The wound healing, chronic fibrosis, and cancer progression triad

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Rybinski B, Franco-Barraza J, Cukierman E. The wound healing, chronic fibrosis, and cancer progression triad. Physiol Genomics 46: 223–244, 2014. First published February 11, 2014; doi:10.1152/physiolgenomics.00158.2013.—For decades tumors have been recognized as “wounds that do not heal.” Besides the commonalities that tumors and wounded tissues share, the process of wound healing also portrays similar characteristics with chronic fibrosis. In this review, we suggest a tight interrelationship, which is governed as a concurrence of cellular and microenvironmental reactivity among wound healing, chronic fibrosis, and cancer development/progression (i.e., the WHFC triad). It is clear that the same cell types, as well as soluble and matrix elements that drive wound healing (including regeneration) via distinct signaling pathways, also fuel chronic fibrosis and tumor progression. Hence, here we review the relationship between fibrosis and cancer through the lens of wound healing.

wound healing; regeneration; fibrosis; cancer; tumor stroma; myofibroblasts; tumor- or cancer-associated fibroblasts; desmoplasia; epithelial-to-mesenchymal transition

The sheer complexity of the tumor stroma constitutes a remarkable discerning challenge for investigators. However, the tumor niche resembles a site of chronic wound healing (16, 84, 317). Out of the many similarities between wound healing and tumor progression, the mutual presence of myofibroblastic cells has emerged as a particularly common hallmark (56). Myofibroblasts are contractile spindle-shaped and stress forming mesenchymal cells that are known to express alpha smooth muscle actin (α-SMA), contain excessive structural rough endoplasmic reticulum, and produce copious amounts of extracellular matrix (ECM) (56, 90, 183). Myofibroblasts are also seen during fetal development (183) and are transiently present at sites of acute wound healing to repair injured ECM (i.e., to renew and/or to crosslink ECM fibers) and facilitate wound closure. Nonetheless, their constant presence in tumor-activated stroma (i.e., desmoplasia) is known to encourage neoplastic progression (16, 56). Interestingly, similar persistent myofibroblastic populations are also common occurrences during chronic tissue fibrosis (56, 317, 390). Hence, we propose to review the triad “wound healing/chronic fibrosis/cancer” (WHFC) through the lens of wound healing while highlighting their numerous common and few dissimilar traits.

The wound healing response to injury is known to trigger functional (i.e., proliferative) and phenotypic changes in fibroblasts, lymphocytes, and epithelial and endothelial cells (212). For example, the changes in epithelial and Stromal cells respectively correspond to the epithelial-mesenchymal transition (EMT) and myofibroblastic differentiation processes (212). Both EMT and myofibroblastic differentiation are common occurrences during wound healing (or tissue regeneration) but also throughout development, inflammation, and fibrosis, as well as during tumor progression. In EMT, epithelial cells acquire a mesenchymal migratory phenotype, switching the expression of known cell-cell adhesion molecules like E-cadherin in favor of mesenchymal proteins such as N-cadherin and vimentin (362). In cancer, EMT facilitates metastasis by promoting ECM proteolysis and enhancing malignant cell motility, thus conferring an ability to invade (362). EMT also renders cancer cells a stem-like character, which is believed to be responsible for therapeutic evasion, as well as a distinctive prolonged latency/survival mechanism known as dormancy (38). Myofibroblastic differentiation, also known as fibroblastic activation, describes the process by which the mesenchymal/fibroblastic components of the stroma (i.e., resident fibroblasts, recruited bone marrow-derived mesenchymal cells, pericytes, endothelial cells, and others) acquire a myofibroblastic phenotype (90). EMT and myofibroblast differentiation are regulated by similar factors and signaling pathways (Supplemental Table S1).1 Moreover, some investigators believe that stromal myofibroblasts arise also from epithelial cells via EMT-like processes (56, 123). Importantly, the healing process during acute injury involves four components: coagulation, inflammation, cellular proliferation, and ECM repair (212). Hence, wound healing (or regeneration) could be regarded as a four-component process that constitutes the foundation for cell proliferation, EMT, and myofibroblast differentiation in the WHFC triad. Similarly to how Champollion deciphered hieroglyphs by understanding Greek and Coptic, wound healing constitutes “the Rosetta Stone” needed for a better understanding of the WHFC triad (Fig. 1).

Chronic fibrosis and tumorigenesis can both result from a prolonged and exacerbated healing response that could stem

1 The online version of this article contains supplemental material.
from chronic injury (195, 317, 390), and as such the similarities that tumors and their stroma have in common with chronic fibrosis are intriguing. In fact, it is well known that chronic fibrosis, such as in liver cirrhosis induced by persistent viral infection (i.e., hepatitis), sustained alcohol consumption or toxin-containing food, constitutes a direct cancer predisposition (86, 91, 117). The epithelia from both fibrotic and tumor tissues are characterized by the presence of hyperproliferating cells (219, 269, 288); however, carcinoma cells carry transformative mutations (as well as epigenetic changes) presumed to be absent in the fibrotic tissue. Also intriguing is the stromal element of both conditions, a dense myofibroblastic component with an excessive deposition of ECM (56). The large population of myofibroblasts at the tumor stroma is referred to as cancer- or carcinoma-associated fibroblasts (i.e., known as CAFs) and also, in this review, as tumor-associated fibroblasts (TAFs). TAFs and fibrotic myofibroblasts drive tumor progression and chronic fibrosis, respectively, through both paracrine effects on surrounding cells and exacerbated synthesis of ECM (16, 56, 286, 389). TAFs express a plethora of protumorigenic growth factors, inflammatory cytokines, proteolytic enzymes, and ECM proteins such as collagen (Col)-I and -III as well as a fibronectin spliced variant known as ED-A and more (56). Hence, it is only logical to assume that effective targeting drugs that interfere with one (i.e., chronic fibrosis) will also stall the other (i.e., desmoplasia). Supple-
mental Table S2 includes a list of drugs targeting both diseases.

While chronic fibrosis predisposes the affected tissues to develop cancer (9, 30, 81, 91, 117, 232), a desmoplastic reaction that correlates with poor prognosis (56, 125, 317) is also believed to enhance tumor progression. In turn, transformed tumor cells also promote TAF activation, thus supporting the desmoplasmic reaction (16, 20, 56). Describing the relationship between cancer and fibrosis in these terms implies that fibrosis could be sparking tumorigenesis and tumor progression might be instigating chronic fibrosis. We suggest that the relationship between cancer and fibrosis (i.e., the WHFC triad) constitutes both correlation and causation (Fig. 1). Therefore, here we review the mechanisms by which the prolonged four component process of wound healing facilitates chronic fibrosis and tumorigenesis through a common mechanistic link: the WHFC triad.

THE COAGULATION CASCADE FUELS THE WHFC TRIAD

Wound healing begins with the process of coagulation where platelets from circulating blood accumulate at the damaged site and, together with increased levels of coagulation factors as
as well as with fibrinogen deposition and cleavage, result in the formation of insoluble fibrin strands building up a clot (212). The clot prevents blood loss and constitutes a short lived scaffold or provisional ECM that serves both for cell migration/invasion and as a growth factor reservoir (212).

In chronic fibrosis as in cancer, a process resembling clotting fuels disease progression. For example, fibrin bundles can accumulate at the tumor stroma as a result of a leaky tumor vasculature (260), and similar fibrin deposition has also been reported in a variety of fibrotic tissues (151, 308). Although the role of fibrin in chronic fibrosis remains rather unclear, it is known that fibrin exerts a mitogenic effect on fibroblasts (337). During tumor progression, fibrin facilitates angiogenesis (93); meanwhile, fibrinogen also promotes angiogenesis as well as oncogenic proliferation and antitumorigenic immune suppression (135). Moreover, elevated plasma fibrinogen correlates with a poor cancer prognosis (110, 203, 225, 290, 296, 321, 356). Intriguingly, fibrinogen may induce a prolonged inflammatory state in several types of cancer and fibrosis (68), suggesting a possible link between chronic inflammation and persistent activity of the coagulation cascade.

The cleavage of fibrinogen is considered the end result of the coagulation cascade signaling pathway (160). Thrombin directly cleaves fibrinogen into fibrin (95). Besides its role in the coagulation cascade, thrombin promotes cancer progression and fibrosis (47, 95). In cancer, thrombin induces cell proliferation, tumor growth, angiogenesis, and metastasis (95). The ability of thrombin to act as a mitogen is not limited to an epithelial context. It also has been reported to enhance fibroblast proliferation in a model of bleomycin-induced pulmonary fibrosis (358). Furthermore, thrombin inhibition reduces the severity of both pulmonary (358) and liver fibrosis (83).

The ability of thrombin to promote cancer and fibrosis is believed to be mediated in part through its ability to activate the protease-activated receptor (PAR)-1 (47, 95). PAR-1 is present in a variety of tumor types (89), and its activation promotes cancer cell proliferation and angiogenesis (95). In the clinic, PAR-1 signaling is suspected to facilitate melanoma metastasis (416), and it correlates with poor prognosis of gastric, prostate, and breast cancers (28, 100, 313). PAR-1 also facilitates pulmonary fibrosis (143), while PAR-1-deficient mice seem to resist bleomycin-induced pulmonary fibrosis (142). PAR-1 is one of four PAR isoforms (PAR-1 to -4), and, while thrombin induces many of its effects via PAR-1, PAR-3 and -4 are also suitable to be activated by thrombin (47). However, PAR-2 can be activated by other molecules from the coagulation cascade such as the tissue factor (TF) and activated factor X (FXa) (47). TF is expressed by many types of cancer cells (169), and TF or FXa-induced PAR-2 signaling has been implicated in both chronic fibrosis and tumor progression (33, 34, 151, 169, 318, 347). Interestingly, TF, FXa, thrombin, and activation of PAR-1 or PAR-2 may possibly promote myofibroblastic differentiation (Supplemental Table S1). This type of common occurrence opens up the possibility that other enzymes from the coagulation cascade could also induce/sustain myofibroblastic populations during chronic fibrosis and tumor desmoplasia.

To this end, the excess of fibrin deposition observed in chronic fibrosis and desmoplastic stroma indicates a possible enhancement in fibrin synthesis but also a likely decreased rate of its degradation. The fibrin/platelet clot formed during wound healing is eventually broken down by plasmin, a cleaved subproduct of plasminogen (111). Plasmin also plays a role in the ECM context due to its proteolytic activity in Col degradation, transforming growth factor beta (TGF-β) activation (see below) and release of other ECM-bound growth factors (111). Urokinase-type plasminogen activator (uPA) and tissue-type plasminogen activator (tPA) can each cleave plasminogen to obtain plasmin, and both enzymes are inactivated by plasminogen activator inhibitor-1 (PAI-1)(111). Interestingly, uPA has been reported to inhibit fibrosis (216, 334, 335) but also to promote tumor progression (335). Similarly, the role of tPA is also unclear as it has been reported both as a promoter as well as inhibitor of chronic fibrosis and cancer (62, 144, 196, 303, 405). Finally, PAI-1 has also been shown to either promote or inhibit these two diseases (111, 155). The above-mentioned facts together provide rationale to suggest that the coagulation cascade constitutes a shared WHFC triad targetable pathway.

**CHRONIC INFLAMMATION IS A DRIVER OF THE WHFC TRIAD**

Although overly simplified, it is generally agreed that, during homeostatic disturbances (i.e., during wound healing), M1 type macrophages drive an inflammatory response known to be tumor suppressive and antiﬁbrotic, while M2 type macrophages promote profibrotic and protumorigenic effects (96, 390). In response to macrophages and to injured epithelial cells, naïve CD4 + T-cells can differentiate into largely antiﬁbrotic and antitumorigenic Th1 cells or profibrotic and protumorigenic Th2, Th17, and Treg cells, while CD8 + T-cells are considered cytotoxic and constitute a positive neoplastic prognostic marker (390). During the acute wound healing response, immunity is triggered to prevent infection and to induce quick healing. Hence, a plethora of acute inflammatory secreted cytokines induce cellular proliferation, EMT, and myofibroblastic differentiation (56, 390).

Importantly, even though EMT has been proved to be difficult to detect in many cancers, chronic inflammation associated with fibrosis (389) and tumor progression (103) could induce persistent EMT as well as myofibroblast differentiation, both of which are known to further fuel the WHFC triad. It has been shown that inflammatory responses promote tumor development and progression including metastasis, and this axis has been thoroughly reviewed (120). In the following sections as well as in Supplemental Table S1, we highlight several inflammatory factors that influence chronic fibrosis and tumor progression because of their effects on cellular plasticity related to the WHFC triad. Please note that many of these factors have also been reviewed elsewhere for their effects in regulating the plasticity of mesenchymal stem cells (343, 397, 406).

**TGF-β**

The TGF-β superfamily of cytokines plays a notorious role in the WHFC triad. TGF-β exerts a dual effect during cancer progression, modulating the behavior of both epithelial and stromal cells imparting both pro- and antitumorigenic effects (238). For example, it has been described that TGF-β plays an antitumoral role on preneoplastic liver lesions by inhibiting epithelial cells’ growth and driving proapoptotic signaling when induced by other cytokines such as interferon-α (70). On the other hand, during more advanced tumorigenic stages,
TGF-β has also been associated with increased invasion and metastasis of malignant cells, correlating with enhanced activity and over expression of several integrins (238) as well as triggering EMT (242). Regarding its effects on stromal cells, TGF-β can also induce both myofibroblastic differentiation (238) and altered ECM production (254). Of note and documented in several models of renal fibrosis (413), TGF-β can, in addition, induce endothelial to mesenchymal transduction (16, 412). Although it acts through different cells and mechanisms and clearly participates as a tumor suppressor at early tumorigenic stages, it is nevertheless clear that TGF-β also promotes fibrosis (238) as well as tumor progression and metastasis (242). Certainly more information is needed in this regard as knockout of the TGF-β receptor II was shown to have tumor-promoting consequences (271), while collective cell migration was shown to improve under TGF-β signaling depletion (243). Nonetheless, TGF-β is evidently the most dominant profibrogenic factor known in all tissues, and anti-TGF-β agents (see Supplemental Table S2) are highly effective in a variety of fibrosis models. Latent TGF-β is secreted by various cell types, such as lymphocytes, platelets, cancer cells, myofibroblasts, and others, during the WHFC triad. For example, latent TGF-β can be secreted by cancer cells (242), inducing myofibroblastic differentiation of stromal cells, contributing to enrich an invasive TAF population (72, 211). Latent TGF-β is the inactive form of TGF-β that is found linked to the ECM and is sequestered by the latency-associated protein (LAP) and latent TGF-β-binding protein 1 (5). Latent TGF-β can be activated by ECM damage and myofibroblastic engagement of LAP via cell-matrix receptors such as integrins αvβ1, αvβ3, αvβ5, αvβ6, αvβ8, and others (257). TGF-β can be released and activated via ECM contraction induced by myofibroblastic cells (5). In fact, it has been suggested that changes in matrix stiffness could regulate the equilibrium between storage and release of matrix-bound factors such as TGF-β (383). In addition, the integrin-mediated binding of LAP may trigger a conformational change in the molecule, releasing the bioactive form of this factor (5). It is noteworthy that myofibroblasts secrete and activate TGF-β, establishing a self-sustained feedback loop, contributing to expand their own population. In addition, TGF-β effects are known to induce an increase in Col-I-rich ECM production (254). Autocrine TGF-β signaling has been shown to drive myofibroblast differentiation during tumor progression (185), suggesting a similar operational process in chronic fibrosis. Interestingly, the activation of TGF-β and subsequent myofibroblast differentiation can be triggered in vitro by a constant interstitial fluidic flow (265), suggesting the possibility of a physical effect of augmented tissue pressure, enhancing and expanding both myofibroblast and TAFs populations at the vicinity of the WHFC triad. To this end, it is not surprising then to recognize that all the WHFC triad diseases present changes in interstitial fluid flow.

### Interleukin-6

An important acute phase mediator, interleukin-6 (IL-6), is also a proinflammatory factor that has a prominent role in stromal activation. IL-6 null mice exhibit delayed wound healing related to a diminished myofibroblast differentiation (105). Moreover, IL-6 null mice exhibit an attenuated lung fibrosis phenotype in response to radiation (311), suggesting a role for IL-6-induced myofibroblast differentiation in fibrosis. In addition, IL-6 induces epithelial cancer cell proliferation (315) while also activating the EMT program (145, 348) and contributing to a preference for bone metastasis (8, 327). It is well documented that IL-6 promotes colitis-associated cancer via a STAT3-dependent mechanism (119). Finally, myofibroblasts from a variety of tissues are known to produce IL-6 (46, 330, 361), suggesting that mesenchymal IL-6 may participate in the WHFC triad by facilitating tissue fibrosis, tumoral desmoplasia, epithelial growth, and metastasis.

### IL-17

IL-17 is a cytokine that is upregulated in response to Th17 effector cytokine IL-23 (121, 379). IL-17 upregulation has been associated, together with other cytokines such as IL-6 and IL-22, as proinflammatory (394) as well as protumorigenic (379). IL-17 is known to act on myeloid and mesenchymal cells to induce expression of IL-6 (and others) (394). In addition, IL-17 is believed to induce expression of CXCR chemokines known for their neurophil chemo-attractive capabilities (394). Interestingly, IL-17 has also been associated with the expression of ECM remodeling enzymes such as matrix metalloproteinases (MMPs) MMP1, MMP3, MMP9, and MMP13, which are linked to both chronic inflammation and cancer (394). In particular, IL-17 has been shown to be activated by local microbial factors that can particularly penetrate neoplastic but not normal tissues. For example, a deterioration of the colorectal cancer tumoral barrier is induced in early lesions, allowing microbial factors to promote local IL-17-dependent inflammation to further drive tumor growth (121). Interestingly, IL-17 has an ability to activate fibroblasts in autoimmune, liver fibrosis, and cancer (246). Together these facts emphasize an important role for IL-17 in the WHFC triad.

### Chemokines

For the success of a wound resolution, it is necessary to coordinate the recruitment of several types of cells (e.g., inflammatory cells, macrophages, fibroblasts, and endothelial and epithelial cells) to the insulted tissue. This recruitment is mediated by chemotactic cytokines (chemokines), which are diffusible molecules that activate cell proliferation and direct cell migration toward the wound (21). The cytokines and chemokines involved in wound healing and their influence in tumor microenvironment (370) have been listed in several publications (21, 191, 411). In this review, we highlight some chemokines that are especially relevant to the WHFC triad.

During wound healing, the CXC chemokine family facilitates angiogenesis as well as lymphocyte recruitment (411). CXC receptors (CXCRs), in combination with chemokine ligands such as CXCL8 (184), play a key role during various cellular key responses (76, 253, 411). Therefore, it is not surprising that deregulation of the CXCRs/CXCL8 axis leads to fibrotic pathologies affecting lung (237, 340), kidney (351), and liver (417). Moreover, CXCR receptors have been proposed as therapeutic targets in some fibrotic conditions such as asthma (48, 175). In cancer, CXCRs/CXCL8 seem to be especially relevant to the WHFC triad.
enronment in colon cancer not only by recruiting inflammatory cells but also by recruiting myeloid-derived suppressor cells into the tumor milieu and therefore obstructing antitumor immune responses (170). The broad spectra of CXCR5/CXCL8 effects on cancer progression remains unclear, and yet CXCL8 expression has been proposed as a chemotherapy effectiveness marker (104).

Additional chemotactic axes relevant to the WHFC triad, which present contentious roles in fibrosis, are CXCR3 and their relevant ligand chemokines such as CXCL10 (411). Activity of CXCL10/CXCR3 promotes fibroblastic maturation, and it also inhibits endothelial cell proliferation and migration into the wound, a crucial step for wound tissue remodeling and maturation (21). Since CXCR3 activity has been associated with myofibroblastic differentiation and ECM maturation, it is expected that CXCR3-associated chemokines will be implicated in fibrosis. Indeed, in liver, CXCR3 is upregulated and is believed to play a profibrotic role in chronic hepatitis C (414). In fact, serological levels of CXCR3-associated chemokines have been proposed to help monitor the progression and complications of chronic liver diseases (352). In contrast, studies in chronic pancreatitis have proposed an anti-fibrotic role for CXCR3 and its associated chemokine CXCL9 via downregulation of Col-1-a1 and TGF-β (329). Additionally in the pancreas, the blockade of CXCL10/CXCR3 promotes a fibrotic condition in renal disease by upregulation of TGF-β (262). Disparities in the CXCR3 activity on different organs during a fibrotic response suggest a more complicated interplay of immune cells for a balanced regulation between pro- and antifibrotic cytokines/chemokines, which needs more investigation.

CXCL10/CXCR3 plays important roles in cancer as well. For example, in murine models of breast cancer, hypoxia inducible factor (HIF)-1α mediates the expression of CXCL10 and CXCR3 in mesenchymal stem cells and breast cancer cells, respectively, promoting metastasis (50). Moreover, in melanoma murine models as well as in colon cancer patients, CXCR3 promotes metastasis to lymph nodes (172, 173), and recent findings have demonstrated a synergism between CXCR3 and CXCR4 to promote metastasis to liver and lungs (258).

Perhaps the best-known chemokine reported in the WHFC triad is CXCL12, also known as stromal-derived factor (SDF)-1, which together with its main receptor CXCR4 induces migration of endothelial cells during wound-induced angiogenesis (411). The chemotactic properties of CXCL12 have also been documented during mobilization of fibrocytes from the bone marrow to the lungs (284), which in concert with other chemokines contribute to aggravate pulmonary fibrosis (230). Moreover, the SDF-1/CXCR4 axis has been shown to trigger prostate myofibroblastic differentiation in vitro, suggesting a role in fibrosis (109).

In cancer, the SDF-1/CXCR4 axis has been implicated in the progression of several types of malignancies, and its expression is associated with poor prognosis (66, 132, 387, 393, 395). An important study almost a decade ago demonstrated that SDF-1 secreted by TAFs (but not normal fibroblastic cells) triggers both angiogenesis and tumor growth via CXCR4 (275). SDF-1/CXCR4 activity is known to induce EMT and promote cell motility, invasion, and metastasis (299, 336), demonstrating a strong relationship between stromal and tumor components (298). Moreover, in breast cancer cells in vitro, CXCR4 signaling has been found to induce endocrine therapy resistance (300).

Several inflammatory stimuli are responsible for the expression of particular sets of chemotactic receptors in cancer cells (371), and more recently we have started to learn about the cooperation between diverse chemotactic receptors driving the metastatic phenotype as in the case of induction of CXCR2 expression via CXCR4 activation in breast cancer (336). The complexity in the chemokines receptor/ligand networks calls for a better understanding of the role of inflammatory signals modulating the evolution of the WHFC triad to develop better therapeutic strategies.

**Cyclo-oxygenase-2**

The enzyme cyclo-oxygenase-2 (COX-2) converts arachidonic acid to prostaglandin endoperoxide H2 (PGE2), and its expression triggers an inflammatory response (6). Nevertheless, the function of COX-2 and PGE2 seems to be contradictory and perhaps cell type specific during chronic fibrotic conditions. For example, PGE2 inhibits hepatic fibrosis (23) as well as pulmonary fibrosis where it has been shown to limit fibroblast proliferation and myofibroblast differentiation (37, 187). Also, although COX-2 is expressed briefly in response to tissue injury (131), the use of COX-2 and prostaglandin inhibitors appears only to impart a modest effect over wound healing (131, 244). Even though these types of inhibitors block inflammation, they exacerbate injury since they prevent wound healing especially in the intestine (354). Conversely, COX-2 inhibition reduces renal fibrosis (129, 302), suggesting that PGE2 may contribute to fibrosis in the kidney. Anti-inflammatory COX-2 inhibitory therapy in combination with chemoprevention and as adjuvant to chemotherapy shows some therapeutic promise (178). The role of COX-2 has been particularly well studied in colon cancer. However, COX-2 inhibition as a therapy may require more investigation, as prolonged COX-2 inhibition promotes drug resistance, resulting in increased intestinal fibrosis and tumor recurrence in mice (44, 69). Intriguingly, the induced drug resistance is believed to be mediated by intestinal myofibroblasts, as chronic COX-2 inhibition promotes TGF-β-induced myofibroblastic differentiation and augmented stromal PGE2 synthesis (44, 69). Moreover, TAFs from colon adenocarcinomas express a variety of factors that also drive tumor progression (188, 231). Hence, it is possible that the persistent myofibroblastic population maintained by chronic COX-2 inhibition is responsible for tumor recurrence as well as its correlation with tumor progression (178).

**Peroxisome Proliferator Activated Receptor Gamma**

While the previously discussed proinflammatory molecules seem to promote the WHFC triad, the role of the peroxisome proliferator activated receptor gamma (PPAR-γ), a transcription factor involved in immunomodulation, is notable for its inhibitory effects over fibrosis and cancer. During the late stages of wound healing, PPAR-γ activation stalls fibroblast proliferation and overthrows the myofibroblast phenotype (248), suggesting a mechanism for the termination of the healing response. PPAR-γ agonists are recognized for their inhibitory effects on TGF-β-induced myofibroblast differentiation (31, 41, 202, 239, 241, 252, 380). This antifibrotic effect...
has been proposed as a strategy to attenuate pulmonary and renal interstitial fibrosis (174, 194, 332). Then again, in cancer, some investigators have proposed an oncogenic, while others have stated a tumor-suppressive, role for PPAR-γ (124). For example, PPAR-γ agonists have been shown to impart anti-cancer effects (22), while a tumor-promoting effect is observed when PPAR-γ is inhibited (236) (see Supplemental Table S2). Nevertheless, PPAR-γ signaling has also been shown to exert an inhibitory effect on TGF-β-induced EMT in lung cancer cells and subsequent metastasis (297). Even more, PPAR-γ ligands can also induce EMT upon intestinal epithelial cells passing through a Rho-dependent activation of ERK1/2 (51), suggesting a tight regulation of the PPAR-γ signaling effects that could be associated with a particular cell type in a particular TGF-β signaling context. Interestingly, there is evidence to suggest an intricate regulation of the tumor stroma in colon adenocarcinomas, where COX-2 and PPAR-γ have been found co-expressed only in myofibroblasts at the tumor-associated but not the normal stroma (372). These findings suggest a PPAR-γ negative regulation of both fibrosis and cancer where its presence at late stages of nonpathological wound healing possibly contributes to prevent the WHFC triad occurrences.

CELL PROLIFERATION: A COMMON AVENUE USED BY THE WHFC TRIAD

A hallmark of developmental growth but also of wound healing is a transient hyperproliferative wave affecting both epithelial and stromal cells (183). This feature results from a temporary burst of diverse mitogenic signals triggered by a disturbance in the homeostasis of a given tissue (i.e., during wound healing). Expectedly, a similar spectrum of mitogens can also be constitutively detected in fibrotic and tumor tissues (Fig. 1), promoting epithelial and mesenchymal cell proliferation. Growth factors have a preponderate role among the large variety of mitogenic signals at the WHFC triad (Supplemental Table S1). Here we emphasize some of them.

Platelet-derived Growth Factor

One of the most abundant molecules released in response to tissue injury is the platelet-derived growth factor (PDGF) (227). PDGF is a key player in the persistence of chronic fibrosis, exerting a strong mitogenic signal upon fibroblasts and myofibroblasts (32). Also, PDGF can stimulate the proliferation of epithelial cancer cells in different types of tumors, exerting its influence through both paracrine and autocrine mechanisms (133, 287). Hence, PDGF-induced cell proliferation could very well sustain a desmoplastic reaction while supporting tumor progression, thus fueling a protumorigenic vicious cycle (20). Some studies suggest that PDGF could sustain or even initiate desmoplasia in breast cancer (148, 326), while others point to the fact that PDGF is a promisory factor (67) as well as an EMT regulator (388). Furthermore, PDGF expression can also affect other growth factors, as inhibition of stromal PDGF receptors in a murine model of cervical cancer was shown to alter the levels of expression of the fibroblast growth factors (FGF)-2 and -7 (287). Moreover, the inhibition of FGF-2 and FGF-7 in cervical cancer restricts their respective mitogenic and angiogenic effects, suggesting PDGFR as an attractive therapeutic target (287).

FGF-2

FGF-2, also known as basic fibroblast growth factor, encompasses a pivotal role during wound healing (220). FGF-2 exerts a robust mitogenic effect upon fibroblasts, and its participation has been linked to multiple fibrotic disorders (26, 152, 162, 177, 344–346). In addition, FGF-2 can be induced by TGF-β (see above), suggesting an important correlation between the two molecules (177, 345). Nonetheless, FGF-2 has also been reported to inhibit TGF-β-induced myofibroblast differentiation (92, 150, 154, 234, 339). Interestingly, during nonpathological wound healing, FGF-2 inhibits excessive scarring by triggering myofibroblastic apoptosis, yet quiescent (i.e., non-activated) fibroblasts appear to be refractory to this molecule (1, 339). These observations suggest that, under physiological conditions, FGF-2 may play a key regulatory role during wound resolution, encouraging fibroblast proliferation to repair damaged tissue but restricting the myofibroblastic population to limit excessive scarring.

Nevertheless, FGF-2 effects have been implicated in the development of several tumors by promoting proliferation and survival of cancer cells and even supporting tumor angiogenesis (368). It is not surprising that the effect of FGF-2 and the FGF receptor family (FGFR) in cancer is considered “complex” as the pathway has been shown to have both protumorigenic and tumor-suppressive roles (368). What is more, FGFR-induced activities can be ligand-independent (via genomic mutations of signaling molecules) or ligand dependent (368). Although the role of FGF-2 in desmoplasia remains rather obscure, its detection and implication at the stroma of prostate and mammary cancers as well as other malignancies (223, 367, 403) suggests a fairly broad participation (267).

Epidermal Growth Factor Receptor

The epidermal growth factor receptor (EGFR) family, also known as the ErbB family, consists of pleiotropic membrane-spanning cell surface receptors with intracellular tyrosine kinase activities that are triggered by homo- or hetero-dimerization when engaged by growth factors. Some of the family growth factor ligands are known to participate in wound healing [i.e., epidermal growth factor (EGF), heparin-binding epidermal growth factor (HB-EGF), and TGF-α] (281, 409). Under physiological conditions, EGFR signaling drives wound healing, and controlled activity of EGFR by HB-EGF has been proposed as a therapeutic approach to prompt and accelerate cutaneous wounds healing (161, 363). Of note, aberrant EGFR activation is known to promote fibrosis in tissues such as lung, heart, and pancreas (128, 214, 229, 245). EGFR activity has been implicated in the progression of malignancies such as colon and lung cancer (94, 134, 314). Nevertheless, EGFR activity has also been implicated in tissue regeneration during acute and chronic colitis where EGFR activity seems to limit fibrosis induced tumorigenesis (80). Then again, EGFR signaling activates EMT in malignant cells (130, 312). Doubly, EGFR activity affects the tumor stroma by inducing HB-EGF overexpression in TAFs, thus supporting tumor progression by direct and indirect effects (259).
Additional Deregulated Developmental Signals Promoting WHFC

The healing of a wounded tissue is known to be influenced by signaling molecules involved in embryonic development (183). Some of these signaling molecules are highly relevant during wound resolution and, when deregulated, are major players in the WHFC triad. To this end, Notch and Hedgehog (Hh) signaling cascades constitute two well-known examples of developmental signaling implicated in these processes (see Supplemental Table S1).

Notch signaling is an evolutionary well-conserved pathway known for regulating embryonic morphogenesis, tissue homeostasis, angiogenesis, and maintenance of the vascular system (25). The role of Notch during wound healing has been demonstrated in vitro as well as by genetic manipulation of murine models where Notch-deficient animals present delayed wound healing (54, 77, 228). Regarding its mechanisms of action, it is known that Notch signaling drives EMT, providing a vast source of fibroblastic cells necessary for wound repair (171). In addition, Notch activity has also been associated with fibrosis promotion in a variety of tissues (such as skin, kidney, lung, and cardiac tissue), influencing cell proliferation, extensive fibrilogenesis, TGF-β production, and α-SMA expression (171). In cancer (but not in regenerating epithelium during wound healing), Notch has also been shown to have an oncogenic role, promoting protein biosynthesis, cell growth, apoptosis resistance, and angiogenesis (305). Moreover, it has also been implicated in triggering EMT (224), fueling cancer invasion and enriching the desmoplastic TAF population. Nevertheless, there is evidence to suggest that Notch, depending on the cellular context, can exert a tumor suppressive role (224).

Another important signaling pathway in the WHFC triad is the Hh cascade. The Hh signaling pathway is triggered by several types of Hh ligands (i.e., Sonic, Desert, and Indian). The ligands bind to the receptor Patched, which in turn releases Smoothened to promote Gli transcription factors (Gli-1, Gli-2, and Gli-3) (58). Gli-1 appears to be the most abundant Hh-activated transcription factor, while Sonic Hh (Shh) is the better characterized ligand in the signaling pathway (58). Shh signaling is essential for wound healing as demonstrated by the inhibitory effect imparted by the topical application of a cyclopamine, a well-characterized inhibitor of smoothened, at all stages of the wound healing process (200). However, Shh signaling has also been shown to promote fibrosis in several organs such as lung, liver, and skin (157). Likewise, Hh has been shown to participate in tumor progression (58) by promoting stromal activation/desmoplasia (149, 359). For example, in pancreatic as well as in prostate cancer, it has been found that Shh is released by cancer cells, affecting stromal cells, causing differentiation and proliferation of α-SMA-positive myofibroblastic TAFs (15, 149, 199, 328, 376). Besides its ability to activate the tumor stroma, Shh ligands also promote EMT in cancer cells (396). Interestingly, while Shh signaling facilitates tumor progression through multiple mechanisms, it is possible that Shh ligands are not the only possible triggers of Shh signaling, as it has been found that TGF-β can also induce the activity of Gli-1 (157). This signaling pathway overlap strongly suggests a common participation in the WHFC triad. Although the relationship between Shh signaling and TGF-β activation merits additional investigation, the synergistic interaction could constitute an interesting therapeutic approach.

PATHOLOGICALLY ALTERED ECMs constitute both CAUSE AND CONSEQUENCE OF CHRONIC FIBROSIS and TUMOR PROGRESSION

Below we discuss the participation of physical ECM (i.e., architecture or stiffness) properties, as well as noteworthy components of pathological ECM common to the WHFC triad.

Topographical/architectural Changes in ECM

A pathological mesenchymal ECM differs from its normal counterpart and is recognized by an altered and constitutive expression of its building molecules, as well as for their characteristic architectural organization (294, 295), which together are believed to impart distinct physical and biochemical properties to the ECM (97, 410). Moreover, the altered architectural composition of a pathological ECM has been associated with clinical outcomes in breast cancer (59). Functionally, it has been suggested that topographical ECM alterations can impact normal stromal cells’ behaviors, thereby supporting a phenotypic switch (i.e., myofibroblastic activation) (4, 139). Concurrently, these newly activated cells continue synthesizing the above-mentioned altered ECM, feeding a vicious cycle affecting additional adjacent stromal (4, 16, 115, 137), as well as epithelial cells (16, 45, 97, 116, 197, 201), in a continuously expanding “field effect.”

ECM Stiffness

Both fibrotic and tumor-associated (i.e., desmoplastic) ECMs are characterized by an increase in stiffness compared with their normal stromal equivalent, which is known to be more flexible or compliant (116, 208, 277, 364). A less flexible environment exerts a greater physical tension to the cell body, which is transduced into multiple intracellular signals, modifying cell behavior. For example, relatively stiff ECMs alter cell growth, modify gene expression, and alter the differentiated status of cells (292, 338, 378). Moreover, ECMs subjected to a higher physical tension induce structural modifications to ECM-associated molecules, modulating their bioactivity (i.e., releasing active molecules of TGF-β), which in turn, for instance, can trigger myofibroblastic differentiation (139) and also further fuel the production of the altered (166) and stiffer ECM. Hence, in chronic fibrosis, tissue stiffness fuels a positive feedback enhancing the chronicity of fibrotic disease (218). Meanwhile in cancer, a rigid ECM induces tumor progression (42, 208, 282, 410) but also an enrichment of activated/myofibroblastic cells (i.e., TAFs), which in turn sustain a desmoplastic reaction and further promote tumorigenesis (16, 20, 64, 116, 399). ECM rigidity regulates the effects of TGF-β upon tumor cells, as compliant matrices facilitate TGF-β-induced apoptosis, while stiffer matrices promote TGF-β-induced EMT (206). Various ECM-related modifiers and a plethora of molecular components participate in the WHFC triad, and a few are listed below as well as in Supplemental Table S1.

Fibronectin

A native well-conserved constituent of the mesenchymal ECM that plays a determinant role in the WHFC triad is...
fibronectin (FN). Plasma FN (307) is commonly found in the circulation, while cellular FN (400) is the glycoprotein that is expressed by fibroblastic cells and is incorporated into the fibrillar ECM mesh. From a single human gene and as product of alternative mRNA splicing, several cellular FN variants can be expressed (384). Two spliced exons called “extra domains A” and “B” (EDA and EDB) are particularly and abundantly expressed during embryonic developmental stages, while an important (i.e., normal) reduction is observed in adult tissues (384). Nevertheless, during the physiological ECM restructuring of wounded tissue, EDA-FN is upregulated by myofibroblastic cells (118). Moreover, in a positive feedback regulation, EDA-FN has been shown to exert a permissive action during myofibroblastic activation (82). EDA-FN overexpression has been found to occur in response to increased ECM tension (364), and it is influenced by high levels of TGF-β (82). EDA-FN also influences the tumor microenvironment, affecting both stromal and tumor cells. At the stromal compartment, EDA-FN seems to be intimately related to the bioavailability of active TGF-β for the subsequent sustainability of the reactive stroma (384). EDA-FN is known for promoting tumor growth, EMT, and tumor-induced lymphangiogenesis (349, 391). EDB-FN has also been associated with tumor progression, in particular with tumor angiogenesis and is being tested as a potential tumor target in the clinic (320). Hence, alternative spliced forms of FN seem to play important roles in the WHFC triad.

Collagen

As the major ECM component in the connective tissue and the most abundant protein in mammals, collagen has been described as a heterotrimeric triple helix glycoprotein that can be assembled into de novo fibrous matrices during healing, playing a crucial role during the repair of tissue defects. Collagen deposition increments the ECM tension during the restoration of the vessels network and the recovery of tissue architecture during wound healing (118). Among the collagen family, Col-I is the most abundant interstitial protein of the ECM (107) often seen in fibrotic tissue and desmoplasia. Then again, Col-IV is the main component of epithelial and endothelial basal membrane (167). The activated/myofibroblastic phenotype seen in the WHFC triad is commonly described as containing abundant Col-I and -III, although reports showing changes in Col-IV and -V are also evident (136). In addition, Col-VI spiked some attention due to its selective expression in some fibrotic conditions (i.e., kidney and myocardial fibrosis) as well as in hepatocellular carcinoma (136). In fact, its level of expression has been recently proposed to serve as a clinical prognostic marker (204, 205). Carcinomas that typically present an aggressive progression (i.e., liver and pancreas) are characterized by a large deposition of interstitial FN and collagen. These occurrences are blamed for augmented tumor cell proliferation that is accompanied by an intense desmoplastic reaction (12). For example, it was reported that pancreatic cancer cells can trigger fibroblastic activation while promoting increased deposition of Col-I and -III in a nude murine model (12). In breast cancer, Col-I cross-linking is increased, showing a reduction on its degradation rate, resulting in excessive deposition (168). In sum, Col-I and -III changes are typically described in diseases associated with the WHFC triad.

Lysyl Oxidase

An important regulator of ECM rigidity is the activity of the enzyme lysyl oxidase (LOX), which catalyzes the cross-linking of collagen (see above) and elastin bundles, modifying the tensile strength of the ECM (165). LOX is essential for maintaining ECM strength during normal development (233) and becomes highly expressed during the course of several fibrotic diseases (164, 392). Throughout cancer progression, LOX facilitates Col-I cross-linking, promoting ECM stiffening and therefore altering known integrin-regulated signaling pathways (208). In fact, increased LOX activity has been associated with a poor prognosis in several carcinomas (392). LOX and LOX-like enzyme 2 (LOXL2) are downstream targets of HIF-1α and, under hypoxic conditions, promote EMT by repressing E-cadherin (319). In a xenograft tumor model, the inhibition of LOX2 decreased the number of α-SMA-positive TAFs (17). Furthermore, LOXL2 has been proposed as a prognostic biomarker for squamous cell carcinomas (283). Together these data support the correlation between increased cross-linked Col, stiffer ECM, an enriched TAF population, and the exacerbation of tumor desmoplastic reaction. Interestingly, the same LOX inhibitory antibody hinders murine liver and pulmonary fibrosis (17), suggesting a compelling similarity as proposed in the WHFC triad (Supplemental Table S2).

MMPs

Even though, intuitively, MMPs, are ECM-cleaving proteases, they do not antagonize fibrosis, cancer, or fibrosis-induced cancer. Hence, these enzymes are regarded as ECM remodeling proteins as opposed to ECM-degrading proteins (278). MMPs play a crucial role during inflammation, re-epithelialization, and ECM remodeling associated with regenerative wound healing (113). Specifically, MMPs mediate ECM remodeling during scar resolution, cleave and activate ECM-associated growth factors, regulate chemokine activity, and also facilitate keratinocyte migration into the wound (113, 278). MMPs can be expressed by inflammatory cells, epithelial cells, fibroblasts, and myofibroblasts. The sources and functions of MMPs implicated in the context of cancer (176) and fibrosis (278) are very diverse and beyond the scope of this review. Nonetheless, we highlight two gelatinsases, MMP-2 and MMP-9 (19, 176), as these enzymes seem to convey a recurrent presence in the WHFC triad.

The participation of MMP-2 and MMP-9 in chronic fibrosis appears to be contentious. For example, a clear upregulation of these MMPs expressed by myofibroblasts is evident during the development of idiopathic pulmonary fibrosis (322). Also, MMP-2 has been found to be highly expressed in fibrotic liver myofibroblasts (293), which incidentally has been proven to constitute a noteworthy predisposition to developing hepatic cell carcinoma (86). Nonetheless, a downregulation of MMP-2 has been reported during in vitro myofibroblastic differentia-
tation of rat embryonic fibroblasts (141), while its inhibition has been shown to accelerate murine renal fibrosis (268).

In cancer, MMP-2 and MMP-9 are known to promote tumor progression by facilitating the release of stored latent growth and other factors (i.e., TGF-β) from the ECM, thus enhancing respectively cell proliferation and migration (36). MMPs can also facilitate cancer cell migration (385) by interaction with cell-matrix adhesion molecules, such as integrins, promoting degradation of cancer cells from cell-cell and cell-matrix adhesions (36). To further fuel the recurring cycle, EMT enhances the expression of MMP-2 and MMP-9 (36). Interestingly, MMP cleaved ECM peptides (i.e., soluble ECMS) are known to induce an integrin-dependent TGF-β-like signaling response (106). Also, CD147, a cell-surface glycoprotein upregulated in tumor cells, can stimulate both stromal MMP production and myofibroblast differentiation (147). In sum, MMPs produced by cancer cells promote tumor invasion as well as myofibroblast differentiation, reinforcing a tumor-supportive stroma, which in turn further secretes and activates additional MMPs to further fuel this vicious cycle.

**Hyaluronan**

A particular component of the ECM, with no inflammatory activity but related to inflammation, is the glycosaminoglycan hyaluronan (HA). This macromolecular molecule contains multiple unit repetitions of N-acetyl-glucosamine as well as of glucuronic acid, and it binds and stores large amounts of water molecules conforming a viscous gel (355). HA has been suggested to function as a pliable substrate for tissue remodeling necessary for wound healing (355). It also binds fibrinogen, supporting clotting, and acts as a mesh that traps inflammatory cells (342). Large HA molecules of ~25,000 disaccharide units (355) encourage cellular quiescence, while small HA fragments ranging from 4 to 25 units encourage proliferation and angiogenesis (342). It has been proposed that HA breakdown at the injury site renders fragments that participate in the initiation of the healing response (342).

HA accumulation is associated with exacerbation of fibrosis (27, 159). The expression of HA synthesizing enzymes “hyaluronan synthase (HAS) 1–3” during chronic fibrosis has been associated with an increased inflammatory reaction (52, 213). Knockdown of HAS2 reduces the effectiveness of TGF-β-stimulated EMT (291), and elevated HAS2 expression is sufficient to induce EMT in normal epithelial cells (418). Moreover, inhibition of HA synthesis antagonizes TGF-β-induced myofibroblastic differentiation (381), and inhibition of HAS2 impairs myofibroblast accumulation in mouse models of pulmonary fibrosis (213). Since elevated HA has been observed in a variety of fibrotic diseases (210, 213, 357) and HAS2 regulates TGF-β-dependent functions (291), their accumulation and increase in expression are respectively suggestive (i.e., markers) of fibrotic myofibroblast differentiation and EMT.

With regards to cancer, increased HA is linked to poor cancer outcomes (355), while upregulation of HAS2 is a common occurrence in highly metastatic breast cancer cells (273). Nevertheless, the role of HA in cancer seems to be tissue specific. In general, accumulation of HA in tissues where it is normally absent facilitates tumor growth (i.e., gastric cancers), while reduced HA is associated with cancer in normally HA-rich stratified epithelia containing tissues such as skin, mouth, and larynx (355). Interestingly, epithelial HA accumulation in colon cancer constitutes a bad prognostic occurrence (304), while stromal HA builds up are associated with an unfavorable prognosis in ovarian, prostate, breast, and nonsmall cell lung cancers (11, 217, 289, 382). In the case of liver, the main organ in charge of processing HA (98), a large synthesis of the glycosaminoglycan and increasing circulating levels of HA have been found to correlate with liver fibrosis (cirrhosis) (280). In addition, hepatic cirrhosis is known to predispose for hepatocarcinoma, and HA deposition has also been associated with cancer progression (285, 341). Most of the mechanisms of HA’s driven tumor progression deal with the promotion of cell proliferation, NF-κB activity, and angiogenesis (333). HA also binds to the cellular receptor CD44, which encouages motility and invasion (333). Intriguingly, HA expression is increased via growth factors such as EGF, keratinocyte growth factor, and PDGF (355), suggesting a more complicated role for certain growth factors, which could promote both cancer cell proliferation and HA accumulation. Interestingly, the antiproliferative and proapoptotic effect of HA inhibition, affecting both the tumor and surrounding stroma (285), highlights the importance of designing anticancer strategies focused on attacking both the tumor and its associated fibrotic stroma.

**Periostin**

Periostin is a matricellular protein that increases ECM stiffness by direct binding to fibrillar ECM proteins such as Col-I (270) and by interacting with the TGF-β-related protein BMP-1, which is needed to activate LOX (240). In addition, periostin functions as a ligand for integrins αvβ3, αvβ5, and α6β4 (102, 306). In general, periostin expression seems to play a main role during wound healing and tissue regeneration (reviewed in Ref. 192). For example, periostin-deficient mice present a reduction in the number of cutaneous wound healing/repair myofibroblasts (88). Periostin has been associated with myofibroblastic differentiation in vivo and in vitro via an integrin-dependent mechanism as well as with ECM remodeling (60, 87). As a consequence, periostin increases are also associated with pathologies marked by a fibrotic condition such as idiopathic pulmonary fibrosis, renal fibrosis, asthma, and skin sclerosis in scleroderma (261, 323, 331, 401).

Regarding cancer, periostin has been found to be expressed by cancer epithelial cells (255, 306), and recently it has been associated with increased esophageal cancer invasion of p53 mutants via STAT1 induction (386). Moreover, periostin can also be expressed by stromal cells and, as such, has been identified in desmoplastic pancreatic cancer (102), colon cancer, cholangiocarcinoma (179, 369), and other types of cancer (193, 222, 249, 255, 306). Consequently, periostin seems to facilitate both tumoral and stromal progressions. Periostin integrin-dependent signaling activates focal adhesion kinase (FAK), as well as the PI3K/AKT pathway, thus generally promoting malignant cell survival, proliferation, and angiogenesis (255, 306). Moreover, activation of integrins by periostin promotes EMT in cancer cells (255). Intriguingly, in bladder cancer, periostin seems to function as a tumor suppressor by in vitro inhibition of invasion and reduction of in vivo metastasis (180). Moreover, in many cancer cell lines in vitro periostin seems to suppress anchorage-independent growth, while low
periostin mRNA levels are observed in lung cancer in vivo (408).

Tenascin C

Tenascin C is another ECM-related glycoprotein expressed in response to tissue injury known to modulate the wound healing response (251). Tenascin C levels decrease subsequently to wound healing resolution (251). This decrease in tenascin C is also associated with a natural reduction in myofibroblasts (251). Hence, it is not surprising that a mis-regulated and continuous expression of tenascin C is a common occurrence, which is believed to drive fibrosis and cancer via increased angiogenesis, ECM alterations, and immunomodulation (250). For example, tenascin C-deficient mice are protected from pulmonary and liver fibrosis (43, 85). In addition, tenascin C has been shown to promote tumorigenic progression by supporting cell motility, metastasis, and more (101, 127, 207, 274, 279). Tenascin C is synthesized by both tumoral and stromal cells (71, 276). Then again, tenascin C could also indirectly promote the WHFC triad by its effect on the down-regulation of tPA, which facilitates fibrin accumulation, thus triggering a mitogenic response (40).

Osteonectin/SPARC

The secreted protein and rich in cysteine (SPARC), also known as osteonectin, is a matricellular protein that is known to affect ECM remodeling/dynamics. SPARC is often highly expressed during wound healing, cancer, and fibrosis (18, 55, 365). For example, the upregulated expression of SPARC seems to be a common event during several chronic fibrotic conditions such as in the cases of heart, lungs, kidneys, liver, dermis, intestine, and eyes (365). SPARC is produced by fibroblasts in response to TGF-β, but it can also promote Col-I and TGF-β synthesis (365). In a SPARC null murine model, a decreased deposition of dermal collagen constitutes a predominant trait accompanied by an enhanced contractile behavior of dermal fibroblasts (39). By affecting collagen deposition and reducing the number of myofibroblasts, decreased SPARC expression reduces liver fibrosis (10). Nonetheless, despite the implication of SPARC in the control of wound healing, its precise role remains rather unclear. For instance, it has been reported that SPARC can both induce and inhibit the wound healing process (18, 39).

Similarly, SPARC has also been implicated in tumor progression where it is believed to sometimes promote, and at other times prevent, this process apparently in a tissue- and cell-specific manner (55). Among other functions, SPARC participates both in carcinoma EMT and in TAFs’ myofibroblastic differentiation, thus promoting both tumorigenesis and desmoplasia (55). In prostate cancer, for example, the expression of SPARC by malignant cells can be predictive of metastasis, while in pancreatic and nonsmall cell lung cancers, elevated expression of SPARC at the tumor stroma indicates poor prognostics (29, 74, 189). Regarding SPARC’s tumor-suppressing effects in breast, ovarian, colon, and bladder carcinomas, SPARC has been associated with inhibition of proliferation and induction of apoptosis (55, 309). Intriguingly, a positive feedback between SPARC and TGF-β has been observed, as both can induce the expression of each other (365). Nevertheless, a comprehensive comparative proteomic analysis has demonstrated that not all the cellular responses to SPARC signaling depend on TGF-β (114), and, although there is a certain parallelism between them promoting neoplasia (242), a balance of both SPARC and TGF-β activity could be responsible for tumor progression (114). These facts suggest that an intricate regulation of the SPARC/TGF-β signaling axis also constitutes a common occurrence of the WHFC triad (Supplemental Table S1 and Fig. 1).

Proteoglycans

Comprising a diverse group of members, the proteoglycan family includes both cell surface proteins, such as heparan sulfate proteoglycans, and ECM components, such as small leucine-rich proteoglycans (SLRPs), and large chondroitin sulfate proteoglycans (153). Proteoglycans participate in immunity regulation (57, 99), ECM assembly (53), and cancer modulation (153). However, as a complete description of proteoglycans is beyond the scope of this review, we highlight a selection of ECM proteoglycans most relevant to the WHFC triad.

Versican, a large chondroitin sulfate proteoglycan, is up-regulated during both wound repair and cancer progression (63, 112, 301, 353). During normal conditions, myofibroblasts from human granulation tissue express elevated levels of versican (126). There is a lack of studies investigating the role of versican in the context of chronic fibrosis. However, the expression of versican mRNA has been detected as increased in rat lungs after bleomycin-induced injury, and TGF-β signaling increases versican synthesis in fibrotic lung in vivo (373). On the other hand, versican expression correlates with a poor prognosis in many cancer types (301, 325). This proteoglycan interacts with multiple ECM and cell surface components, promoting cell proliferation, cell motility, and metastasis (301, 310, 382). Intriguingly, in breast cancer, versican has been associated with chemotherapy resistance related to EGFR hypersignaling and enhanced cancer cell self-renewal (78, 79). Moreover, versican synthesis appears to be upregulated by TAFs in the ovarian cancer stroma (407) where TGF-β signaling triggers versican expression, and together they contribute to promote ovarian cancer aggressiveness (407).

The SLRP biglycan expression is enhanced in myofibroblasts from human granulation tissue (126). The role of biglycan in fibrosis may be tissue specific since biglycan does not affect TGF-β-induced pulmonary fibrosis (186), and biglycan mRNA appears not to be increased in mercuric chloride-induced renal tubulointerstitial fibrosis (350). However, biglycan expression and serological levels of its processed byproducts correlate with the extent of liver fibrosis in murine models (108, 204). Biglycan is also involved with progression and poor prognosis in a variety of malignancies such as gastrointestinal and endometrial cancers (7, 122, 221, 377, 415). In contrast, biglycan expression correlates with a favorable prognosis in bladder cancer where it seems to have an inhibitory effect on tumor cell proliferation (266).

In addition to biglycan, the SLRP decorin also plays an active role in the WHFC triad, although its participation in chronic fibrosis and tumor progression seems to have an inhibitory effect. Animal models have shown that absence of decorin exacerbates fibrosis (13, 247, 316). Moreover, interesting data suggest that decorin itself could be a beneficial...
antifibrotic therapeutic alternative (156, 186, 402). A suggested antifibrotic mechanism of decorin deals, at least partially, with the inactivation of TGF-β signaling (14), impairing myofibroblastic differentiation in liver fibrosis models (14). Another mechanism described is the direct interaction with the cytokine known as connective tissue growth factor or CTGF, inhibiting its fibroblastic activation role (374). In cancer, decorin gene therapy or delivery of decorin protein inhibits tumor growth, tumor progression, and metastasis (153). Besides the attenuation of TGF-β signaling by decorin described in fibrosis models (14, 402), another mechanism of action has been found in the tumor microenvironment where it could also be inhibiting EGFR signaling (153). Decorin binds to EGFR overlapping with the EGF binding domain and inducing internalization of EGFR via caveolar endocytosis, resulting in decreasing EGFR accessibility on the cell surface (153). Together, these effects evoke a downregulation of EGFR signaling in tumor xenografts and apoptosis induction (153). Decorin has also shown additional antitumor effects including the inhibition of angiogenesis and induction of p21 (153, 264). Although reduced decorin expression in the tumor stroma indicates poor prognosis in models of breast cancer (264), decorin can be found in both the tumor stroma and fibrotic tissue (14, 153). This could be explained as a protective response (153), although it has also been observed that collagen-bound decorin found in the ECM could not be effectively protective (14). Future work may seek to clarify the influence of decorin on the myofibroblastic cells during chronic fibrosis and tumor desmoplasia.

CHRONIC FIBROSIS AND CANCER DIFFER FROM TRANSIENT WOUND HEALING IN THAT THEY BOTH ENCOMPASS A SUSTAINED MYOFIBROBLASTIC POPULATION

While regenerative healing wounds, fibrosis, and tumor-associated desmoplasia all contain metabolically active myofibroblasts and EMT cells, it is only under normal healing/regenerative (i.e., nonpathological) conditions that the activity of these cells eventually ceases. This may result from activated cells’ (i.e., EMT or myofibroblasts) senescence and subsequent elimination. This can occur through p53- and/or integrin-regulated apoptosis or potentially by cell clearance via natural killer (NK) cells and/or by interferon gamma secretion (146, 181, 198, 209, 360, 398). From the one side, myofibroblastic apoptosis could promote pathologic scarring (75). Then again, the persistence of myofibroblasts during fibrosis and cancer-associated desmoplasia suggests that these cells escape from their natural elimination process by alterations in the myofibroblastic cells-clearance mechanisms. Alternatively, persistent activated cells may “sense” chronic injury or tumor-associated microenvironment (i.e., desmoplasia) and consistently respond “normally.” In addition, fibroblastic cells naturally progress and become senescent in response to a variety of signals. Nevertheless, under pathological circumstances when clearance mechanisms (i.e., wound resolution) are evaded, cells can develop what is known as the “senescence associated secretory phenotype” or SASP, thus turning them into sustained and proinflammatory senescent cells (61). In response to SASP, affected fibroblastic cells produce a variety of diffusible factors that in turn can drive both fibrosis and tumorigenesis (61), further fueling the WHFC triad’s fibrosis/tumorigenesis vicious cycle (see Fig. 1).

To further complicate things, although SASP seems to fuel the WHFC triad, evidence also suggests that the sole escape of fibroblasts from senescence can also support WHFC. For example, during normal wound healing, the matricellular protein CCN1 (also known as cysteine-rich protein 61 or CYR61) is expressed by myofibroblasts and tends to accumulate along with the myofibroblastic cell population (198). High concentrations of CCN1 induce myofibroblast senescence (198) and promote downregulation of collagen and TGF-β as well as upregulation of MMPs (198). Together these changes induce a senescent matrix-degrading cell state (198). During wound healing resolution, myofibroblast senescence serves to prevent pathological scarring (198). Hence, senescence is generally recognized to inhibit fibrosis (163, 190), so it is not surprising that mice expressing defective CCN1 suffer from exacerbated fibrosis in response to injury (198). Furthermore, recent findings have shown that restoring lost levels of CNN1 in a hepatic fibrosis murine model can induce myofibroblastic senescence and reduce chronic tissue-damaging fibrosis (182). Nevertheless, the role that CCN1 plays in both tumor-associated stroma (i.e., desmoplasia) and in tumorigenesis remains rather obscure. CCN1 has been shown to present opposing effects on tumor progression, as some data indicates that CCN1 inhibits tumor growth by inducing apoptosis and senescence, while other reports suggest it promotes tumor growth via the stimulation of cancer cell proliferation and angiogenesis (198).

In addition, it is known that CCN1 induces senescence by activating the p16(INK4A)/pRb pathway (198). Interestingly, TAFs are hyperproliferative cells that express diminished levels of p16(INK4A) (3). Also, p16(INK4A) downregulation in normal human breast stromal fibroblasts is accompanied by an increase in α-SMA expression, suggesting that absence of p16(INK4A) could promote myofibroblastic differentiation (3). Additionally, p16(INK4A)-deficient fibroblasts enhance xenograft tumor growth compared with effects imparted by wild-type cells (3). Finally, fibroblasts from oral squamous cell carcinoma with a p53 and p16(INK4A) inactive background have been shown to induce the upregulation of α-SMA expression, which correlates with poor patient prognosis (215). All these data strongly suggest that inactive p16(INK4A) is a hallmark of TAFs and, therefore, of cancer progression. Regarding p53, it has recently been shown that stromal p53 decrease in chronic liver damage promotes tumor development by a noncell autonomous mechanism that upregulates a tumor promoting M2 macrophage microenvironmental state (226). Hence, there is strong possibility that at least two distinctive populations of TAFs that drive tumorigenesis coexist at the tumor stroma: a hyperproliferative p16 low population and a rather quiescent SAPS positive and presumably p16 high population. This type of TAF heterogeneity reveals a high level of complexity at the tumor-stromal interaction niche, which requires a better understanding to improve the design of increasingly effective stroma targeting therapeutics.

If one considers the fact that cell death, for example during injury, causes fibrosis, then another important fact of the WHFC triad is that some types of fibrotic tissues are often characterized by regions of cellular death (219, 269, 288), while, similarly, tumors contain noteworthy areas of necrotic tissue (226). Cell death is well known to activate an inflam-
matory response, which when sustained and prolonged, in turn, would facilitate both fibrosis and tumorigenesis (35, 195, 389). Moreover, whether fibrotic myofibroblasts and TAFs are senescent or hyperproliferative, they also must evade stromal cell clearing mechanisms, such as apoptosis, which promote wound resolution (Fig. 1). In hypertrophic scars, the presence of apoptosis-resistant myofibroblasts high in antipapoptotic protein Bcl-2 has been reported (256). Although the mechanisms remain unclear, TGF-β may induce myofibroblast resistance to apoptosis (138). Intriguingly, the fact that tumors release a variety of molecules that can interfere with the immune response as well as with NK cell infiltration and activation (209) suggests the prevalence of an immunosuppressive microenvironment that may protect fibrotic myofibroblasts and desmoplastic TAFs from active NK cell elimination. For example, it has been shown that TGF-β is a potent inhibitor of NK (366). Although there is not much information about this mechanism in cancer and specifically on the tumor stroma, research on the chronic fibrosis field has shown a relationship between the persistence of myofibroblasts with an immunosuppressed microenvironment profile (i.e., myofibroblasts attract immune-suppressive cells) (38, 181, 375). Hence, facilitating the NK cell-mediated elimination of myofibroblastic and tumoral cells is expected to be a feasible approach to counteract the WHFC triad (38).

CONCLUDING REMARKS

Possible Approaches to Clinically Interfere With the WHFC Triad

Since a convergence between wound healing, cancer, and fibrosis is compelling, its implications for fibrosis and cancer treatment are profound. To achieve effective drug delivery, it is crucial to comprehend the challenge that a fibrotic microenvironment represents. For example, due to a dense and altered ECM deposition, the free access for drugs and other molecules, including glucose and oxygen, is severely impaired in desmoplastic malignancies (65). In this context, it is necessary to develop novel drug delivery strategies to obtain better results while targeting both fibrosis (to obtain stromal normalization) and cancer (see Supplemental Table S2). This is true in particular with regards to carcinomas displaying an intense desmoplastic reaction such as in pancreatic cancer (263). Importantly, it is troubling that current approaches aimed at treating fibrosis and cancer also counteractively promote the loss of homeostatic equilibrium, which in turn further promotes the WHFC triad (Fig. 1). For example, surgical procedures and radiation are effective in resecting and eliminating cancerous and chronically fibrotic masses. Nevertheless, the same treatments also create wounds that must be healed, hence possibly further promoting fibrosis and hyperplastic growth (2, 49, 73). Moreover, while surgery triggers a wound healing response, chemotherapy also activates the same mechanisms that induce fibrosis (49, 272). Above we stated that fibrotic disease fuels cancer progression and vice versa (Fig. 1). Since it is possible that radiation and chemotherapy-induced fibrosis also stimulate relapse or latent escape of secondary tumors, a more optimistic perspective will rely on the use of antifibrotic therapies in cancer treatments. This approach could have a great impact on those malignancies where fibrosis constitutes a clear predisposition, such as in the cases of pancreatic or hepatic cell carcinomas, where studies have shown promising results targeting and repressing both tumor and stroma (285). These may offer an advantage when used against cancer, while some chemotherapies and targeted cancer treatments could also have great impact on resolving fibrotic chronic diseases. For this we have put together Supplemental Table S2, which lists some of the current common WHFC triad treatments under investigation or in clinical use today.

Reflecting Upon the WHFC Triad

This review attempts to highlight the importance of understanding wound healing mechanisms, as a pathway to comprehend two subsequent conditions, derived from the persistence and exacerbation of the healing process. In the absence of wound resolution, continuous alterations to tissue homeostasis engage both stroma and epithelia for the onset of chronic fibrosis and/or cancer. We propose the consolidation of commonalities from these three conditions under the concept of the WHFC triad. In other words, similar mechanisms operate in this triad to sustain a vicious cycle where a fibrotic stroma could trigger a malignant behavior of epithelial cells, while the progression of carcinomas also promotes a fibrotic/desmoplastic stroma fueling the conditions for both (Fig. 1).

The WHFC triad is assembled by common biological responses from the three described conditions. Several proteins and signaling pathways encompass a remarkable degree of overlap amongst wound healing, pathological chronic fibrosis, and cancer (317). The mentioned players can be clustered based on their biological mechanisms, in a coagulation/fibrinolysis-like response, inflammation signaling processes, as well as ECM remodeling mechanisms. These biological occurrences are responsible for triggering hyperproliferative cell behavior and phenotypic/activating modifications such as EMT and myofibroblastic differentiation (Fig. 1 and Supplemental Table S1).

Specifically for cancer, the WHFC triad constitutes a rather fertile ground facilitating genetic mutations and epigenetic hyperplastic occurrences, which eventually constitute the malignant cell population (91). Moreover, an additional difference between chronic fibrosis and cancer is that, although both expand in an immediate field-like effect through the affected tissue, only cancer is known to metastasize to tissues localized at considerable distances from the primary lesion, while secondary neoplastic niches are actively preconditioned to facilitate metastatic cancer cell adaptation (120). Importantly, crucial aspects of the WHFC triad that have already started to receive attention, such as genetic and epigenetic signatures as well as microRNA profiles (24, 404), although in need of being fully understood, are already being proposed to serve as predictors of the outcome of some fibrosis-related malignancies. Hence, we conclude by stating that a better recognition and a deeper understanding of the commonalities and disparities within the WHFC triad will surely provide insights for a more effective therapeutic targeting of these interconnected pathologies (Supplemental Table S2).

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