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Stroma in normal and cancer wound healing

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Abbreviations.

αSMA	alpha Smooth Muscle Actin
bFGF	basic Fibroblast Growth Factor
CAF	Cancer-Associated Fibroblast
ECM	ExtraCellular Matrix
EMMPRIN	Extracellular Matrix MetalloPRoteinase Inducer
FAPα	Fibroblast Activation Protein alpha
FSP-1	Fibroblast Specific Protein 1
HGF	Hepatocyte Growth Factor
MMP	Matrix MetalloProteinase
MAPK	Mitogen Activated Protein Kinase
PAI-1	Plasminogen Activator Inhibitor-1
PDGF	Platelet-Derived Growth Factor
PDGFR	Platelet-Derived Growth Factor Receptor
TGFβ	Transforming Growth Factor beta
TIMP	Tissue Inhibitor of Matrix Proteinase

VEGFR Vascular Endothelial Growth Factor Receptor

Keywords.

Fibroblasts

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Cancer-Associated Fibroblasts

EMMPRIN

FAPα

TGFβ

Abstract

It is currently believed that stroma, the connective framework of biological tissues, plays a central role in normal wound healing and in cancer. In both these contexts, stromal cellular components such as activated fibroblasts interact with complex protein networks that include growth factors, structural protein or proteinases in order to initiate and sustain an extensive remodelling process. However, although this process is usually spatially and temporally self-limited, it is unregulated in the case of cancer and leads to uncontrolled cell proliferation and invasion within tissues, metastasis and therapeutic resistance. In this review, we outline the role of stroma in normal healing, cancer and post radiotherapy, with a particular focus on the crosstalk between normal or cancer cells and fibroblasts. Understanding these mechanisms is

particularly important as several stromal components have been proposed as potential therapeutic targets.

Introduction

Following tissue injury, the host response consists of a reparative process involving the action of cells from the connective tissue as well as their ExtraCellular Matrix (ECM) products. In a general way, the damaged tissue will be restored in a normal and functional state [1]. However, there are conditions under which repair is excessive, resulting in chronic non-healing wounds and fibrosis that can virtually affect every tissue and organ system. Fibrosis, occurring, for example, after radiation therapy in cancer treatment, can be considered as the “dark side” of normal tissue repair [2]. This physio-pathological process starts just after the injury and involves several components such as migratory and local cells, ECM proteins and soluble factors. To rapidly and efficiently repair the wound, a large and complex number of cellular responses is set in motion with acute and tight regulations [1]. This process is spatially and temporally self-limited and the mechanisms underlying it include inflammatory and growth factors, cell-matrix and cell-cell interactions, hence controlling cell proliferation, migration and differentiation, and, ultimately, stages of re-epithelialization, fibroplasia, angiogenesis, wound contraction and ECM remodelling.

Similarities between the healing process and tumour development have previously been noted with Alexander Haddow suggesting that "tumour production is a possible over-healing" [3] and a decade later, it was postulated by Dvorak that "tumours are wounds that do not heal" [4]. Dvorak further suggested that tumour stroma composition strongly resembles stroma found in the granulation tissue of healing wounds. However, this process is not self-limited in cancer and is characterised by uncontrolled cell proliferation, invasion and

metastasis [5]. Therefore, better understanding of the mechanisms involved in normal wound healing could impact our understanding of the biology of tumours especially of aggressive ones.

In this review, we will explore the stroma in normal wound healing and cancer, where crosstalk between cells (normal or cancer) and fibroblasts exists with a focus on the main factors involved. Healing in radiotherapy will be also addressed.

Normal wound healing

Adjacent to the damaged area following an injury, local cells initiate a cascade of events that are pre-requisite for restoring tissue function [6]. Cellular and molecular biology studies have shown that several pathways are called into play to terminate bleeding at the wound site as an initial response [7]. Reflex vasoconstriction temporarily minimises blood loss until homeostasis is achieved by the formation of a plug composed of insoluble fibrin-based materials after platelet aggregation and initiation of the plasma coagulation cascade. The presence of activated platelets within this fibrin-based plug provides an abundant source of factors involved in the wound repair process including vasoconstrictive substances, such as thromboxane A, or growth factors, such as Transforming Growth Factor beta (TGFβ). Most importantly, a temporary matrix, due to the formation of the fibrin-based plug, is available for all cellular responses [1,6].

Once blood lost is controlled, inflammatory cells initiate their migration into the wound as a result of the increased in the permeability of blood vessels adjacent to the injury site. The expansion of fibroblasts into the wound represents the beginning of the proliferative phase. This phase is characterised by the replacement of the previous established wound matrix with a type III collagen-rich granulation tissue. Fibroblasts migration and proliferation into the wound are, mainly, regulated by tissue growth factors such as Platelet-Derived Growth Factor

(PDGF), TGFβ and/or basic Fibroblast Growth Factor (bFGF). Two days after the injury, fibroblasts initiate their migration into the wound and after four days they represent the most predominant cells in the granulation tissue. Indeed, in both injured and normal connective tissues, fibroblasts are one of the most abundant cell populations. They can be characterised by the expression of Fibroblast Specific Protein 1 or FSP-1 (S100A4), a filament-associated, calcium-binding protein found in the cytoplasm of fibroblasts (Figure 1), but not of epithelial cells, thus allowing fibroblast identification [8]. Fibroblast primary functions are to form ECM, maintain the tissue microenvironment and sustain cell growth. Specifically, in the last stages of wound healing, they are involved in wound contraction, ECM remodelling by proteases such as Matrix MetalloProteinases (MMPs) and in the substitution of type III collagen for type I collagen (Figure 1) [1,6].

Wound healing in radiotherapy

Ionizing radiation exposure can induce a variety of cellular death processes, such as apoptosis. Even though this therapy is used to kill cancer cells, it also damages nearby healthy cells, leading to acute and chronic side effects such as excessive scarring [9]. The main difference between radiation injury during fractionated radiotherapy and a "normal" wound is the repetition of the injury induced by each fraction of irradiation that finally induces different stages of acute and late effects according to tissue type and its intrinsic radio sensitivity. As such, at the end of the treatment, irradiated tissue is profoundly different from non-irradiated tissue. In fact, following radiotherapy, radiation-induced inflammatory reaction may impact radiation response by increasing endothelial dysfunction and levels of cytokines or growth factors such as TGFβ [10]. The overexpression of TGFβ in irradiated tissues has been suggested to induce fibroblast proliferation and differentiation [11]. In irradiated patients, ionizing radiation exposure of health tissues is associated with an increase

of type I and III collagen synthesis and density, an alteration of ECM remodelling by MMPs and sequential activation of growth factors, including TGFβ itself (Figure 2). Such deregulation following radiotherapy can also be accountable for complications appearing several decades later. It is worth to note that the involved molecular pathways are common with mechanisms underlying other fibrotic responses [12].

The fibrotic response

The inappropriate repair of connective tissue is defined pathologically as fibrosis. This process is increasingly recognized as an important feature of many chronic diseases [2]. Fibrosis can affect virtually every organ system, most notably the lung, liver, kidney, heart, blood vessels, vascular, skin or eye. Many of the key morphological features of fibrosis affecting these organs are similar to those of normal scarring. Traditionally, fibrosis has been viewed as an irreversible, end-stage sequel to multiple diverse disease processes. Typical examples include skin and soft tissue scarring and contraction after burn, hypertrophic scars, keloids after physical injury [13], pulmonary fibrosis, skin fibrosis myelofibrosis after radiation, cardiac fibrosis post-myocardial infarction, brain scarring post-stroke [14], systemic sclerosis, chronic graft versus host disease [15], retroperitoneal fibrosis, asthma, sarcoidosis, atherosclerosis, inflammation, schistosomiasis or hepatitis C-associated cirrhosis and HIV-associated lymphoid fibrosis [16]. The pathogenesis of fibrosis remains poorly understood but it generally involves a "fibrogenic cascade" involving multiple molecular pathways and cellular targets [17]. The role of inflammation in this process has been historically highlighted, providing the rationale for considering anti-inflammatory or immunosuppressive therapies in fibrotic disease. However, it has become increasingly evident that these types of interventions are generally ineffective [18]. The lack of effective treatments, and the high mortality and increasing morbidity attributed to chronic fibrotic

diseases, has stimulated research on the cellular, molecular, and genetic basis of fibrosis [18]. One of the ways fibrotic progression can be simply assessed is through the measurement of type I / type III collagen ratio and change in such ratios has been reported in fibrosis that follows radiation therapy for cancer [19].

Cancer wound healing

Similarities are known to exist between wound healing and carcinogenesis [3,4]. Indeed, during the initial phase of tumour development, the basal membrane surrounding tumour cells is rapidly degraded by proteinases such as MMPs that are secreted either by cancer or stromal cells. The loss of this physical barrier allows the contacts of tumour cells with stroma, leading to interactions reminiscent of stromal-epithelial interactions found in wound healing [20]. Such stromal-epithelial interactions are crucial for maintaining functional differentiation and growth/quiescence of organ homeostasis, whereas their deregulation plays a critical role in the initiation and promotion of carcinogenesis [21]. Aberrant interactions between cancer cells and its microenvironment significantly change the composition of the stroma and contribute to the progression of several human cancers including that of the prostate, breast, and ovary [22–25]. The tumour microenvironment includes a mixture of resident fibroblasts, myofibroblasts, endothelial cells as well as innate and adoptive immune cells embedded within the ECM [26]. Apart from direct cell contacts, stromal-epithelial interactions are influenced by soluble factors such as cytokines (IL-6), growth factors (TGFβ), hormones, ECM molecules or extracellular vesicles (EVs) [27–31]. EVs refers to microvesicles, exosomes or even oncosomes and are involved in communications between cancer cells themselves but also between cancer cells and cells from the microenvironment. Recently EVs were shown to actively organizing the ECM and to interact with the ECM molecules. They are now considered as components of the ECM [32]. EVs represent

promising diagnostic and therapeutic tools in cancer since they have also been involved in radio- or chemo-therapies and resistance to treatment [33].

All those different nodes of communication between tumour cells and the microenvironment are expressed both by tumour and stromal cells and lead to a global increase in ECM remodelling by MMPs, angiogenesis, protease activity and immune cell infiltration. Over the last decades, tumour stroma has gained a lot of interest because of its potential as a therapeutic target and its role in the resistance of tumours to therapy (Figure 3). The latter is conferred by altered oxygen availability, environmental acidity and ECM protein content around tumour cells, thus creating a barrier that prevents delivery of therapeutic molecules but allows metastasis [22,23]. Targeting various components of the tumour stroma represents an alternative approach which is radically different traditional therapies that target cancer cells. Consequently, the modulation of the crosstalk between cancer and stromal cells are being tested in clinical trials for several drugs such as Src kinase or TGFβ inhibitors, or angiogenesis inhibitors against the Vascular Endothelial Growth Factor Receptor (VEGFR) [29].

Reactive stroma in normal and cancer wound healing

Stroma is traditionally perceived as a connective tissue consisting of type I collagen and fibroblasts. However, it is closely associated with other ECM components of the such as fibronectin, laminin, MMPs and glycosaminoglycans [34]. This tissue is found in a very large number of organs where it plays a major role during embryonic development and tissue homeostasis. The stromal-epithelial interactions occurring during embryonic development favour the structural and spatial organization of tissues in respect to their surrounding structures and participate in the temporal regulation of genes expressed at different phases of the process [35]. Stroma is also an important player in tissue homeostasis since it contributes

to the regulation of epithelial and stromal cells growth and differentiation as well as to ECM turn-over [35].

To understand the importance of the stroma in tumour development, it is essential to understand the differences between "normal" stroma and reactive stroma found at the periphery of the tumour tissue (Figures 1 and 3). For example, stroma found within a breast carcinoma is profoundly different from the stroma of the normal mammary gland [36]. Healthy stroma contains a limited number of fibroblasts, most often in a quiescent state and characterised by the expression of FSP-1 [8]. This is associated with a physiological ECM consisting of fibrous proteins such as type I collagen and organized into a controlled and loose fibrillar network that incorporates low amounts of MMPs [6,34]. In contrast, tumour stroma is associated with ECM rich in type I and type III collagens, MMPs and fibrous proteins such as fibronectin (Figure 3). Those types of collagen dominate the tumour microenvironment and are pro-carcinogenic by regulating tumour density and stiffness [37–39]. Increased density of type I collagen in tumour stroma is associated with decreased dispersion of anti-cancer drugs within the tumour that reduced their bioavailability and attenuates their absorption by tumour cells, thus diminishing their therapeutic effect [40]. In addition, collagen fibres within the tumour stroma are organised differently compared to healthy tissue. For example, in pancreatic cancer, collagen fibres are arranged in a long linear network that forms "paths" for the rapid migration of tumour cells to the nearest blood vessels [40,41]. Tenascin C, an ECM glycoprotein that is constitutively expressed by fibroblasts within the tumour stroma, has been shown to correlate with tumour invasiveness. Tenascin C and type I collagen were found to form similar tubular "network" structures for tumour cell invasion [42]. Fibronectin is another ECM glycoprotein that is synthesized predominantly by fibroblasts and is abundant in most connective tissues [43,44]. Fibronectin is involved in regulation of cell adhesion, migration and differentiation by interacting with

specific cell receptors such as integrins and proteoglycans. Like type I collagen, fibronectin density is increased within the tumour stroma and correlates with an increase in tumour growth and malignant cell dispersion as well as with decreased responses to anti-inflammatory substances [45,46]. Different fibronectin isoforms can be synthetized through the alternative splicing of 3 regions (ED-A, ED-B and IIICS) of the primary transcript. The expression level of the ED-B isoform is relatively low in normal adult tissues, but high expression levels can be found in foetal and tumour tissues particularly during angiogenesis [47]. Fibronectin isoforms can also be used as prognostic indicators: the presence of the ED-A isoform in urine is associated with poor prognosis in patients with bladder cancer [48].

There is no one single, predefined tumour stroma since its compositions and quantity of its components differ depending on the type of tumour studied. Some breast, pancreas and stomach carcinomas primarily consist of stroma that is rich in elastic and collagen fibres forming up to 90% of the tumour mass, while others e.g. bone marrow tumours, have very little stroma that is heavily infiltrated by lymphocytes [49]. Even though numerous studies suggest that alterations in tumour stroma are associated with poor cancer progression, no specific stromal markers have been found to strongly predict clinical outcome. However, tumour tissue can be grossly evaluated with stains such as Masson's trichrome (MT) stain [50]. Such coarse quantification of stromal alterations could be an informative marker of cancer progression (Figure 3) and could have prognostic value in patients with highly aggressive cancer [25,51].

Stromal-epithelial interactions are involved in a wide range of tumour-promoting effects, most notably the stimulation of several proteinases by stromal fibroblasts. Stroma remodelling can be mediated by several MMPs such as interstitial collagenase (MMP-1), collagenase 3 (MMP-13), stromelysin 1 (MMP-3), gelatinases A and B (MMP-2, -9) or MT1-MMP (MMP-14). Such proteolytic enzymes of ECM proteins can regulate cell behaviour in

normal and tumour stroma by remodelling ECM proteins and by regulating the activation and activity of several growth factors or cytokines such as TFGβ, IL-1 and IL-6, as well as their bioavailability [52,53]. During the wound healing process, MMPs facilitate cell migration or wound remodelling. However, these proteolytic enzymes must be tightly controlled by their natural inhibitors, the Tissue Inhibitor of Matrix Proteinases (TIMPs), the lack of which results in MMP overactivity, as is the case in chronic wounds [54]. Increased MMP activity is also a common feature observed in carcinogenesis, leading to primary tumour invasion and metastasis [55,56]. Even though differences in ECM proteins and remodelling enzymes can be distinguished in the tumour stroma, the most important feature is a large number of activated fibroblasts that predominate over quiescent fibroblasts as compared to normal stroma [42].

Fibroblast activation and TGFβ

Wound repair can be characterized by the activation and differentiation of quiescent fibroblasts into myofibroblasts, a key event in wound healing allowing ECM remodelling and wound contraction [57]. Both *in vitro* and *in vivo*, fibroblast differentiation into myofibroblast can be monitored by the expression of alpha Smooth Muscle Actin (αSMA), the actin isoform typical of vascular smooth muscle cells [58]. The presence of αSMA stress fibres characterizes myofibroblasts and is related to their contractile propriety since a direct correlation could have been demonstrated between αSMA expression and ECM contraction. By stimulating αSMA expression in fibroblasts, TGFβ is considered to be the major factor contributing to their differentiation into myofibroblasts [59]. Through receptor binding, TGFβ triggers a cascade of signalling events that lead to the phosphorylation of Smad proteins and their translocation to the nucleus where they regulate the expression of targeted genes [60]. TGFβ-dependent fibroblast activation results in morphological changes and

deregulations of their functions through changes in the expression of several proteins. One such major change is the induction of αSMA expression by fibroblasts, which consequently acquire contractile properties similar to smooth muscle cells. TGFβ-activated fibroblasts in turn synthesize a set of factors such as Tenascin C and Hepatocyte Growth Factor (HGF), which will promote tumour cell migration and invasion [61,62].

In addition to up-regulating αSMA expression, TGFβ is also well known as a potent stimulator of connective tissue formation. TGFβ controls both the expression of ECM proteins, including fibronectin or type I and III collagens, and the expression of proteinase inhibitors, including TIMPs or Plasminogen Activator Inhibitor-1 (PAI-1) [63]. Conversely, connective tissue turnover and remodelling are controlled by the activity of proteolytic enzymes such as MMPs. Therefore, the outcome of the healing process, whether normal, hypertrophic or ulcerated, will be dictated by the balance between ECM formation and deposition by growth factors like TGFβ and its degradation by MMPs. Moreover, in addition to its effect on ECM proteins and protease inhibitors synthesis, TGFβ can also regulate the expression of several MMPs. TGFβ has been shown to inhibit the synthesis of interstitial collagenase (MMP-1) and stromelysin (MMP-3) and to increase MMP-2 (gelatinase A) or MMP-9 (gelatinase B) expression in different cell types [63]. Some MMPs such as MMP-2, MMP-9 and MT1-MMP are also involved in the processing of TGFβ from its latent form to the active one, hence demonstrating a complex interplay between ECM formation and degradation. Consequently, since myofibroblasts are considered as key cells for the connective tissue remodelling associated with wound healing, it would be expected that they encompassed an elaborate equipment to control ECM formation and turnover in a refined way (Figure 1).

Stroma cells with differentiated myofibroblast phenotypes are also found in primary and metastatic carcinoma tumours, where it is believed that they play a central role in cancer cell

growth, proliferation, and metastatic conversion [51,57]. These fibroblastic cells adjacent to neoplastic cell nests express significant amounts of αSMA, are the most abundant type found in tumour stroma (almost 50% of the cell population in tumour tissues) and are normally referred to as Cancer-Associated Fibroblasts (CAFs) [64]. Exposure to growth factors or chemokines/cytokines transforms resident fibroblasts into CAFs through the acquisition of mesenchymal properties, which is commonly termed mesenchymal-to-mesenchymal transition. Those cells may be involved in the resistance of malignant cells to therapeutic agents [65].

CAFs are characterized by the expression of several proteins such as αSMA and other common fibroblast markers like vimentin, Tenascin C and Fibroblast Activation Protein alpha (FAPα or seprase), a homodimeric integral membrane gelatinase involved in ECM remodelling, tumour growth, and metastasis [66,67]. FAPα possesses two peptidase activities: a dipeptidyl-peptidase activity, specific for N-terminal Xaa-(Pro/Ala) sequences, and an endopeptidase activity. Currently, not much is known about the regulation of its expression in cancer. TGFβ, 12-o-tetradecanoyl phorbol-13-acetate or retinoic acid were shown to increase its expression [68]. Although the physiologic substrates of FAPα have yet to be fully determined, it has been proposed that FAPα plays a role in matrix invasion through the degradation of gelatine and denatured type I collagen including MMP-1-cleaved type I collagen. Recently, it has been shown that cancer cell migrates on elongated protrusions of fibroblasts within the extracellular matrix [69]. Since FAPα is a transmembrane proteinase, it is tempting to speculate that it can allow such cancer cell migration on fibroblast scaffold.

In patient biopsies, CAFs can be distinguished from other cell types by their co-expression of vimentin and αSMA without expression of calponin as well as by the expression of Platelet-Derived Growth Factor Receptor (PDGFR) [51]. CAF presence is considered to be a

response of the host cells to inductive stimuli exerted by tumour cells, which in return actively participate in disease progression by secreting proteolytic enzymes like MMPs, thus promoting tumour invasion and metastasis (Figure 3). It is currently postulated that an important proportion of these enzymes is produced by stromal myofibroblasts as part of the host response to tumour presence. EMMPRIN (Extracellular Matrix MetalloPRoteinase INducer), a membrane glycoprotein significantly enriched on the surface of tumour cells, has been shown to stimulate the synthesis of MMPs in fibroblasts [70].

Dual role of EMMPRIN in stroma activation

The major function of MMPs has always been considered to be ECM degradation as part of normal tissue remodelling [52,71]. In physiological conditions, MMPs are only expressed when needed and, under pathological circumstances, their aberrant expression is often noticed [72]. Consequently, tight regulation is required to maintain the proper turnover of ECM and involved numerous physiological regulators including soluble factors such as cytokines, growth factors and hormones. However, the identification of transmembrane glycoprotein inducing MMP expression, EMMPRIN, revealed a new mechanism of MMP regulation through direct cell-cell interactions [70]. Since EMMPRIN is particularly enriched in tumour cells at the plasma membrane, its functional role was at first limited to cancer progression due to its MMP induction ability (Figure 3). However, recent studies have evidenced a broader involvement of EMMPRIN in many physio-pathological situations where deregulated tissue degradation occurs through deregulated MMP activities [21,73]. EMMPRIN over-expression is also associated with tumour radio-resistance (Figure 2) and can be considered as an adverse predictor of clinical outcome [74,75].

EMMPRIN, also named basigin or CD147, belongs to the immunoglobulin super family and is expressed at the plasma membrane of numerous cell type including epithelial cells and

fibroblasts [72,73]. It is a single-pass type I membrane protein composed of two C2-like immunoglobulin domains, a transmembrane domain and a short cytoplasmic tail. Three conserved N-glycosylation sites, with variable glycosylation states, are presents in the N-terminal extracellular region. Whether EMMRIN is glycosylated determines its MMP stimulating activity with the non-glycosylated form of EMMPRIN unable to induce MMPs and even antagonizing the activity of the native glycosylated form. In the transmembrane domain, a sequence of 29 amino acids is highly conserved among several species including human, chicken and mouse, thus indicating the importance of this region for EMMPRIN functions. One glutamic acid, a charged amino acid, and leucine zipper-like sequences have also been evidenced within the hydrophobic stretch of the transmembrane domain, suggesting that interactions with other transmembrane proteins could occur through this specific intramembrane site [76].

Until the discovery of EMMPRIN, soluble paracrine mediators such as cytokines and growth factors were considered as the major regulators of MMP expression. EMMPRIN has been shown to stimulate the production of almost all MMPs but has no effect on their physiological inhibitors, the TIMPs [73]. Furthermore, EMMPRIN modifies the proteolytic balance not only by increasing MMPs production but also by modulating their activation, since MMPs-14 and -15, two membrane type MMPs, expression can be up-regulated by EMMPRIN, thus leading to the activation of other MMPs such as MMP-2 [77,78]. In fibroblasts, EMMPRIN induction of MMPs involves the regulation at the mRNA level through the Mitogen Activated Protein Kinase (MAPK) p38 signalling pathway since SB203580, a p38 inhibitor, can block MMP-1 stimulation [79]. However, other studies have evidenced the implications of phospholipase A2 and 5-lipoxygenase in the induction of MMP-2. The different signalling pathways used by EMMPRIN to regulate MMP expressions remains to be determined [80].

EMMPRIN has also been suggested to play an important function in cell differentiation due to the developmental defects observed in the knock-out mice. Several studies in different biological systems could also supported such an involment in cell differentiation [76,81,82]. In many epithelia, including the epidermis, the actively differentiating basal cells exhibit a particularly strong and constitutive expression of EMMPRIN, whereas, in the superficial terminally differentiated cells, a weak expression could be seen [83]. Increased EMMPRIN expression, with a parallel MMP stimulation, is also noticed when human monocytes differentiate into macrophages

Recent studies have also shown the involvement of EMMPRIN in αSMA expression and myofibroblast differentiation [84,85] and an age associated increased expression in cardiac tissue [86]. Altogether, these data expand on the known functions of EMMPRIN and suggest that stromal EMMPRIN is an important regulator of fibroblast activation that may represent an interesting therapeutic target (see following section). In fact, EMMPRIN was shown to actively participate in myofibroblasts differentiation **(**Figure 1**).** Increasing EMMPRIN expression by adding exogenous recombinant EMMPRIN or by cDNA transfection results in the stimulation of αSMA expression [84]. Using the same conditions, EMMPRIN also increased the contractile properties of fibroblasts as shown by the augmented contraction of collagen lattices and by the appearance of αSMA stress fibres using immunohistochemical analysis. Additionally, EMMPRIN and αSMA have been demonstrated to colocalize within to the same cells in the stroma of pathological tissues, confirming the implication of EMMPRIN in myofibroblasts differentiation *in vivo* [84].

Altogether these data suggest a dual function of EMMPRIN during normal wound healing (Figure 1) or tumour stroma formation (Figure 3) since EMMPRIN can regulate the degradative potential of activated fibroblasts and can also influence the contractile phenotype of these activated cells in an MMP-independent manner. Other studies in EMMPRIN knock-

out mice have shown that EMMPRIN inhibition correlated with a strong reduction of MMP expression during tissue repair following injury [21,87,88]. This effect can be partially explained by the absence of EMMPRIN which is known to be involved in MMP induction. However, cytokines and growth factors released from the damaged epithelium such as TGFβ should still induce MMP expression. Based on all these observations, it is tempting to suggest that EMMPRIN could play a role in the action and activities of such cytokines and growth factors and that tumour cells could use EMMPRIN-related pathways in order to signal neighbouring fibroblasts towards differentiation to myofibroblasts and MMP secretion.

Therapeutic targets and concluding remarks

Stroma in normal wound healing and cancer share many characteristics and similar pathways appear to be implicated. Stroma in both contexts contains activated fibroblasts within a complex ECM (Figures 1 and 3). In normal tissue, these components maintain the tissue microenvironment and sustain cell growth in various ways with spatial and temporal self-limitations. In tumour stroma, fibroblast activities promote tumour progression and impede response to therapy. Abnormal wound healing can also occur in cancer patient after radiation therapy. A better understanding of the different mediators involved in stroma activation such TGFβ or EMMPRIN during this physio-pathological process could lead to the development of new therapies for controlling stroma activation in physiopathological wound healing and cancer.

TGFβ is considered as the main inducer of fibroblast activation that occurs in wound healing, tumour initiation and progression. After binding to its receptors, TGFβ induces signalling pathways leading to the upregulation of targeted genes such αSMA [59,89]. The expression of αSMA has also been shown to be controlled by EMMPRIN. Moreover, fibroblasts lacking EMMPRIN (siRNA) are less responsive to TGFβ and do not differentiate

into fully activated cells since their αSMA level is reduced [84]. As EMMPRIN and TGFβ receptors are transmembrane proteins expressed at the extracellular surface of fibroblasts, one would expect that these proteins could interact and, therefore, regulate intracellular signalling pathways leading to fibroblast activation. This activation is a key process in fibrosis and tumour stroma formation and could potentially be inhibited by modulating EMMPRIN and TGFβ receptor interactions.

Additional research aiming at identifying specific markers for activated fibroblasts could also facilitate the development of targeted therapies that control fibroblast activation and limit their activity in fibrosis or in cancer proliferation and metastasis. Such new biomarkers, with the help of imaging technology [90], could also help to better differentiate indolent cancer from metastatic, aggressive and lethal tumours.

Author Contributions

EH, CJ, HQN, YB, ADLT, VS and HW wrote the review and EH prepared the figures.

Conflicts of Interest.

The authors confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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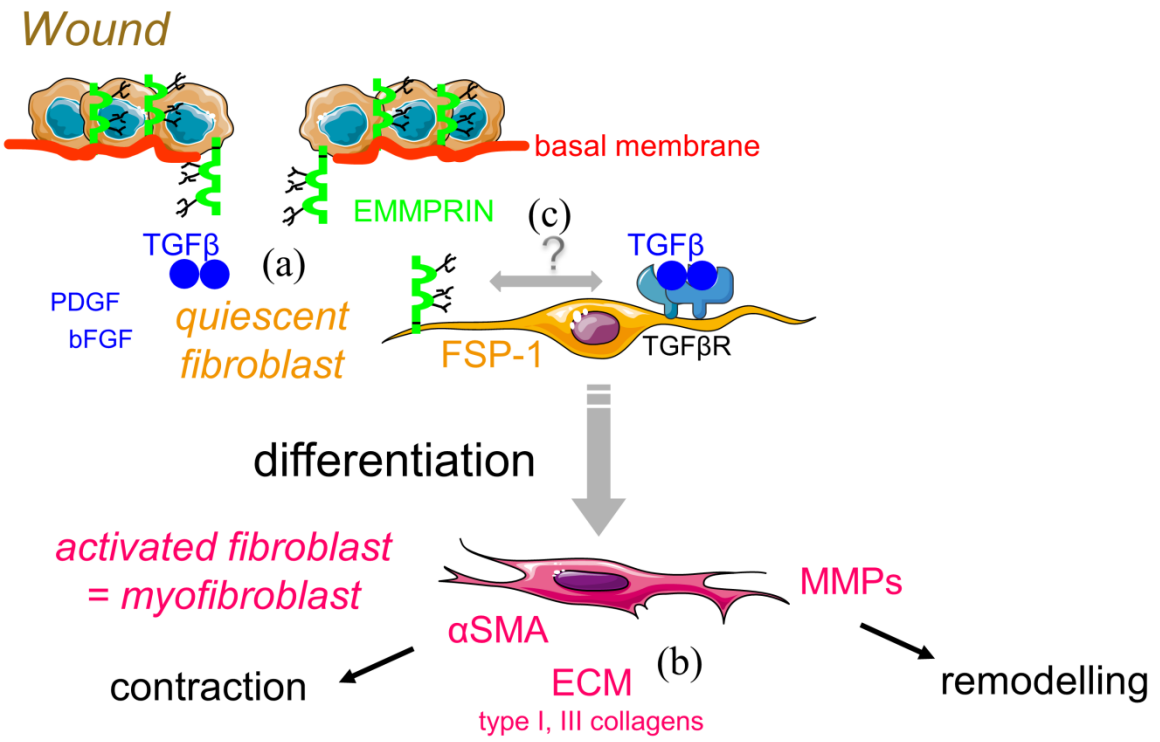

Figure 1 Normal wound healing

(a) Injuries which cause basal membrane disruption would lead to stromal-epithelial interactions and release of growth factor such as PDGF, bFGF, or TGFβ, inducing fibroblast migration, proliferation and differentiation from quiescent (FSP-1 positive) to activated (αSMA positive) fibroblast. (b) Such activated fibroblasts are named myofibroblasts due to their expression of αSMA and their wound contraction abilities. TGFβ is well known as a potent inducer of fibroblast differentiation as it allows αSMA and ECM molecules expression. In addition to its MMP inducing activity, EMMPRIN can also trigger fibroblast differentiation and αSMA expression. (c) Since EMMPRIN and TGFβ receptors are both expressed at the fibroblasts plasma membrane, it is tempting to speculate on their possible interactions.

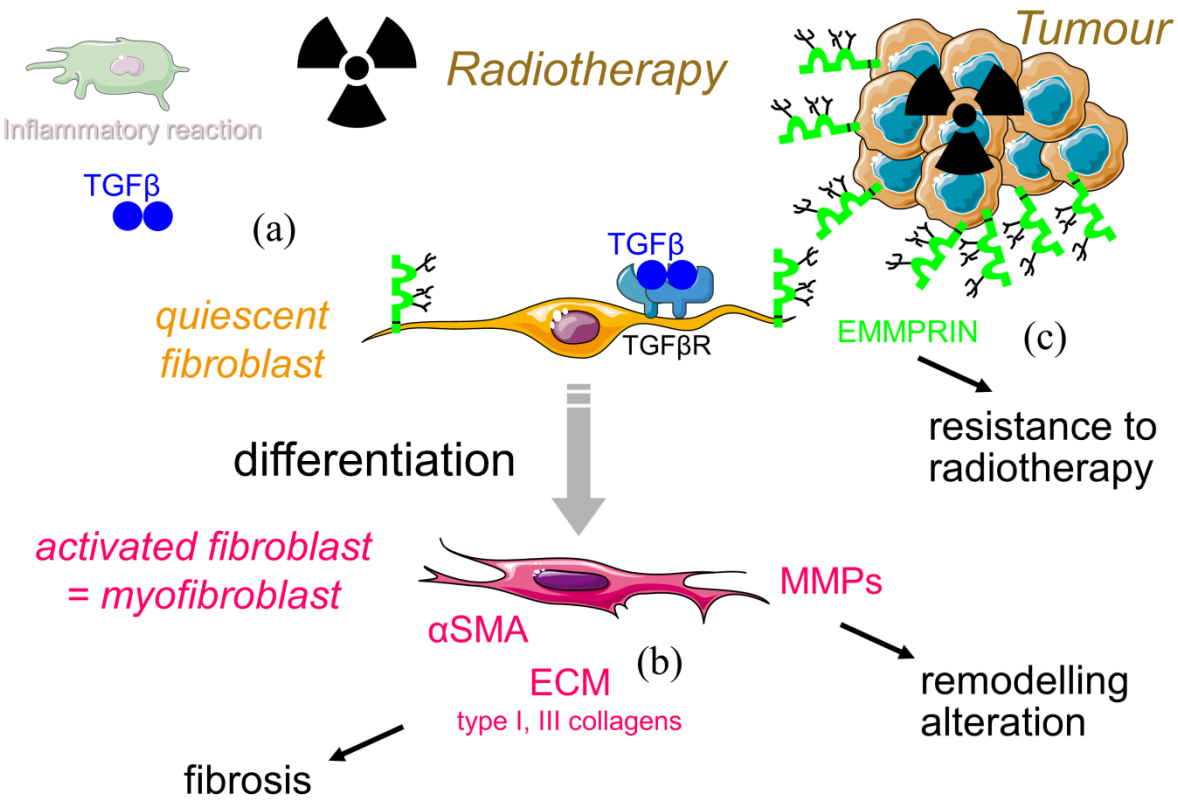

Figure 2 Wound healing in radiotherapy

(a) After fractionated radiotherapy, the inflammatory reaction induces TGFβ overexpression and, consequently, fibroblast proliferation and differentiation from quiescent cells to myofibroblasts. (b) The continuous TGFβ release is suggested to be responsible of the increase in type I and III collagen synthesis and density, as well as in the alteration of ECM remodelling by MMPs, common mechanisms found with other fibrosis responses. (c) Resistance to radiotherapy has been associated with unfavourable clinical outcomes and EMMPRIN over-expression by cancer cells.

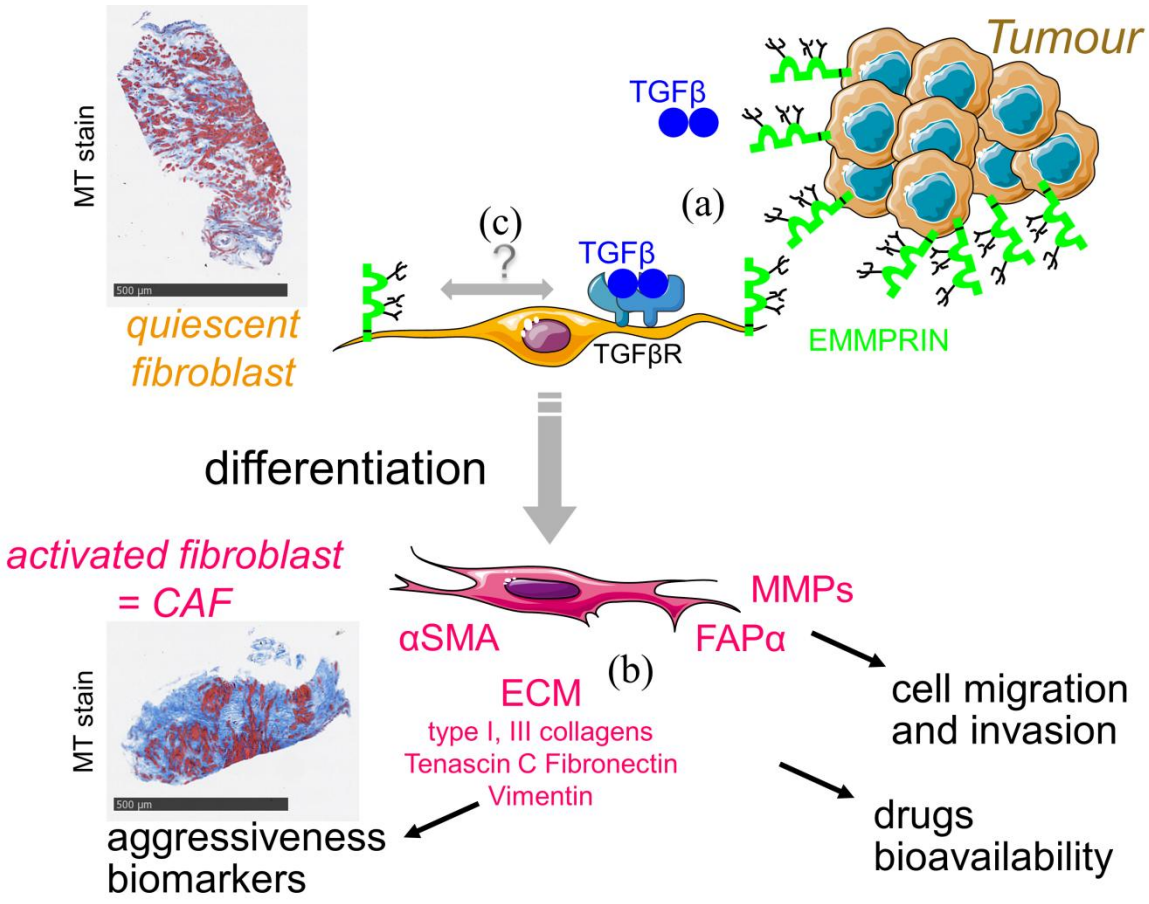

Figure 3 Cancer wound healing

(a) After basal membrane disruption, contacts between tumour cells and fibroblasts occur both through EMMPRIN and TGFβ, thus leading to fibroblast differentiation from quiescent to activated cells, referred to as Cancer-Associated Fibroblast (CAF). Such modifications in stroma nature can be evidenced by Masson's trichrome (MT) as displayed by the two histological staining performed on malign and benign prostate biopsies obtained from the same patient. Stromal alterations can be revealed by change in the aniline blue staining which can be of prognostic value in patients with highly aggressive cancer. (b) Stromal-epithelial interactions involve a wide range of tumour-promoting effects such as FAPα and MMP

stimulations by CAF allowing cancer cell migration and invasion. As evidenced by MT staining, ECM protein expressions increase within the tumour stroma, thus participating to tumour growth and metastasis as well as to drug resistance. (c) As for normal wound healing (Figure 1), it is tempting to speculate on possible interactions between EMMPRIN and TGFβ receptors in fibroblasts. Hampering such interactions could represent a promising mode of control in fibroblast activation, a key process in fibrosis and tumour stroma formation.
scale bar = 500 μm.