1 (-)/cs-CD34 (+) adipose stromal/progenitor cells. *Stem Cell Research* 22, 1–12.
- Housri, N., Yarchoan, R., & Kaushal, A. (2010). Radiotherapy for patients with the human immunodeficiency virus: Are special precautions necessary? *Cancer: Interdisciplinary International Journal of the American Cancer Society* 116, 273–283.
- Hung, K. Y., Huang, J. W., Chen, C. T., Lee, P. H., & Tsai, T. J. (2003). Pentoxifylline modulates intracellular signalling of TGF- β in cultured human peritoneal mesothelial cells: Implications for prevention of encapsulating peritoneal sclerosis. *Nephrology Dialysis Transplantation* 18, 670–676.
- Hymes, S. R., Strom, E. A., & Fife, C. (2006). Radiation dermatitis: Clinical presentation, pathophysiology, and treatment 2006. *Journal of the American Academy of Dermatology* 54, 28–46.
- Iannuzzi, C. M., Atencio, D. P., Green, S., Stock, R. G., & Rosenstein, B. S. (2002). ATM mutations in female breast cancer patients predict for an increase in radiation-induced late effects. *International Journal of Radiation Oncology* Biology* Physics* 52, 606–613.
- Iekushi, K., Taniyama, Y., Azuma, J., Sanada, F., Kusunoki, H., Yokoi, T., ... Morishita, R. (2010). Hepatocyte growth factor attenuates renal fibrosis through TGF- β 1 suppression by apoptosis of myofibroblasts. *Journal of Hypertension* 28, 2454–2461.
- Jenkins, G. (2008). The role of proteases in transforming growth factor- β activation. *The International Journal of Biochemistry & Cell Biology* 40, 1068–1078.
- Johansson, S., Svensson, H., & Denekamp, J. (2000). Timescale of evolution of late radiation injury after postoperative radiotherapy of breast cancer patients. *International Journal of Radiation Oncology* Biology* Physics* 48, 745–750.
- Jones, R. E., Foster, D. S., Hu, M. S., & Longaker, M. T. (2019). Wound healing and fibrosis: Current stem cell therapies. *Transfusion* 59, 884–892.
- Jung, J. -W., Hwang, S. -Y., Hwang, J. -S., Oh, E. -S., Park, S., & Han, I. -O. (2007). Ionising radiation induces changes associated with epithelial-mesenchymal transdifferentiation and increased cell motility of A549 lung epithelial cells. *European Journal of Cancer* 43, 1214–1224.
- Kalman, N. S., Zhao, S. S., Anscher, M. S., & Urdaneta, A. I. (2017). Current status of targeted radioprotection and radiation injury mitigation and treatment agents: A critical review of the literature. *International Journal of Radiation Oncology* Biology* Physics* 98, 662–682.
- Katsuno, Y., Qin, J., Oses-Prieto, J., Wang, H., Jackson-Weaver, O., Zhang, T., ... Xu, J. (2018). Arginine methylation of SMAD7 by PRMT1 in TGF- β -induced epithelial-mesenchymal transition and epithelial stem-cell generation. *Journal of Biological Chemistry* 293, 13059–13072.
- Kern, S., Eichler, H., Stoeve, J., Kluter, H., & Bieback, K. (2006). Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. *Stem Cells* 24, 1294–1301.
- Keyser, K. A., Beagles, K. E., & Kiem, H. P. (2007). Comparison of mesenchymal stem cells from different tissues to suppress T-cell activation. *Cell Transplantation* 16, 555–562.
- Kilroy, G. E., Foster, S. J., Wu, X., Ruiz, J., Sherwood, S., Heifetz, A., ... Gimple, J. M. (2007). Cytokine profile of human adipose-derived stem cells: Expression of angiogenic, hematopoietic, and pro-inflammatory factors. *Journal of Cellular Physiology* 212, 700–709.
- Kim, J. H., Jenrow, K. A., & Brown, S. L. (2014). Mechanisms of radiation-induced normal tissue toxicity and implications for future clinical trials. *Radiation Oncology Journal* 32, 103.
- Kim, J. H., Kolozsvary, A. J., Jenrow, K. A., & Brown, S. L. (2013). *Mechanisms of radiation-induced skin injury and implications for future clinical trials*. Taylor & Francis.
- Kim, W. S., Park, B. S., Kim, H. K., Park, J. S., Kim, K. J., Choi, J. S., ... Sung, J. H. (2008). Evidence supporting antioxidant action of adipose-derived stem cells: Protection of human dermal fibroblasts from oxidative stress. *Journal of Dermatological Science* 49, 133–142.
- Kim, W. S., Park, B. S., & Sung, J. H. (2009). The wound-healing and antioxidant effects of adipose-derived stem cells. *Expert Opinion on Biological Therapy* 9, 879–887.
- Kim, W. S., Park, B. S., Sung, J. H., Yang, J. M., Park, S. B., Kwak, S. J., & Park, J. S. (2007). Wound healing effect of adipose-derived stem cells: A critical role of secretory factors on human dermal fibroblasts. *Journal of Dermatological Science* 48, 15–24.
- Kinnaird, T., Stabile, E., Burnett, M. S., Shou, M., Lee, C. W., Barr, S., ... Epstein, S. E. (2004). Local delivery of marrow-derived stromal cells augments collateral perfusion through paracrine mechanisms. *Circulation* 109, 1543–1549.
- Koh, H. B., Scraggs, A. M., & Huang, S. K. (2016). Transforming growth factor- β 1 increases DNA methyltransferase 1 and 3a expression through distinct post-transcriptional mechanisms in lung fibroblasts. *Journal of Biological Chemistry* 291, 19287–19298.
- Komi, D. E. A., Khomtchouk, K., & Santa Maria, P. L. (2019). A review of the contribution of mast cells in wound healing: Involved molecular and cellular mechanisms. *Clinical Reviews in Allergy & Immunology*, 1–15.

- Kumar, R., Griffin, M., Adigbli, G., Kalavrezos, N., & Butler, P. E. (2016). Lipotransfer for radiation-induced skin fibrosis. *British Journal of Surgery* 103, 950–961.
- Kumar, V. (2005). Tissue renewal and repair: Regeneration, healing, and fibrosis. *Pathologic Basis of Disease*, 87–118.
- Lahtz, C., Bates, S. E., Jiang, Y., Li, A. X., Wu, X., Hahn, M. A., & Pfeifer, G. P. (2012). Gamma irradiation does not induce detectable changes in DNA methylation directly following exposure of human cells. *PLoS One* 7, e44858.
- Lainé, M., Gupta, N., Kirsh, O., Pradhan, S., & Defossez, P.-A. (2018). Mechanisms of DNA methyltransferase recruitment in mammals. *Genes* 9, 617.
- Lawrence, D. A. (2001). Latent-TGF- β : An overview. *Molecular and Cellular Biochemistry* 219, 163–170.
- Lawrence, M., Daujat, S., & Schneider, R. (2016). Lateral thinking: How histone modifications regulate gene expression. *Trends in Genetics* 32, 42–56.
- Leask, A., & Abraham, D. J. (2004). TGF- β signaling and the fibrotic response. *The FASEB Journal* 18, 816–827.
- Lee, M. H., Arcidiacono, J. A., Bilek, A. M., Wille, J. J., Hamill, C. A., Wonnacott, K. M., ... Oh, S. S. (2009). Considerations for tissue-engineered and regenerative medicine product development prior to clinical trials in the United States. *Tissue Engineering Part B: Reviews* 16, 41–54.
- Lee, S. H., Jin, S. Y., Song, J. S., Seo, K. K., & Cho, K. H. (2012). Paracrine effects of adipose-derived stem cells on keratinocytes and dermal fibroblasts. *Annals of Dermatology* 24, 136–143.
- Lefaix, J.-L., Delanian, S., Leplat, J.-J., Tricaud, Y., Martin, M., Nimrod, A., ... Daburon, F. (1996). Successful treatment of radiation-induced fibrosis using CuZn-SOD and Mn-SOD: An experimental study. *International Journal of Radiation Oncology* Biology* Physics* 35, 305–312.
- Liao, W., Hei, T. K., & Cheng, S. K. (2017). Radiation-induced dermatitis is mediated by IL17-expressing $\gamma\delta$ T cells. *Radiation Research* 187, 464–474.
- Liéart, S., Merceron, R., Vanderaa, C., Lambert, F., Colau, D., Stockis, J., ... Coulie, P. G. (2018). Structural basis of latent TGF- β 1 presentation and activation by GARP on human regulatory T cells. *Science* 362, 952–956.
- Lin, S.-L., Chen, R.-H., Chen, Y.-M., Chiang, W.-C., Lai, C.-F., Wu, K.-D., & Tsai, T.-J. (2005). Pentoxifylline attenuates tubulointerstitial fibrosis by blocking Smad3/4-activated transcription and profibrogenic effects of connective tissue growth factor. *Journal of the American Society of Nephrology* 16, 2702–2713.
- Lindgren, A., Schultz, L., Sinha, I., Cheung, L., Khan, A., Tekle, M., ... Halle, M. (2019). Autologous fat transplantation alters gene expression patterns related to inflammation and hypoxia in the irradiated human breast. *British Journal of Surgery* 106 (5), 563–573.
- Lipson, K. E., Wong, C., Teng, Y., & Spong, S. (2012). CTGF is a central mediator of tissue remodeling and fibrosis and its inhibition can reverse the process of fibrosis. *Fibrogenesis & Tissue Repair*. Vol. 5. (pp. S24) BioMed Central.
- Liu, R.-M., & Desai, L. P. (2015). Reciprocal regulation of TGF- β and reactive oxygen species: A perverse cycle for fibrosis. *Redox Biology* 6, 565–577.
- Liu, S., Zhang, H., Zhang, X., Lu, W., Huang, X., Xie, H., ... Jin, Y. (2011). Synergistic angiogenesis promoting effects of extracellular matrix scaffolds and adipose-derived stem cells during wound repair. *Tissue Engineering. Part A* 17, 725–739.
- Liu, Y. (2004). Hepatocyte growth factor in kidney fibrosis: Therapeutic potential and mechanisms of action. *American Journal of Physiology-Renal Physiology* 287, F7–F16.
- Liu, Y., & Yang, J. (2006). Hepatocyte growth factor: New arsenal in the fights against renal fibrosis? *Kidney International* 70, 238–240.
- Ma, Y., Qi, M., An, Y., Zhang, L., Yang, R., Doro, D. H., ... Jin, Y. (2017). Autophagy controls mesenchymal stem cell properties and senescence during bone aging. *Aging Cell* 17 (1), e12709.
- MacLeod, A. S., Rudolph, R., Corriden, R., Ye, I., Garijo, O., & Havran, W. L. (2014). Skin-resident T cells sense ultraviolet radiation-induced injury and contribute to DNA repair. *The Journal of Immunology* 192, 5695–5702.
- Marconi, R., Serafini, A., Giovanetti, A., Bartoleschi, C., Pardini, M. C., Bossi, G., & Strigari, L. (2019). Cytokine modulation in breast cancer patients undergoing radiotherapy: A revision of the most recent studies. *International Journal of Molecular Sciences* 20, 382.
- Martin, M., Lefaix, J.-L., & Delanian, S. (2000). TGF- β 1 and radiation fibrosis: A master switch and a specific therapeutic target? *International Journal of Radiation Oncology* Biology* Physics* 47, 277–290.
- Martin, M., Vozenin, M., Gault, N., Crechet, F., Pfarr, C., & Lefaix, J. (1997). Coactivation of AP-1 activity and TGF- β 1 gene expression in the stress response of normal skin cells to ionizing radiation. *Oncogene* 15, 981.
- Massagué, J. (1990). The transforming growth factor-beta family. *Annual Review of Cell Biology* 6, 597–641.
- Massagué, J. (1998). TGF- β signal transduction. *Annual reviews 4139 El Camino way, PO Box 10139, Palo Alto, CA 94303-0139, USA*.
- Massagué, J. (2012). TGF β signalling in context. *Nature Reviews Molecular Cell Biology* 13, 616.
- Matsuda, K., Falkenberg, K. J., Woods, A. A., Choi, Y. S., Morrison, W. A., & Dille, R. J. (2013). Adipose-derived stem cells promote angiogenesis and tissue formation for in vivo tissue engineering. *Tissue Engineering. Part A* 19, 1327–1335.
- McIntosh, K., Zvonic, S., Garrett, S., Mitchell, J. B., Floyd, Z. E., Hammill, L., ... Storms, R. W. (2006a). The immunogenicity of human adipose-derived cells: Temporal changes in vitro. *Stem Cells* 24, 1246–1253.
- McIntosh, K., Zvonic, S., Garrett, S., Mitchell, J. B., Floyd, Z. E., Hammill, L., ... Gimble, J. M. (2006b). The immunogenicity of human adipose-derived cells: Temporal changes in vitro. *Stem Cells* 24, 1246–1253.
- McQuestion, M. (2011). Evidence-based skin care management in radiation therapy: Clinical update. *Seminars in oncology nursing*. Vol. 27. (pp. e1–e17). Elsevier.
- Mendelsohn, F. A., Divino, C. M., Reis, E. D., & Kerstein, M. D. (2002). Wound care after radiation therapy. *Advances in Skin & Wound Care* 15, 216–224.
- Meneses, A. G. D., Reis, P. E. D. D., Guerra, E. N. S., Canto, G. D. L., & Ferreira, E. B. (2018). Use of trolamine to prevent and treat acute radiation dermatitis: A systematic review and meta-analysis. *Revista Latino-Americana de Enfermagem* 26.
- Meng, X.-M., Nikolic-Paterson, D. J., & Lan, H. Y. (2016). TGF- β : The master regulator of fibrosis. *Nature Reviews Nephrology* 12, 325.
- Meziani, L., Deutsch, E., & Mondini, M. (2018). Macrophages in radiation injury: A new therapeutic target. *Oncimmunology* 7, e1494488.
- Miller, E. R., Pastor-Barriuso, R., Dalal, D., Riemersma, R. A., Appel, L. J., & Guallar, E. (2005). Meta-analysis: High-dosage vitamin E supplementation may increase all-cause mortality. *Annals of Internal Medicine* 142, 37–46.
- Minteer, D., Marra, K. G., & Rubin, J. P. (2013). Adipose-derived mesenchymal stem cells: Biology and potential applications. *Advances in Biochemical Engineering/Biotechnology* 129, 59–71.
- Mizuno, H., Itoi, Y., Kawahara, S., Ogawa, R., Akaishi, S., & Hyakusoku, H. (2008). In vivo adipose tissue regeneration by adipose-derived stromal cells isolated from GFP transgenic mice. *Cells, Tissues, Organs* 187, 177–185.
- Mohan, A., & Singh, S. (2017). Use of fat transfer to treat a chronic, non-healing, post-radiation ulcer: A case study. *Journal of Wound Care* 26, 272–273.
- Monceau, V., Pasinetti, N., Schupp, C., Pouzoulet, F., Opolon, P., & Vozenin, M.-C. (2010). Modulation of the rho/ROCK pathway in heart and lung after thorax irradiation reveals targets to improve normal tissue toxicity. *Current Drug Targets* 11, 1395–1404.
- Morgan, K. (2014). Radiotherapy-induced skin reactions: Prevention and cure. *British Journal of Nursing* 23, S24–S32.
- Mori, T., Kawara, S., Shinozaki, M., Hayashi, N., Kakinuma, T., Igarashi, A., ... Takehara, K. (1999). Role and interaction of connective tissue growth factor with transforming growth factor- β in persistent fibrosis: A mouse fibrosis model. *Journal of Cellular Physiology* 181, 153–159.
- Mukherjee, D., Coates, P. J., Lorimore, S. A., & Wright, E. G. (2014). Responses to ionizing radiation mediated by inflammatory mechanisms. *The Journal of Pathology* 232, 289–299.
- Müller, K., & Meineke, V. (2007). Radiation-induced alterations in cytokine production by skin cells. *Experimental Hematology* 35, 96–104.
- Müller, K., & Meineke, V. (2011). Radiation-induced mast cell mediators differentially modulate chemokine release from dermal fibroblasts. *Journal of Dermatological Science* 61, 199–205.
- Murphy-Ullrich, J. E., & Suto, M. J. (2018). Thrombospondin-1 regulation of latent TGF- β activation: A therapeutic target for fibrotic disease. *Matrix Biology* 68, 28–43.
- Nguyen, A. V., & Soulika, A. M. (2019). The dynamics of the skin's immune system. *International Journal of Molecular Sciences* 20.
- Nunes, I., Munger, J., Harpel, J. G., Nagano, Y., Shapiro, R., Gleizes, P.-E., & Rifkin, D. B. (1998). Structure and activation of the large latent transforming growth factor-beta complex. *Journal of the American Optometric Association* 69, 643–648.
- O'Donovan, A., Coleman, M., Harris, R., & Herst, P. (2015). Prophylaxis and management of acute radiation-induced skin toxicity: A survey of practice across Europe and the USA. *European Journal of Cancer* 24, 425–435.
- Ogaly, H., Eltablawy, N., El-Behairy, A., El-Hindi, H., & Abd-Elsalam, R. (2015). Hepatocyte growth factor mediates the antifibrogenic action of Ocimum bacilium essential oil against CCl4-induced liver fibrosis in rats. *Molecules* 20, 13518–13535.
- Olshchewski, T., Bajor, K., Lang, B., Lang, E., & Seegenschmiedt, M. H. (2006). Radiotherapy of basal cell carcinoma of the face and head: Importance of low dose per fraction on long-term outcome. *JDDG: Journal der Deutschen Dermatologischen Gesellschaft* 4, 124–130.
- Ostrau, C., Hülsenbeck, J., Herzog, M., Schad, A., Torzewski, M., Lackner, K. J., & Fritz, G. (2009). Lovastatin attenuates ionizing radiation-induced normal tissue damage in vivo. *Radiotherapy and Oncology* 92, 492–499.
- Otsuka, M., Egawa, G., & Kabashima, K. (2018). Uncovering the mysteries of Langerhans cells, inflammatory dendritic epidermal cells (IDEC) and monocyte-derived Langerhans cells-like cells (LC-like cells) in the epidermis. *Frontiers in Immunology* 9, 1768.
- Panel, B. R. (2016). *Cancer moonshot blue ribbon panel report*. (2016).
- Panetti, P., Marchetti, L., & Accorsi, D. (2009). The serial free fat transfer in irradiated prosthetic breast reconstructions. *Aesthetic Plastic Surgery* 33, 695–700.
- Pannkuk, E. L., Laiakis, E. C., Fornace, A. J., Jr., Fatanmi, O. O., & Singh, V. K. (2018). A metabolomic serum signature from nonhuman primates treated with a radiation countermeasure, gamma-tocotrienol, and exposed to ionizing radiation. *Health Physics* 115, 3–11.
- Pardali, E., Sanchez-Duffhues, G., Gomez-Puerto, M., & ten Dijke, P. (2017). TGF- β -induced endothelial-mesenchymal transition in fibrotic diseases. *International Journal of Molecular Sciences* 18, 2157.
- Patan, S. (2004). Vasculogenesis and angiogenesis. *Angiogenesis in brain tumors* (pp. 3–32). Springer.
- Patel, V., & McGurk, M. (2017). Use of pentoxifylline and tocopherol in radiation-induced fibrosis and fibroatrophy. *British Journal of Oral and Maxillofacial Surgery* 55, 235–241.
- Petit-Frère, C., Capulas, E., Lyon, D. A., Norbury, C. J., Lowe, J. E., Clingen, P. H., ... Arlett, C. F. (2000). Apoptosis and cytokine release induced by ionizing or ultraviolet B radiation in primary and immortalized human keratinocytes. *Carcinogenesis* 21, 1087–1095.
- Philips, B. J., Grahovac, T. L., Valentin, J. E., Chung, C. W., Biley, J. M., Pfeifer, M. E., ... Rubin, J. P. (2013). Prevalence of endogenous CD34+ adipose stem cells predicts human fat graft retention in a xenograft model. *Plastic and Reconstructive Surgery* 132, 845–858.
- Philips, B. J., Marra, K. G., & Rubin, J. P. (2012). Adipose stem cell-based soft tissue regeneration. *Expert Opinion on Biological Therapy* 12, 155–163.
- Phulpin, B., Gangloff, P., Tran, N., Bravetti, P., Merlin, J.-L., & Dolivet, G. (2009). Rehabilitation of irradiated head and neck tissues by autologous fat transplantation. *Plastic and Reconstructive Surgery* 123, 1187–1197.
- Pinheiro, C. H., de Queiroz, J. C., Guimaraes-Ferreira, L., Vitzel, K. F., Nachbar, R. T., de Sousa, L. G., ... Curi, R. (2012). Local injections of adipose-derived mesenchymal

- stem cells modulate inflammation and increase angiogenesis ameliorating the dystrophic phenotype in dystrophin-deficient skeletal muscle. *Stem Cell Reviews* 8, 363–374.
- Plock, J. A., Schneider, J. T., Schweizer, R., Zhang, W., Tsuji, W., Waldner, M., ... Gorantla, V. S. (2017). The influence of timing and frequency of adipose-derived mesenchymal stem cell therapy on immunomodulation outcomes after vascularized composite Allotransplantation. *Transplantation* 101, e1–e11.
- Plock, J. A., Schneider, J. T., Zhang, W., Schweizer, R., Tsuji, W., Kostereva, N., ... Gorantla, V. S. (2015). Adipose- and bone marrow-derived mesenchymal stem cells prolong graft survival in vascularized composite allotransplantation. *Transplantation* 99, 1765–1773.
- Puissant, B., Barreau, C., Bourin, P., Clavel, C., Corre, J., Bousquet, C., ... Blancher, A. (2005). Immunomodulatory effect of human adipose tissue-derived adult stem cells: Comparison with bone marrow mesenchymal stem cells. *British Journal of Haematology* 129, 118–129.
- Quan, T. E., Cowper, S. E., & Bucala, R. (2006). The role of circulating fibrocytes in fibrosis. *Current Rheumatology Reports* 8, 145–150.
- Rabbani, Z. N., Anscher, M. S., Folz, R. J., Archer, E., Huang, H., Chen, L., ... Vujaskovic, Z. (2005). Overexpression of extracellular superoxide dismutase reduces acute radiation induced lung toxicity. *BMC Cancer* 5, 59.
- Randall, K., & Coggle, J. (1995). Expression of transforming growth factor- β 1 in mouse skin during the acute phase of radiation damage. *International Journal of Radiation Biology* 68, 301–309.
- Rehman, J., Traktuev, D., Li, J., Merfeld-Clauss, S., Temm-Grove, C. J., Bovenkerk, J. E., ... March, K. L. (2004). Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. *Circulation* 109, 1292–1298.
- Richter, K., & Kietzmann, T. (2016). Reactive oxygen species and fibrosis: Further evidence of a significant liaison. *Cell and Tissue Research* 365, 591–605.
- Richter, K., Konzack, A., Pihlajaniemi, T., Heljasvaara, R., & Kietzmann, T. (2015). Redox-fibrosis: Impact of TGF β 1 on ROS generators, mediators and functional consequences. *Redox Biology* 6, 344–352.
- Rigotti, G., Marchi, A., Galie, M., Baroni, G., Benati, D., Krampera, M., ... Sbarbati, A. (2007). Clinical treatment of radiotherapy tissue damage by lipoaspirate transplant: A healing process mediated by adipose-derived adult stem cells. *Plastic and Reconstructive Surgery* 119, 1409–1422.
- Rinkevich, Y., Walmsley, G. G., Hu, M. S., Maan, Z. N., Newman, A. M., Drukker, M., ... Lorenz, H. P. (2015). Identification and isolation of a dermal lineage with intrinsic fibrogenic potential. *Science* 348, aaa2151.
- Roberts, A. B., Russo, A., Felici, A., & Flanders, K. C. (2003). Smad3: A key player in pathogenetic mechanisms dependent on TGF- β . *Annals of the New York Academy of Sciences* 995, 1–10.
- Rodemann, H. P., & Bamberg, M. (1995). Cellular basis of radiation-induced fibrosis. *Radiation and Oncology* 35, 83–90.
- Rosenthal, R. A., Fish, B., Hill, R. P., Huffman, K. D., Lazarova, Z., Mahmood, J., ... Doctrow, S. R. (2011). Salen Mn complexes mitigate radiation injury in normal tissues. *Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents)* 11, 359–372.
- Ryan, J. L. (2012). Ionizing radiation: The good, the bad, and the ugly. *Journal of Investigative Dermatology* 132, 985–993.
- Saharinen, J., Taipale, J., & Keski-Oja, J. (1996). Association of the small latent transforming growth factor-beta with an eight cysteine repeat of its binding protein LTBP-1. *The EMBO Journal* 15, 245–253.
- Saito-Fujita, T., Iwakawa, M., Nakamura, E., Nakawatari, M., Fujita, H., Moritake, T., & Imai, T. (2011). Attenuated lung fibrosis in interleukin 6 knock-out mice after C-ion irradiation to lung. *Journal of Radiation Research* 52, 270–277.
- Salvo, N., Barnes, E., Van Draanen, J., Stacey, E., Mitera, G., Breen, D., ... De Angelis, C. (2010). Prophylaxis and management of acute radiation-induced skin reactions: A systematic review of the literature. *Current Oncology* 17, 94.
- Schultze-Mosgau, S., Blaese, M. A., Grabenbauer, G., Wehrhan, F., Kopp, J., Amann, K., ... Rödel, F. (2004). Smad-3 and Smad-7 expression following anti-transforming growth factor beta 1 (TGF β 1)-treatment in irradiated rat tissue. *Radiation and Oncology* 70, 249–259.
- Sciubba, J. J., & Goldenberg, D. (2006). Oral complications of radiotherapy. *The Lancet Oncology* 7, 175–183.
- Serini, G., Bochaton-Piallat, M. -L., Ropraz, P., Geinoz, A., Borsi, L., Zardi, L., & Gabbiani, G. (1998). The fibronectin domain ED-A is crucial for myofibroblastic phenotype induction by transforming growth factor- β 1. *The Journal of Cell Biology* 142, 873–881.
- Serra-Renom, J. M., Muñoz-Olmo, J. L., & Serra-Mestre, J. M. (2010). Fat grafting in post-mastectomy breast reconstruction with expanders and prostheses in patients who have received radiotherapy: Formation of new subcutaneous tissue. *Plastic and Reconstructive Surgery* 125, 12–18.
- Shang, Q., Bai, Y., Wang, G., Song, Q., Guo, C., Zhang, L., & Wang, Q. (2015). Delivery of adipose-derived stem cells attenuates adipose tissue inflammation and insulin resistance in obese mice through remodeling macrophage phenotypes. *Stem Cells and Development* 24, 2052–2064.
- Sheppard, D. (2015). Epithelial-mesenchymal interactions in fibrosis and repair. Transforming growth factor- β activation by epithelial cells and fibroblasts. *Annals of the American Thoracic Society* 12, S21–S23.
- Shi, H. -X., Lin, C., Lin, B. -B., Wang, Z. -G., Zhang, H. -Y., Wu, F. -Z., ... Luo, X. (2013). The anti-scar effects of basic fibroblast growth factor on the wound repair in vitro and in vivo. *PLoS One* 8, e59966.
- Shrshirval, S., Kosmacek, E. A., & Oberley-Deegan, R. E. (2019). Reactive oxygen species drive epigenetic changes in radiation-induced fibrosis. *Oxidative Medicine and Cellular Longevity* 2019, 4278658.
- Simone, N. L., Soule, B. P., Ly, D., Saleh, A. D., Savage, J. E., DeGraff, W., ... Mitchell, J. B. (2009). Ionizing radiation-induced oxidative stress alters miRNA expression. *PLoS One* 4, e6377.
- Spiekman, M., Przybyt, E., Plantinga, J. A., Gibbs, S., van der Lei, B., & Harmsen, M. C. (2014). Adipose tissue-derived stromal cells inhibit TGF- β 1-induced differentiation of human dermal fibroblasts and keloid scar-derived fibroblasts in a paracrine fashion. *Plastic and Reconstructive Surgery* 134, 699–712.
- Sterodimas, A., de Faria, J., Nicaretta, B., & Pitanguy, I. (2010). Tissue engineering with adipose-derived stem cells (ADSCs): Current and future applications. *Journal of Plastic, Reconstructive & Aesthetic Surgery* 63, 1886–1892.
- Stoecklein, V. M., Osuka, A., Ishikawa, S., Lederer, M. R., Wanke-Jellinek, L., & Lederer, J. A. (2015). Radiation exposure induces inflammasome pathway activation in immune cells. *The Journal of Immunology* 194, 1178–1189.
- Strem, B. M., Hicok, K. C., Zhu, M., Wulur, I., Alfonso, Z., Schreiber, R. E., ... Hedrick, M. H. (2005). Multipotential differentiation of adipose tissue-derived stem cells. *Keio Journal of Medicine* 54, 132–141.
- Strong, A. L., Cederna, P. S., Rubin, J. P., Coleman, S. R., & Levi, B. (2015). The current state of fat grafting: A review of harvesting, processing, and injection techniques. *Plastic and Reconstructive Surgery* 136, 897.
- Stubblefield, M. D. (2011). Radiation fibrosis syndrome: Neuromuscular and musculo-skeletal complications in cancer survivors. *PM&R* 3, 1041–1054.
- Suga, H., Eto, H., Shigeura, T., Inoue, K., Aoi, N., Kato, H., ... Yoshimura, K. (2009). IFATS collection: Fibroblast growth factor-2-induced hepatocyte growth factor secretion by adipose-derived stromal cells inhibits postinjury fibrogenesis through ac-Jun N-terminal kinase-dependent mechanism. *Stem Cells* 27, 238–249.
- Suga, H., Glotzbach, J. P., Sorkin, M., Longaker, M. T., & Gurtner, G. C. (2014). Paracrine mechanism of angiogenesis in adipose-derived stem cell transplantation. *Annals of Plastic Surgery* 72, 234–241.
- Sun, W., Ni, X., Sun, S., Cai, L., Yu, J., Wang, J., ... Cao, X. (2016). Adipose-derived stem cells alleviate radiation-induced muscular fibrosis by suppressing the expression of TGF- β 1. *Stem Cells International* 2016.
- Szumacher, E., Wighton, A., Franssen, E., Chow, E., Tsao, M., Ackerman, I., ... Ung, Y. (2001). Phase II study assessing the effectiveness of Biafine cream as a prophylactic agent for radiation-induced acute skin toxicity to the breast in women undergoing radiotherapy with concomitant CMF chemotherapy. *International Journal of Radiation Oncology* Biology* Physics* 51, 81–86.
- Tang, W., Zeve, D., Suh, J. M., Bosnakovski, D., Kyba, M., Hammer, R. E., ... Graff, J. M. (2008). White fat progenitor cells reside in the adipose vasculature. *Science* 322, 583–586.
- Tomasek, J. J., Gabbiani, G., Hinz, B., Chaponnier, C., & Brown, R. A. (2002). Myofibroblasts and mechano-regulation of connective tissue remodelling. *Nature Reviews Molecular Cell Biology* 3, 349.
- Toshkov, I. A., Gleiberman, A. S., Mett, V. L., Hutson, A. D., Singh, A. K., Gudkov, A. V., & Burdelya, L. G. (2017). Mitigation of radiation-induced epithelial damage by the TLR5 agonist entolimod in a mouse model of fractionated head and neck irradiation. *Radiation Research* 187, 570–580.
- Travis, E. L. (2001). Organizational response of normal tissues to irradiation. *Seminars in radiation oncology. Vol. 11*. (pp. 184–196). Elsevier.
- Tsai, C. -C., Wu, S. -B., Kau, H. -C., & Wei, Y. -H. (2018). Essential role of connective tissue growth factor (CTGF) in transforming growth factor- β 1 (TGF- β 1)-induced myofibroblast transdifferentiation from Graves' orbital fibroblasts. *Scientific Reports* 8, 7276.
- Tsuji, W., Rubin, J. P., & Marra, K. G. (2014). Adipose-derived stem cells: Implications in tissue regeneration. *World J Stem Cells* 6, 312–321.
- Vallée, A., Lecarpentier, Y., Guillemin, R., & Vallée, J. -N. (2017). Interactions between TGF- β 1, canonical WNT/ β -catenin pathway and PPAR γ in radiation-induced fibrosis. *Oncotarget* 8, 90579.
- Vandeweyer, E., & Deraemaeker, R. (2000). Radiation therapy after immediate breast reconstruction with implants. *Plastic and Reconstructive Surgery* 106, 56–58 (discussion 59–60).
- Varma, M. J., Breuls, R. G., Schouten, T. E., Jurgens, W. J., Bontkes, H. J., Schuurhuis, G. J., ... van Milligen, F. J. (2007). Phenotypic and functional characterization of freshly isolated adipose tissue-derived stem cells. *Stem Cells and Development* 16, 91–104.
- Venkatesulu, B. P., Mahadevan, L. S., Aliru, M. L., Yang, X., Bodd, M. H., Singh, P. K., ... Abe, J. -i., & Krishnan, S. (2018). Radiation-induced endothelial vascular injury: A review of possible mechanisms. *JACC: Basic to Translational Science* 3, 563–572.
- Verhoekx, J. S., Mudera, V., Walbeehm, E. T., & Hovius, S. E. (2013). Adipose-derived stem cells inhibit the contractile myofibroblast in Dupuytren's disease. *Plastic and Reconstructive Surgery* 132, 1139–1148.
- Waldner, M., Zhang, W., James, I. B., Allbright, K., Havis, E., Bliley, J. M., ... Washington, K. M. (2018). Characteristics and immunomodulating functions of adipose-derived and bone marrow-derived mesenchymal stem cells across defined human leukocyte antigen barriers. *Frontiers in Immunology* 9.
- Wei, J., Bhattacharyya, S., Jain, M., & Varga, J. (2012). Suppl 1: Regulation of matrix remodeling by peroxisome proliferator-activated receptor- γ : A novel link between metabolism and Fibrogenesis. *The Open Rheumatology Journal* 6, 103.
- Wei, J., Meng, L., Hou, X., Qu, C., Wang, B., Xin, Y., & Jiang, X. (2019). Radiation-induced skin reactions: Mechanism and treatment. *Cancer Management and Research* 11, 167.
- Weigel, C., Schmezer, P., Plass, C., & Popanda, O. (2015). Epigenetics in radiation-induced fibrosis. *Oncogene* 34, 2145.
- Werb, Z. (1997). ECM and cell surface proteolysis: Regulating cellular ecology. *Cell* 91, 439–442.
- Willis, B. C., Dubois, R. M., & Borok, Z. (2006). Epithelial origin of myofibroblasts during fibrosis in the lung. *Proceedings of the American Thoracic Society* 3, 377–382.
- Wynn, T. (2008). Cellular and molecular mechanisms of fibrosis. *The Journal of Pathology* 214, 199–210.
- Xavier, S., Piek, E., Fujii, M., Javelaud, D., Mauviel, A., Flanders, K. C., ... Yarkoni, S. (2004). Amelioration of radiation-induced fibrosis inhibition of transforming growth factor- β signaling by halofuginone. *Journal of Biological Chemistry* 279, 15167–15176.

- Yamamori, T., Sasagawa, T., Ichii, O., Hiyoshi, M., Bo, T., Yasui, H., ... Inanami, O. (2017). Analysis of the mechanism of radiation-induced upregulation of mitochondrial abundance in mouse fibroblasts. *Journal of Radiation Research* 58, 292–301.
- Yang, X., Walton, W., Cook, D. N., Hua, X., Tilley, S., Haskell, C. A., ... Kirby*, S. L. (2011). The chemokine, CCL3, and its receptor, CCR1, mediate thoracic radiation-induced pulmonary fibrosis. *American Journal of Respiratory Cell and Molecular Biology* 45, 127–135.
- Yarnold, J., & Brotons, M. -C. V. (2010). Pathogenetic mechanisms in radiation fibrosis. *Radiotherapy and Oncology* 97, 149–161.
- Yi, M., Liu, B., Tang, Y., Li, F., Qin, W., & Yuan, X. (2018). Irradiated human umbilical vein endothelial cells undergo endothelial-mesenchymal transition via the Snail/miR-199a-5p axis to promote the differentiation of fibroblasts into myofibroblasts. *BioMed Research International* 2018.
- Yu, Q., & Stamenkovic, I. (2000). Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF- β and promotes tumor invasion and angiogenesis. *Genes & Development* 14, 163–176.
- Yun, I. S., Jeon, Y. R., Lee, W. J., Lee, J. W., Rah, D. K., Tark, K. C., & Lew, D. H. (2012). Effect of human adipose derived stem cells on scar formation and remodeling in a pig model: A pilot study. *Dermatologic Surgery* 38, 1678–1688.
- Zackrisson, B., Mercke, C., Strander, H., Wennerberg, J., & Cavallin-Staahl, E. (2003). A systematic overview of radiation therapy effects in head and neck cancer. *Acta Oncologica* 42, 443–461.
- Zhang, Y., Jiang, X., & Ren, L. (2019). Optimization of the adipose-derived mesenchymal stem cell delivery time for radiation-induced lung fibrosis treatment in rats. *Scientific Reports* 9, 5589.
- Zhong, Y., Lin, Z., Lu, J., Lin, X., Xu, W., Wang, N., ... Chen, Z. (2019). CCL2-CCL5/CCR4 contributed to radiation-induced epithelial-mesenchymal transition of HPAEpic cells via the ERK signaling pathways. *American Journal of Translational Research* 11, 733.
- Zimmerlin, L., Donnenberg, V. S., Rubin, J. P., & Donnenberg, A. D. (2013). Mesenchymal markers on human adipose stem/progenitor cells. *Cytometry. Part A* 83, 134–140.
- Zuk, P. A., Zhu, M., Ashjian, P., De Ugarte, D. A., Huang, J. I., Mizuno, H., ... Hedrick, M. H. (2002). Human adipose tissue is a source of multipotent stem cells. *Molecular Biology of the Cell* 13, 4279–4295.
- Zwierzina, M. E., Ejaz, A., Bitsche, M., Blumer, M. J., Mitterberger, M. C., Mattesich, M., ... Hörl, S. (2015). Characterization of DLK1 (PREF1)+/CD34+ cells in vascular stroma of human white adipose tissue. *Stem Cell Research* 15, 403–418.