Stromal Dynamic Reciprocity in Cancer: Intricacies of Fibroblastic-ECM Interactions

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Abstract:

Stromal dynamic reciprocity (SDR) consists of the biophysical and biochemical interplay between connective tissue elements that regulate and maintain organ homeostasis. In epithelial cancers, chronic alterations of SDR result in the once tumor-restrictive stroma evolving into a “new” tumor-permissive environment. This altered stroma, known as desmoplasia, is initiated and maintained by cancer associated fibroblasts (CAFs) that remodel the extracellular matrix (ECM). Desmoplasia fuels a vicious cycle of stromal dissemination enriching both CAFs and desmoplastic ECM. Targeting specific drivers of desmoplasia, such as CAFs, either enhances or halts tumor growth and progression. These conflicting effects suggest that stromal interactions are not fully understood. This review highlights known fibroblastic-ECM interactions in an effort to encourage therapies that will restore cancer-restrictive stromal cues.

Introduction

Centuries of research corroborate the mesenchymal-stroma accompanying epithelial cancers to be more than an impartial bystander providing mechanical and nutritional support to tumors [1,2]. The stromal compartment is vastly diverse in cellular and extracellular composition across all tissue environments. It is comprised of fat, immune, endothelial and fibroblastic cells as well as a fibroblastic-derived extracellular matrix (ECM). This ECM is rich in fibrous proteins such as type I collagen and fibronectin. It contains proteoglycans, cytokines, chemokines, matricellular proteins such as tenascins, secreted protein acidic and rich in cysteine (SPARC), periostin, and many others which become accessible to cells upon enzymatic and mechanical alterations to the ECM [3-6].

The natural (innate) role of the mesenchymal-stroma is to impart a tightly controlled environment, which in consequence maintains a tumor-repressive homeostatic equilibrium regulated by local fibroblastic cells. The innate stroma accomplishes this by reinstating balance within the tissue in response to growth or temporary assaults [1,5,7-9]. However, instances such as chronic inflammation and cancer impede restoration of an innate mesenchymal homeostatic state [3,10]. When this occurs, stromal components collectively evolve to establish a “new” equilibrium that consequently preserves a pathological condition [11-13]. This type of homeostatic stromal shift is evident in most carcinomas in which the stroma compartment becomes heavily enriched in a well-maintained fibrosis-like reaction known as desmoplasia (**Figure 1**). Desmoplasia is the pathological term that is commonly used to describe the fibrous mesenchymal reaction that usually creates a stiff stromal environment in numerous epithelial cancers. This desmoplastic stroma is enriched in activated fibroblastic cells (myofibroblast) that are responsible for distinct architectural changes in the ECM, such as increased type I collagen deposition, selective inclusion of fibronectin splice variants (e.g., ED-A), hyaluronic acid, tenascin-C and

more [10]. Hence, desmoplastic ECMs are typically stiff and organized in parallel-aligned fiber patterns (i.e., anisotropy) [14,15]. Eventually, these cellular and ECM alterations generate an environment that is no longer tumor-repressive.

It is well established that malignant (e.g., teratoma) cells are capable of tumor formation within permissive environments. However, when these cells are introduced into a tumor-restrictive microenvironment, the malignant characteristics are soon reverted to that of normal cells. These findings suggest that the plasticity of malignant cells tolerates “reversal to normalcy,” allowing cells to differentiate in a non-pathological manner and incorporate into functional tissues/organs [16]. These types of studies demonstrated that unaltered natural mesenchyme can be restrictive of tumor growth while ECM modifications, such as fiber degradation via matrix metalloproteinases (MMPs), can alter the stroma to become tumor permissive [17].

Likewise, stromal influences became evident during experiments that resulted in tumor formation in the absence of malignant epithelial cell contributions. During these experiments, mammary fat pads were pre-cleared from epithelial components and subjected to ionizing radiation. Subsequent to radiation, treated fat-pads were re-populated with un-irradiated non-tumorigenic epithelial cells which consequently developed tumors. Results revealed that radiation-activated stroma promotes tumor formation in otherwise non-tumorigenic cells [18]. Similarly, chronic treatment of stroma with chemical irritants also established tumor permissive capabilities [19]. These and similar studies suggest that neoplastic development and progression is tolerated and often enabled by permissive microenvironmental settings such as the rather stable desmoplastic reaction [20-24].

In accordance, decades of research determined that alterations to innate stromal tissue organization akin to chronic wound healing favor (i.e., predispose) cancer onset [3,25,26]. As such, ECM remodeling in response to environmental insults correlates with loss of homeostatic tissue-specific coordination that is known to be driven by actomyosin cytoskeletal reorganization [14,27-30]. Taken together, these facts suggest interdependence between stromal architecture and function whereby maintenance of tissue homeostasis relies on the dynamic and reciprocal influences imparted amongst stromal elements [11,16,20,22,31-33].

The focus of this review is to discuss the complex array of relationships that transpire between stromal components and to highlight the microenvironmental interplay that prompts desmoplasia [10,34-38]. Throughout the text in this review, the sum of signaling exchanges, as they solely relate to fibroblastic cells-ECM interactions, are referred to as “stromal dynamic reciprocity” or SDR. We postulate that reversion of the tumor-permissive desmoplastic features to the innate tumor-restrictive stage may halt tumor progression. Therefore, a better understanding of SDR could potentially unveil new avenues of therapeutic intervention in cancer.

Desmoplastic SDR

“Dynamic reciprocity” (DR) was first described in the early 1980’s by Dr. Bissell and colleagues to explain the persistent biochemical and biophysical processes that occur between cells and the ECM [39]. In this manner, it was proposed that the ECM affects cellular activity via changes to the cytoskeleton and subsequently drives the expression and secretion of matrix remodeling molecules, such as collagen cross-linkers and MMPs, which re-structures ECM. It is currently understood that this “mechano-transduction” is modulated through transmembrane cell surface receptors (e.g. integrins) which interact

with both the ECM and the cytoskeleton [40-43]. The engagement of these bi-directional ECM-cell receptors serves as a link that enables the transmission of physical signals (and not solely chemical cues [44]) from the extracellular environment to the nucleus [43,45].

With this understanding and by introducing the term “biotensegrity,” based on Dr. Ingber’s original tensegrity definition [46,47], researchers described the role of the actomyosin cytoskeleton as a bridge or “conduit” that enables mechanical changes in the ECM to drive cellular behavior (e.g., myofibroblastic cell activation). Concurrently, this same “conduit” facilitates cell-induced tissue remodeling, such as in the case of ECM alignment, due to cytoskeleton dependent cell contraction [43,48]. These intracellular and extracellular events allow cells to mechanically and biochemically “sense” the changing environment thereby effectively addressing the dynamic needs of the tissue. This is accomplished by modifications in cell shape, polarity, differentiation, survival, motility, gene expression and more [22,39]. As such, SDR is possibly best conceptualized when contrasting acute vs. chronic wound healing processes [3,21,25] or innate (i.e. non-pathologic) vs. tumor-associated (i.e., desmoplastic) microenvironmental influences [3,4,25,26,37,49] (**Figure 2**).

In the case of acute wound healing, stromal cells are temporarily “activated” into myofibroblastic cells capable of engaging in tissue restoration functions such as *de novo* ECM remodeling, production and contraction. This provisional change in cellular activity is prompted by mechanical cues as well as by cytokines and chemokines released from the damaged tissue’s ECM and/or by immune cells recruited to the site of injury. Soon after the insult is repaired, the newly acquired healing state (acute SDR) is once again altered as myofibroblastic cells either senesce or are cleared by natural killer cells, thus ending the transitory myofibroblastic cell functions. Hence, clearing of these cells achieves wound resolution and thus shifts the system back to its innate homeostatic equilibrium [3,21,50]. However, the constant presence of instigating events or insults, as is common in chronic inflammatory wound healing, prompts stromal myofibroblastic cells to sustain healing signals and consequently prevents wound resolution [51]. Under these chronic conditions, the tissue is continuously remodeled and progressively loses its ability to restore the natural/innate tissue function [3,52]. These same sustained SDR processes take place in cancer-associated desmoplasia [3,53].

During desmoplastic onset, innate stromal features, such as an isotropic ECM landscape and the quiescent state of stromal cells, are vigorously modified such that the ability to halt cancer initiation and progression is diminished. These continuous repair mechanisms result in persistent anisotropic type I collagen matrix deposition that is believed to be caused by increased contractile YAP and Rho actomyosin cytoskeletal changes known to control cell contraction [54]. These, in addition to increased ECM density, constitute a desmoplastic trademark reminiscent of tissue scarring [3,49,51,55,56] (**Figure 2**). Overall, these observations are in accord with a model whereby desmoplasia is indeed an active participant in cancer initiation, progression, metastasis and maintenance of pro-tumoral inflammation and immunosuppression [33,51,57-59].

Taken together, there are two apparent dynamic and reciprocal processes that transpire throughout the vicious and expanding SDR cycle. The first process establishes the stromal (i.e., myofibroblastic) cell-imposed effect that is responsible for remodeling the ECM landscape [60,61]. The second process involves an ECM-imposed cellular influence. During both SDR processes, biochemical signaling cascades are regulated through cell-ECM receptors which stimulate intracellular changes mediated by cytoskeletal reorganization [22,43]. Although literature reports suggest that the alterations incurred

during desmoplastic progression fuel the tumorigenic features of cancer cells [58,62,63], many others imply that desmoplasia stalls cancer aggressiveness [64-69]. Together, these findings support the notion that desmoplastic SDR encompasses a complex phenomenon, which necessitates inclusive multidisciplinary and improved research efforts to fully comprehend these dynamic processes in the tumor microenvironment.

Desmoplastic SDR: cancer-associated fibroblasts-ECM interactions

Within the innate stromal environment of soft connective tissues (e.g., dermis in skin), fibroblastic cells are stem-like, quiescent and sparsely dispersed mesenchymal cells that reside within a self-derived mesh-like fibrillar ECM. These innate fibroblasts (i.e., vimentin expressing and pan-keratin null mesenchymal cells) are responsible for maintaining tissue topography upon an ever-changing mechanical load [70]. In response to cytokines such as TGF- β and/or ECM mechanical and biochemical changes, innate/quiescent fibroblastic cells transition into proto-myofibroblastic cells and subsequently evolve into fully activated myofibroblasts. In fact, TGF- β is known to induce the increased deposition of type I collagen during desmoplastic onset by driving this fibroblastic cell transition [71]. As cells transition to activation, they undergo morphological changes that are accompanied by fibrotic ECM production and heterogeneous expression of assorted myofibroblastic markers such as α -smooth muscle actin (α -SMA), desmin, palladin, podoplanin, fibroblast specific protein-1 FSP1/S100A4, fibroblast activation protein (FAP), platelet-derived growth factor receptor alpha (PDGFR) and more [52,72-74]. Hence, the role of myofibroblasts which are often induced by injury is to mend and contract the ECM as well as restore innate tissue characteristics [3,7,29,52,70,75-77].

The signaling cues that promote fibroblastic transition, such as TGF- β , do so by rearranging the cytoskeleton. This rearrangement in turn affects the nucleus (i.e., nucleoskeleton) in a way that indirectly regulates changes in chromatin structure [43]. This ultimately leads to increased expression of additional growth factors, cytokines and matrix remodeling molecules (e.g., MMP 9, MMP14, etc.) while also influencing ECM assembly [43,48,78-81]. Collectively, this posits that the “cytoskeletal conduit” functions as the principal mechano-driver by which cell-induced ECM remodeling and ECM-regulated cellular behaviors are conveyed [20,43,48,82]. A prime example of the influence imparted by this cytoskeletal conduit can be perceived during the nuclear translocation of myocardin-related transcription factor A (MRTF-A). Upon cell stretching, changes in actin polymerization regulate MRTF-A nuclear translocation [83]. In turn, the accumulation of nuclear MRTF-A triggers expression of α -SMA, [84] and together with myosin these modified stress fibers induce cell contraction resulting in ECM rearrangement.

To achieve acute wound resolution following repair or other non-pathological circumstances, myofibroblasts are prone to undergo apoptosis and/or clearance via the recruitment of specialized lymphocytes (e.g. NK cells) [3,52]. However, as previously described, when the myofibroblastic cell population is sustained, it constitutively maintains tissue regeneration responses that suppress immune cell influences. In the case of desmoplasia, this contributes to neoplastic development and progression while simultaneously inhibiting inherent immune tumor-resolution [3,7,51,52,85,86].

In regards to epithelial cancers, persistent stromal myofibroblastic cells are typically referred to as cancer-associated fibroblasts (CAFs) or as tumor-associated fibroblasts (TAFs) [87,88]. The phenotypic features of CAFs are believed to be sustained through epigenetic modifications influenced by paracrine signaling triggered by chronic inflammatory and ECM-prompted alterations [89,90]. This idea was

established using sequence-based genome wide DNA methylation profiling of innate fibroblasts and CAFs from human breast, prostate, lung and other organs [91-93]. These observations are consistent with the vast body of literature proposing the need for sustained (spanning several days) inflammatory factors, such as TGF- β or changes in particular ECM proteins (i.e., inclusion of fibronectin splice variant ED-A and increase in type I collagen fibrillogenesis), in order to effectively achieve myofibroblastic activation [52,94-97]. These details suggest CAF reversion to its innate tumor-suppressive state may require epigenetic regulation.

On the other hand, fibrotic and desmoplastic stromal environments undergo changes in architecture (i.e., force-dependent fiber alignment) and in composition (i.e., increased type I collagen deposition), which further fuel and sustain myofibroblastic activation [97]. As cells become contractile, there is an increased mechanical strain that stretches ECM fibers in a way that ligands become more or less accessible to receptors such as integrins [98]. For example, the major fibronectin receptor, $\alpha_5\beta_1$ -integrin, engages fibronectin most efficiently when its critical bindings sites (i.e. RGD and PHSRN) are positioned at an optimal distance from each other. This positioning results from mechanical stretching of the globular protein during early fibrillogenesis and strengthens the ligand-receptor interaction forming a “catch bond”. However, as stretching further increases, the optimal distance of these fibronectin domains no longer sustains this type of integrin bond, switching instead to a “slip bond” interaction. Under these conditions, $\alpha_5\beta_1$ -integrin is promptly released enabling additional integrins such as $\alpha_v\beta_3$ to engage [99,100].

Furthermore, fibronectin splice variants such as ED-A are increasingly incorporated in fibrous and desmoplastic ECMs. These ED-A-rich ECMs present a preference for $\alpha_4\beta_1$ -integrin binding, which in turn induces Rho-dependent cellular contraction that results in fibrosis maintenance and expansion [101]. In addition, when matrix stiffness is increased (i.e., in aligned anisotropic collagen fibers), activation of latent TGF- β is effortlessly accomplished by even the slightest of cellular contractions [102]. Also, Li, et. al. demonstrated that mesenchymal cells produce (i.e., de novo) ECMs of diverse characteristics when the stiffness of their underlying substrate is altered [103]. The above-mentioned and other ECM mechanical properties are therefore paramount in both imposing and maintaining CAF activation [14,29,39,60,104-110]. Hence, interfering with ECM-induced CAF responses may constitute a therapeutic option that could restore innate (i.e., tumor-restrictive) stroma. Nonetheless, in order to attain a comprehensive understanding of desmoplastic SDR, there is a conceptual need to distinguish between mechanisms responsible for ECM remodeling and ECM-induced cellular responses (i.e., activation and/or maintenance of active myofibroblasts) **Figures 2 & 3.**

It is widely understood that, in addition to other stromal and tumoral cells, CAFs abundantly express and secrete TGF- β . TGF- β is stored within stromal ECM in its latent form and becomes activated/processed by both biochemical and mechanical cues resulting from alterations in ECM anisotropy [97]. This constitutive secretion, processing and receptor engagement of TGF- β is known to trigger cytoskeletal cell-contractile rearrangements that mediate variations in gene expression (i.e., α -SMA [111]), while also maintaining constant mechanical tension at the ECM-cell interface (i.e., via receptor engagement) [43,112-115]. The mechanical strain imposed by the altered ECM transmitted via cytoskeletal conduits leads to additional modifications in nuclei shape and chromatin organization [39,45,116]. Consistent with these observations, mathematical models investigating the links between communal regulation of ECM and nuclear characteristics (e.g., rigidity) via cytoskeletal conduits accurately predicted fluctuations in cell-matrix adhesion structures [116]. Taken together, these fibroblastic-ECM interactions constitute

the SDR maintenance cycle that either retains the stroma environment in an innate homeostatic equilibrium or promotes a distinct desmoplastic stromal state/environment (**Figures 2 & 3**).

This ECM-induced cytoskeletal rearrangement (i.e., conduit) is known to occur via Rho family small GTPases regulation and to participate in cell migration, polarization and differentiation [117-120]. Moreover, molecules involved in the regulation of Rho activity such as caveolin1 have been associated with desmoplastic and pro-metastatic architectural ECM rearrangements [14]. Recent evidence has shown that TGF- β -induced myofibroblastic (i.e., CAF) activation is dependent on Rho activity and nuclear translocation of Yes-associated protein-1 (YAP1). This TGF- β -mediated process is known to transcriptionally regulate proteins involved in proliferation and apoptosis which ultimately culminates in altered ECM production [121]. In fact, YAP has been shown to play a role in both ECM remodeling and in cellular responses to altered ECMs. Hence, in this context, YAP signaling constitutes a major regulator that both triggers and sustains CAF activity [54]. Moreover, changes in CAFs' secreted proteases (i.e. MMPs) or matrix cross-linkers, such as lysyl oxidase, as well as cell-membrane tethered proteases like FAP [122] or CAF-secreted inflammatory matricellular and other components such as periostin [123,124], osteonectin/SPARC, hyaluronic acid [125], tenascin-c [124] and Wnt-5a signaling [123], have all been shown to directly or indirectly modify ECM characteristics such as fiber anisotropy, stiffness and biochemical composition. These dynamic and reciprocal examples support the proposed premise conveyed in **Figure 3** suggesting that diverse stromal equilibrium states are maintained during non-pathological conditions (i.e., innate stroma) or during temporary (acute) or sustained (chronic) injury, growth and/or other circumstances (e.g., neoplasia).

In support of these observations, investigators demonstrated that simple manipulation of the physical features of the ECM *in vitro* dramatically alters gene expression profiles and reprograms cellular phenotypes [126]. Similarly, CAF-induced ECM modifications, which incidentally show high anisotropic features *in vitro*, can activate naïve/innate fibroblasts overnight [29]. Additionally changes to the topographical and/or mechanical characteristics of substrates (i.e. acrylamide gels, hydrogels, etc.) also affect fibroblastic activation [54], which too depends on actin-generated Rho activity [127]. Thus, stiffness and serum factors contribute to the transition of innate stromal cells into an active (i.e., myofibroblastic) phenotype [106,107,128]. When considering the *in vivo* tumor microenvironment, the ECM is classically much stiffer than its innate counterpart. Typically the ECM is aligned in quantifiable organized type I collagen fiber patterns [14,15,129,130]. It also includes increased ECM cross-linking and fiber density [30,106,131-135].

Many studies have aimed to associate notable stromal phenotypic changes such as desmoplastic markers with clinical prognostics. For example, using predictors based on the CAF-to-collagen deposition ratio or type I collagen topographical (anisotropic) ECM features, investigators observed a correlation with unfavorable patient survival [76,129,130,136-139]. Others found that actin binding proteins such as palladin, which regulates stromal pro-metastatic CAF and desmoplastic ECM features (e.g., alignment) [140], can be used as a predictive marker of cancer recurrences subsequent to tumor surgical resection [137]. These types of microenvironmental reactions should be distinguished from tumor-restrictive stroma signatures [141] where, for example, increases in collagen deposition per se, in the absence of increased cellular α -SMA levels, correlates with better patient outcome [76]. Similarly, innate type I collagen topographic signatures in which ECM fibers are isotropic (disorganized) were also determined to correlate with improved patient survival [129,130]. Overall, these findings suggest that increased isotropic-collagen ECM deposition in the absence of activated stromal cellular markers

correlates with better patient outcomes. Collectively, these observations appear to provide rationale for ablating (or reprogramming) desmoplastic stroma as an effective means for tumor control.

Restoration of innate stroma characteristics promises a feasible therapeutic strategy

It is well known that the innate stromal environment is tumor suppressive [3,7,16,67,142] and that the onset of desmoplasia disrupts its natural inhibitory role via induction of a new system that no longer restricts, and perhaps even supports, tumorigenic behaviors [68,82,143,144]. Therefore, it is logical to conclude that desmoplasia inspires tumor growth and metastasis, while it is known that it also confers immunosuppression [51] as well as chemo- and radiotherapy resistance due to massive ECM deposition [85,145,146]. With this in mind, targeting desmoplastic stroma appears to be a promising therapeutic approach for treating neoplasias that are rich in desmoplastic stroma (**Figure 1**) [57,146]. In line with this idea, researchers sought to decrease desmoplasia by eliminating activated stromal cells and/or pathways known to significantly contribute to disease onset and progression.

An attempt to interfere with stromal activation involved targeting the sonic hedgehog (Shh) pathway, which participates in several cellular processes such as stem cell maintenance, cell proliferation, differentiation and tissue polarity as well as desmoplastic induction [147,148]. Hence, constitutive activation of the Shh pathway is considered a common pro-tumorigenic event that has gained much attention as a potential therapeutic target. Indeed, inhibition of this pathway, either by targeting Shh ligand directly or its downstream effectors *smoothed and gli1*, decreases tumor growth and metastasis in a plethora of mouse models [146,148-151]. The role of Shh in tumor-stroma interactions via genetic deletion and long-term (i.e., chronic) pharmacologic Shh inhibition has also been examined. Although inhibition of the pathway indeed accomplished ablation of pancreatic fibrous stroma (i.e., desmoplasia), it unexpectedly accelerated undifferentiated tumor growth and vessel restoration perhaps due to loss of homeostatic SDR regulation in the tissue environment [64]. Accordingly, a clinical trial designed to chronically ablate Shh signaling in pancreatic cancer was prematurely terminated due to its detrimental effects in gemcitabine-Shh combinatorial treatment in comparison to gemcitabine alone [69].

Another approach aimed at negating desmoplastic production sought to target cells primarily responsible for its development: the α -SMA positive CAFs. However, depletion of these cells resulted in increased immunosuppression and advanced tumor progression. After further investigation into patient cohorts and contrary to previous conclusions, it was determined that the accumulation of α -SMA expressing CAFs correlates with improved patient outcomes [65].

When considering these results, it is critical to appreciate the role of the innate stroma in maintaining the tumor-restrictive homeostatic equilibrium via processes related to SDR. For example, removing or ablating key regulators of SDR's cyclic interplay may facilitate tumor progression by concomitantly and inadvertently removing the tumor-counteracting functions of SDR. Ideally, this scenario is reminiscent of an enzymatic reaction in which the addition of any catalyst that lowers the threshold of activation simultaneously accelerates the rate of the reaction. In comparison to this biological phenomenon, the destruction of SDR regulation dramatically increases tumorigenic features by decreasing innate tumor restrictive components. Hence, ablating this microenvironmental setting negates the proposed possibility of "shifting the homeostatic equilibrium" to restore innate stromal tumor-restrictive functions (**Figure 3**).

In light of this rationale, shifting back the desmoplastic stromal system to a tumor-restrictive state could serve as a conceivable therapy. Simply put, restoring the effects that prevent tumor progression should, at minimum, serve as combinatorial therapeutics aimed at targeting the tumor while simultaneously reprogramming the stroma. These, together with novel immune checkpoint inhibitors that reinstate the natural anti-tumoral immune effects, should render the finest results, especially since desmoplasia is known to impart a pro-tumorigenic immunosuppressive microenvironment [125,152].

Another study in pancreatic cancer aimed to eliminate the biophysical barriers created by desmoplastic stroma. The desmoplastic stroma is commonly thought to limit therapeutic perfusion via collapse of resident blood vessels due to apparently high interstitial pressure [153]. In this approach, the researchers explored the contentious role of the ECM component, hyaluronic acid (HA). HA is known to be abundantly expressed in dynamic processes such as embryogenesis and oncogenesis and has been linked to tumor-supportive roles involved in increasing metastatic potential in some instances while functioning as a tumor suppressor in others [154-157]. When investigators targeted HA via enzymatic degradation (using PEGPH20), which excitingly restored previously collapsed blood vessels by decreasing interstitial pressure (or as later suggested solid stress [158]), there was an improved response to gemcitabine treatment [153]. Interestingly, as treatment ceased, HA levels replenished which were speculated to be due to α -SMA positive CAFs and ECM dynamics that notably drive HA expression. Nonetheless, the authors successfully demonstrated that the physical barrier imposed by the desmoplastic stroma can be overcome via enzymatic degradation of HA without supporting tumor growth [153]. Excitingly, this study served as basis for a phase-Ib clinical trial [159] and, after overcoming some adverse drug thromboembolic events, it is currently being followed by additional trials in which PEGPH20 is to be added to front line drug combinations such as nab-paclitaxel/gemcitabine and more. In addition, more recently it was shown that HA targeting increases collagen and fibronectin deposition during fibroblastic activation in a (benign) way that may differ from the more common TGF- β induction [157]. These results suggest that the subsequent ECM production may convey changes in *de novo* ECM production [129,130]. In accordance with this notion, researchers have demonstrated that anisotropic levels of collagen fibers observed in desmoplastic biopsies promotes metastatic breast and other cancers [14,130,160], although isotropic ECM (disordered ECM arrangement) influences the effectiveness of chemotherapeutic treatments [160].

As mentioned previously, desmoplastic onset instigates an immune privileged (i.e., immunosuppressive) effect, which inhibits anti-tumoral T-cells from penetrating the stroma enough to reach the tumor [125]. This observation has been proposed to explain why, even though immune inhibitory checkpoints such as those impeding the Programmed Death-Ligand 1 (PD-L1) should facilitate an adequate immune reaction, these often fail to impart significant responses (i.e., in prominently desmoplastic-rich pancreatic cancer). As such, investigators targeted FAP and CXCL12/SDF1 expressing desmoplastic fibroblasts and were successful in reinstituting an effective anti tumoral T-cell penetration. In consequence, these two approaches were combined and demonstrated a synergistic effect in treating desmoplastic rich cancers such as in the case of pancreatic ductal adenocarcinoma [152].

During early cancer development, TGF- β is established as a tumor suppressor. However, as the tumor progresses, it becomes refractory to TGF- β suppression. When this occurs, the tumor milieu is gradually enriched in TGF- β now instigating, as opposed to repressing, desmoplastic development and tumor progression [161,162]. In light of this, inhibition of TGF- β has become an obvious strategy for targeting cancer. Inhibition of TGF- β is being developed via various strategies such as obstructing ligand or

receptor binding and/or blocking TGF- β downstream effectors [161,162]. Nonetheless, a number of preclinical concerns became evident when hemorrhagic, degenerative and inflammatory lesions developed due to either high dosage or continuous TGF- β inhibitory treatments. Hence, intermittent low dosages were successful at the preclinical setting, which served as basis for designing clinical trials [163]. In fact, some of these drugs were demonstrated to be safe using specific dosage regimens and have proceeded into phase II and III clinical trials while others are still in preclinical development (i.e., Trabedersen [164-167] and Galunisertib [168-172]). Studies attempting to inhibit TGF- β function suggested that understanding and monitoring the long-term effects associated with targeting this pathway are crucial in effectively combating cancer progression and possibly even preventing its development.

Another approach designed to overcome desmoplasia involved transcriptional reprogramming of desmoplastic processes by stabilizing the Vitamin D receptor (VDR) [173]. VDR is cited as the master regulator of the genomic-driven activation of fibroblasts, although its inhibition has been suggested to re-sensitize pancreatic cancer cells that became resistant to gemcitabine [174]. In any case, VDR activation achieves stromal restoration by inhibiting the TGF- β /Smad pathway that, as previously discussed, constitutes a key driver of fibroblastic activation [125,173,175]. With the goal of ramping up the activity of VDR, investigators introduced an analog of its ligand, which resulted in the suppression of inflammatory cytokines and growth factors, enhanced angiogenesis, and increased efficacy of therapeutics while significantly improving overall survival [176]. Together these studies reinforce the feasibility of stromal remodeling and serve as premises behind several planned and ongoing clinical trials [177,178].

Conclusion

Evolutionarily, the body is physiologically conditioned to address and resolve insults that prompt temporary deviations from innate tissue functions through both local and systemic repair processes. These temporary occurrences are typically ended when the acutely altered SDR is reverted back to an innate SDR equilibrium. Nonetheless, when environmental insults such as cancer or chronic fibrosis persist, the acute SDR balance is “shifted” towards a new equilibrium as seen in desmoplastic SDR. This pathological adaptation favors (or permits) the aggressive nature of the disease state whereas ablation of compensatory elements in the stroma impairs the possibility of restoring an innate tumor-restrictive status (**Figure 3**). Similar concepts have been proposed in conditions involving dormant viruses, such as HSV-1, that become re-activated once the threshold for viral tolerance is lowered (i.e physiological and physical changes, immunosuppression, etc.) [179]. Additionally, this notion is conceptualized in circumstances in which SDR processes are lowered/altered such that communal bacteria or yeast are permitted to overtake the host microenvironment [180-183]. In both cases, therapeutic options that restore and maintain the environmental balance of an innate system are most effective in both treatment and prevention of disease progression [179,182-185]. The evidence provided within this review collectively points to a similar adage in cancer in which our limited understanding of balancing SDR interplay impedes substantial advances in current therapies. Thus, a thorough understanding of key regulatory components that influence the balance of SDR processes (**Figure 3**) will surely provide improved therapeutic strategies, and perhaps even preventive therapies to effectively treat cancer, as well as its accompanying microenvironmental components.

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Figure Legends:

Figure 1: Example of an epithelial cancer presenting high levels of desmoplastic stroma. Formalin-fixed and paraffin-embedded surgical normal (non-pathological) and lung cancer (neoplastic) samples were subjected to indirect immunofluorescence. Cellular vimentin is shown in red and is intended to denote both normal and desmoplastic stromal cells. Pan-cytokeratin is in green highlighting epithelial as well as neoplastic cells. DNA intercalating agent, Hoechst, was used to stain nuclei of all cell types and is shown in blue. Note that the neoplastic tissue is enriched with (red) dense stromal cells in comparison to its non-pathological counterpart.

Figure 2: Stromal Dynamic Reciprocity amid fibroblastic cell-ECM interactions in innate vs. desmoplastic homeostatic states. This model highlights the relationship between the cellular and ECM components of the mesenchymal-stroma demonstrating a dependence on this interplay to drive and maintain homeostatic states. The maintenance of the two homeostatic states is portrayed by the orbit-like arrows. The model depicts the phenotypical co-evolution of fibroblastic cells and their self-derived ECMs under the influences of a chronic assault (i.e. inflammation, cancer, etc.) suggesting possible restorative instances (i.e., therapies) of the innate, tumor-restrictive, stroma. Note that the cartoon includes a graphic representation of the cellular cytoskeleton which links, conveys and is modified in response to changes in both ECM and cellular states.

Figure 3: Stromal Dynamic Reciprocity model illustrating the likely peril of desmoplastic ablation. Innate SDR is the natural homeostatic stromal state in which tumor onset is repressed. When this environment is provisionally threatened (e.g., during infection or wounding), the innate homeostatic equilibrium transitions to a new, albeit short lived, homeostatic state: the acute SDR. In this homeostatic state, tissue repair is maintained until restoration is achieved. Following successful repair, and often after an acellular scar is formed, the system shifts or “tilts” back to the innate (tumor

suppressive) homeostatic equilibrium. However, in the absence of wound resolution such as in the case of chronic inflammation and/or cancer, the acute SDR system is further sustained thus shifting the balance towards a third (chronic SDR) homeostatic equilibrium. During each of these transitions, extracellular and intracellular interactions are biophysically conveyed via the actomyosin cytoskeleton, which serves as a “conduit” transferring these mechanical cues amid cells and ECM. Note that this model suggests that a threat to the environment weakens the tumor restrictive (innate) capabilities in which, if not repaired (tilted back) in a timely fashion, cancer will be allowed to ensue (i.e., accompanied by a chronic/desmoplastic state). Additionally, this model proposes that ablation of chronic/desmoplastic SDR eliminates the possibility of shifting the system back to the innate tumor restrictive state.

Figure 1

Figure 2

Figure 3