

# *Fibroblastic Cell-derived Extracellular Matrices: A Cell Culturing System to Model Key Aspects of the Tumor Microenvironment*

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## 16.1 Introduction

Physiological processes require cells to be able to respond to and modulate the environment in which they reside. This dynamic behavior is largely influenced by the extracellular matrix (ECM), which is continuously secreted and modulated by local cells. Proteomic analyses of tissue samples revealed that despite the ECM's origin-dependent composition, ECMs

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are largely comprised of various types of fibronectin, laminin, elastin, collagen, proteoglycan, and other polymer extracellular glycoproteins.<sup>1-3</sup> ECM networks signal to cells *via* discrete cell-matrix receptors, such as integrin heterodimers, “sensing” biochemical as well as mechanical variations of these natural substrates.<sup>4-6</sup> Additionally, cell-secreted signaling molecules, such as growth factors, cytokines and chemokines, can be stored, activated and/or blocked within the ECM.<sup>7</sup> As such, microenvironmental mechanical and biochemical changes can regulate local signal transduction cascades,<sup>7</sup> making the ECM an active modulator of many organismal processes like development, homeostatic equilibrium, acute wound healing, and chronic disease progression.<sup>8-11</sup> Overall, the ECM controls and is reciprocally modulated by resident cells and is thus considered much more than a physical scaffold.

Within the context of solid epithelial cancers, tumor masses are not only comprised of mutated epithelial cells but also include a neighboring environment, or stroma. The stroma constitutes a mixture of cell types (*i.e.* endothelial, neural, fat, immune, fibroblastic and others)<sup>12</sup> and together with the cancer-associated ECM are identified as the tumor microenvironment (TME). Among these cell types, fibroblastic cells (*i.e.*, local and/or recruited mesenchymal cells) are the major producers of the ECM and collectively provide the backbone to healthy tissues. Under physiological conditions, these ECM fibers often appear as a disorganized mesh of fibrillar proteins that work to maintain organs at homeostatic equilibrium. In contrast, fibroblastic cells in the TME, known as cancer-associated fibroblasts (CAFs),<sup>13</sup> are initially transformed by tumor cells and the resulting altered ECMs act as major tumor regulators.<sup>8,14-16</sup> Here, the CAF-produced ECM is noticeably rearranged and becomes highly aligned (and often stiff); providing a similar supportive role to the tumor as the physiological matrix does for organ homeostasis.<sup>14,16-20</sup> It is therefore not surprising that primary and secondary cancer masses can also be regarded as “tumor organs”.<sup>21</sup>

Early reports have suggested that cancer is analogous to seeds sown in soil, in that “cancer seeds” can only populate within a nourishing “fertile organ soil”.<sup>22,23</sup> This concept was validated in the 1970s when researchers like Mintz and Illmensee provided experimental evidence demonstrating that cancer cells are enabled or restricted by the “organ soil” of the local microenvironment.<sup>24,25</sup> Similarly, additional studies in mice indicated that injecting human epithelial cells into normal fibroblast pre-conditioned mammary fat pads enabled normal mammary gland development. Interestingly, when mammary fat pads were pre-conditioned using “activated” fibroblastic cells [*i.e.*, fibroblasts that were both irradiated and engineered to overexpress stromal activators like tumor growth factor beta 1 (TGFβ1)], tumors developed in select cases.<sup>26</sup> Moreover, additional reports indicated that *bona fide* tumor initiating cells fail to yield tumors when grafted together with normal stromal cells; as opposed to when tumor initiating cells were grafted with activated stromal cells.<sup>27</sup> Collectively, these types of studies led researchers

to suggest that it is possible for solid cancers to grow despite the absence of mutated epithelial cells when provided a “tumor permissive” stroma (*i.e.* stroma containing activated fibroblasts and altered ECM).<sup>28</sup>

Stemming from these ideas, the goal of targeting the TME emerged as an attractive therapeutic strategy. However, there is also experimental evidence indicating that ablation of key stromal elements, such as CAFs, can lead to unrestricted tumor growth.<sup>29,30</sup> Such results potentially explain why chronically exposing patients to stroma ablating therapies has not always been beneficial.<sup>31–34</sup> As such, it is now well accepted that the TME can act as a double-edged sword; where healthy homeostatic stroma is naturally tumor-restrictive, while the TME can foster or limit neoplasia development.<sup>35–37</sup>

This rationale potentially underlies the proposal that cancers, akin to chronic wounds, operate *via* persistent regenerative cues.<sup>38–40</sup> The rapid expansion of the TME, defined by pathologists as desmoplasia, is known to be exacerbated by a “field effect”; where local CAFs activate neighboring normal fibroblastic cells through the secretion and activation of cytokines (*e.g.* TGF $\beta$ 1)<sup>41</sup> and the remodeled desmoplastic ECM.<sup>42</sup> Similar to chronic wound healing, activating signals are continuous and perpetuated thus simultaneously converting more normal fibroblastic (local and/or recruited) cells into CAFs and preventing present CAFs from reverting to a tumor-restricting “normal” quiescent state.<sup>8,39</sup>

Considering cancers as chronic wounds opened the door to better understand how human behaviors or inflammatory conditions, such as obesity, aging, and smoking, among others, can affect cancer onset and progression *via* alterations to the various types of stromal cells, the ECM, and pro- *vs.* anti-cancer immune responses.<sup>43–45</sup> It has been reported that changes in the ECM, particularly in collagen composition, can have a profound effect on cancer cell functions and tumor progression.<sup>7,46–53</sup> As such, *in vivo* structural ECM traits, based on collagen signatures, have been defined and proposed to predict cancer cell invasion.<sup>14</sup> Indeed, using the fibroblastic cell-derived matrix culturing system, known as the CDM,<sup>54,55</sup> the Weeratna lab recently established that age-related changes in the expression of fibroblastic cell ECM regulators, such as hyaluronan and proteoglycan link protein 1 (HAPLN1), are responsible for some of the architectural changes seen in matrix proteins like collagen. These changes in turn influence cell motility and the permeability of lymphatic vessels, indicating that the ECM can simultaneously regulate both metastatic routes as well as the composition of tumor immune cell infiltrates.<sup>47,56</sup> Earlier studies also demonstrated that ECMs can be used to study normal and cancerous traits *in vitro*,<sup>57,58</sup> and predict tumor aggressiveness,<sup>14</sup> and could be modulated to enable or restrict neoplasia.<sup>59</sup> Thus, investigators have begun to emphasize the importance of ECM biology and are now studying its composition, production, and remodeling dynamics within the context of cancer.

It is not surprising then, that this necessitated the development of experimental models to investigate the possibility of harnessing the ECM's tumor

restrictive properties, blocking its tumor-enabling abilities, and preserving the *in vivo* characteristics of primary cells *in vitro*. This can be accomplished by exploiting CAFs' ability to constantly synthesize and remodel CDMs. The rest of this chapter will focus on selective aspects of the *in vitro* fibroblastic CDM model, which has been vetted as an effective *in vivo*-like mesenchymal cell culturing system to study TME biology and tumor–stroma interactions.<sup>17,42,50,54,60–64</sup>

## 16.2 The History of Using Fibroblastic CDMs as an *In Vitro* System

Historically, the ability to maintain viable cells after isolation from their native organs has provided researchers with a useful tool to study various cell types in the laboratory. Identifying and maintaining a cells' natural phenotype, however, provided the opportunity to study physiological *vs.* pathological events. The latter has become particularly important when questioning how assorted microenvironmental stimuli modulate cellular functions such as cell growth, differentiation, motility, *etc.* For example, Gail and Boone followed the movement and displacement of fibroblastic cells for the first time, after effectively maintaining the cells in culturing flasks.<sup>65</sup> Elsdale and Bard then demonstrated that fibroblasts adopt a “bipolar spindle form” during movement on collagen fibers; as opposed to the single lamella-based membrane ruffling observed during flat cell culturing.<sup>66</sup> Following these studies, Yamada and colleagues used single ECM proteins, like fibronectin, as substrates to examine whether particular matricellular proteins could enable cell–matrix adhesions and influence fibroblastic cell spread.<sup>67</sup> Additionally, epithelial cells cultured on top of a fibroblastic cell layer (where the fibroblasts secrete nutrients for the continued support of the epithelial cells) or a collagen-coated surface, were used to enable *in vitro* responsiveness to natural mitogens like epithelial growth factor (EGF);<sup>68</sup> highlighting the importance of the ECM in maintaining a more biologically native cell behavior *in vitro*.

It was almost unavoidable that investigators discovered that ECM materials deposited by cells in culture provide an *in vitro* cell culturing advantage, where shape and function (*i.e.*, cell growth) could be regulated.<sup>69</sup> Bissell's team demonstrated that single cells embedded in a collagen-based gel could generate a lumen-contained cellular structure.<sup>57</sup> Next, Kleinman and colleagues isolated the basement membrane protein complex known as Matrigel® (*i.e.* epithelial-like ECM extracted from Engelbreth–Holm–Swarm mouse sarcoma),<sup>70</sup> which was used to enable *in vitro* functional and three-dimensional (3D) differentiation of primary cells.<sup>71</sup> Collectively, these types of studies provided the rationale and justification needed for the use of particular substrates for cell culture; with the goal of maintaining or imparting

discrete effects, architectural features, and gene expression on both single and small-clustered cell colonies *in vitro*.<sup>72</sup> Since then, it has become clear that well-justified rationales are needed for selecting particular 3D ECMs as substrates, and importantly, the type of matrix used should be considered when interpreting experimental data.<sup>9,73</sup>

A similar concern quickly arose for choosing the cell type that would produce the CDMs *in vitro*, as well as the optimization of effective decellularization methods for these matrices. For instance, the search for ideal conditions that could effectively mimic the *in vivo*-like fibroblastic cell–matrix adhesion structures *in vitro*, provided the rationale behind establishing fibroblastic CDMs. These CDMs facilitated uncovering of the 3D matrix adhesion structures, known today as “3D-adhesions”.<sup>54</sup> Detection of 3D-adhesions in tissue samples, as well as in fibroblastic cells cultured in fresh decellularized *ex vivo* murine tissue slices, established how CDMs are suitable natural substrates for culturing fibroblastic and other cell types *in vitro*.<sup>54</sup> While *ex vivo* tissue decellularization has become an important tool used in bioengineering approaches,<sup>74–78</sup> the repopulation of decellularized CDMs *in vitro* has been adapted to study characteristics of the matrix, such as biochemical composition, elasticity or pliability, architectural topography and more.<sup>19,20,42,48,60,79–82</sup> Thus, by using freshly-isolated organ-specific fibroblastic cells (or well-established fibroblastic cell lines) to generate CDMs *in vitro*, researchers have successfully demonstrated the ability to mimic relevant *in vivo* ECM characteristics.

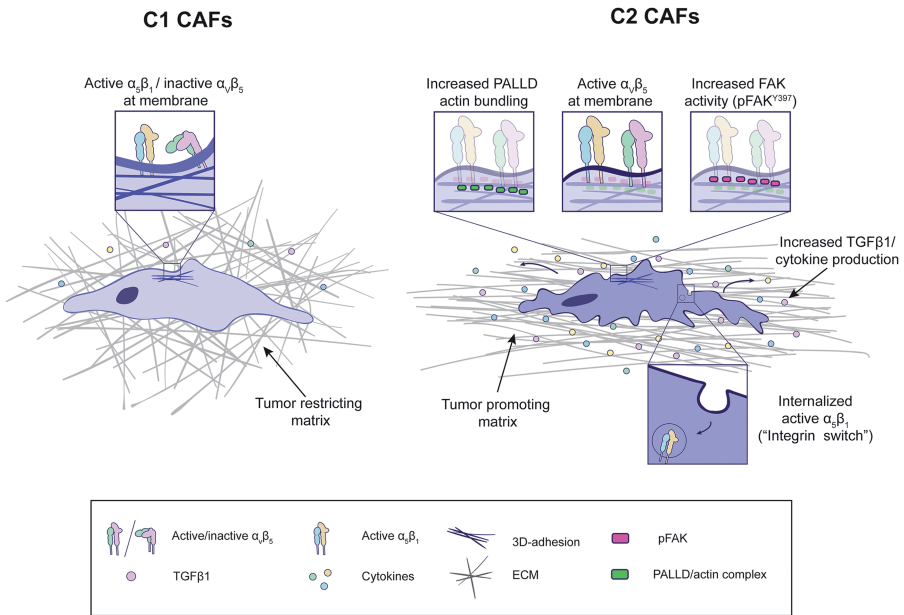
To date, numerous groups have reported the use of a variety of fibroblastic cells, including mesenchymal stem cells and CAFs, to produce CDMs.<sup>42,60,62,64,83</sup> In fact, the CDM culturing model has been used to study dynamic matrix remodeling<sup>63,84</sup> and matrix-induced cellular functions.<sup>47,62,64,85–88</sup> For example, CDMs have been shown to not only direct cell movement,<sup>19,47,54,89–93</sup> but also regulate nerve guidance,<sup>76,83</sup> modulate differential gene expression, alter cellular proliferation, and even induce or inhibit cellular differentiation and/or activation.<sup>42,85,94</sup>

Understanding the characteristics of physiological *vs.* desmoplastic ECMs will allow researchers to better understand the mechanisms underlying the contextual differences in tumor stromal behavior. Specifically, using fibroblastic cells (*i.e.*, patient-derived CAFs and matching normal samples or young *vs.* aged fibroblasts) harvested from human tissues, investigators have used the CDM culturing system to characterize desmoplastic traits.<sup>17,47,61,62,93</sup> One such example used patient-derived CAFs and patient-matched normal fibroblastic cells, genetically and/or pharmacologically altered *in vitro*, to reveal a functional crosstalk between the integrins  $\alpha_v\beta_5$  and  $\alpha_5\beta_1$ . Specifically, CAF CDMs activate  $\alpha_v\beta_5$  in a phosphorylated focal adhesion kinase (pFAK)-dependent manner. Integrin  $\alpha_v\beta_5$  activation can then facilitate the intracellular relocation of active  $\alpha_5\beta_1$  into endocytic vesicles. This change in integrin activity and location, referred to as “integrin

switch” or “integrin crosstalk” (Figure 16.1), can regulate the production of CDMs that in turn maintain the ability to induce CAF activation.<sup>60</sup> In sum, the advent of CDMs for cell biology has resulted in the discovery of important findings such as *in vitro*-generated traits of cells and the mechanisms responsible for regulating these phenotypes, as well as clinically-valued stromal biomarkers.<sup>17,60,93</sup>

### 16.3 Potential Benefits of Using CDMs as *In Vitro* Culturing Systems

To differentiate between CAFs that have tumor-promoting *vs.* tumor-restrictive properties, we propose to use the nomenclature of Class 2 (C2) and Class 1 (C1) CAFs, respectively. As such, the idea of “reprogramming” the TME to convert C2 CAFs into C1 CAFs and maintain this C1



**Figure 16.1** Graphical depiction of phenotypic traits displayed by “C1” and “C2” CAFs. In comparison to anti-tumor C1 CAFs, tumor-promoting C2 CAFs exhibit increased cytokine, chemokine, and growth factor (*e.g.* TGF $\beta$ 1) secretion, and increased expression of the actin bundling protein palladin (PALLD). 3D-adhesion structures also have increased pFAK<sup>Y397</sup> activity in C2 CAFs. Lastly, C2 CAFs can dynamically modulate the topography of self-derived ECMs to increase fiber alignment. The resulting anisotropic C2 CDMs can convert C1 CAFs into C2 CAFs; which may explain the field effect observed *in vivo*. In response to CDMs produced by C2 CAFs, normal fibroblasts undergo a functional “integrin switch” resulting in an enriched internalization of active  $\alpha_5\beta_1$  integrin.

functionality is an attractive therapeutic strategy. Indeed, this strategy is the basis of current attempts to inhibit stromal immunosuppression, promote anti-tumor immunity, and increase the efficacy of radiation therapy.<sup>35,95–103</sup>

To this end, the use of CDMs to study the dynamic remodeling of the ECM during tumorigenesis (which affects stroma–tumor cell interactions and signal transduction<sup>28,39,104–108</sup>) has been a great advantage. In fact, investigators were able to uncover major differences in signaling networks when moving from classic 2D to 3D culturing methods. For example, the prominent cell–matrix adhesion protein FAK in its auto-phosphorylated form, pFAK, was detected at adhesion structures, such as focal adhesions, in cells cultured under classic 2D conditions.<sup>109,110</sup> However, when normal cells are cultured in either collagen lattices or “C1” CDMs, pFAK levels (detected with antibodies against auto phosphorylated tyrosine 397) were constitutively low, akin to levels detected in healthy tissues *in vivo*.<sup>54,111</sup> In contrast, pro-tumor “C2” CAF generated CDMs maintain constitutively high pFAK in fibroblastic cells<sup>60,61</sup> (Figure 16.1 and Table 16.1). As C2 CAFs have been associated with a highly immunosuppressive TME, it is not surprising that stromal pFAK inhibition has been proposed to overcome v-raf murine sarcoma viral oncogene homolog B1 (BRAF) inhibitor resistance<sup>112</sup> and thus improve the efficacy of immune checkpoint therapy.<sup>113</sup>

Furthermore, CDMs have also provided benefits for studying molecules that depend on ECM characteristics or that directly affect C1 vs. C2 matrix production (Figure 16.1 and Table 16.1). One such molecule is the cytokine TGF $\beta$ 1. TGF $\beta$ 1 is one of several signaling molecules whose effects are closely related to stoichiometric and/or biophysical variations in the microenvironment, such as ECM architecture.<sup>114–119</sup> While TGF $\beta$ 1 has been associated with tumor suppression,<sup>120,121</sup> it is also known to induce myofibroblastic activation; promoting the production of CDMs similar to ECMs made by C2 CAFs.<sup>60,122–128</sup> Moreover, many studies have associated TGF $\beta$ 1 signaling with collagen remodeling and with the induction of F-actin regulatory molecules like alpha smooth muscle actin ( $\alpha$ SMA) and its actin bundling partner palladin. These proteins have become reported markers of myofibroblastic activation,<sup>129–131</sup> with activated cells exhibiting similar traits to C2 CAF CDMs<sup>17,60,93,132–139</sup> (Figure 16.1 and Table 16.1). Interestingly, Biffi and colleagues recently reported that while TGF $\beta$ 1 prompts myofibroblastic activation in CAFs, interleukin 1 can trigger CAF heterogeneity by locally shifting CAFs towards a more immunosuppressive class.<sup>140</sup> This work was corroborated in the fibroblast CDM culturing system where, Avery and colleagues proposed that CDMs are also capable of regulating CAF heterogeneity; distinguishing between CAFs expressing  $\alpha$ SMA and the fibroblast activation protein, FAP.<sup>85</sup> Importantly, effects imparted by TGF $\beta$ 1 differ depending on the cells' culturing conditions. For example, TGF $\beta$ 1 receptor type I expression is increased when cells are cultured in 3D conditions following ligand stimulation.<sup>141</sup> Moreover, activation of TGF $\beta$ 1 is contingent on ECM architecture and dynamics as well as

**Table 16.1** Distinguishing phenotypes of C1 and C2 CAFs. A compilation of characteristics unique to Class 1 (C1) and Class 2 (C2) cancer-associated fibroblasts (CAFs). These factors have been used to identify functional differences in the cells that produce the extracellular matrix that forms the TME.

Factor	C1 vs. C2	Effect	Reference
Tumorigenicity	C1: Tumor-restrictive C2: Tumor-supportive	Enhances tumor growth, proliferation, survival, and metastasis	28, 33, 36, 37, 39, 104–108
Immune Modulation	C2 CAFs are immuno-suppressive	Evasion of anti-tumor surveillance	35, 95–103
FAK	Phosphorylation (tyrosine 397) increases in C2	Cell–ECM interaction prompts C1 to C2 conversion and C2 maintenance	54, 60, 61, 109–113 and 200
TGFβ1	Production increases in C2	Induces myofibroblast activation driving C2 function such as pro-tumoral desmoplastic ECM production	41, 114–119, 122–130
TGFβ1-R	Expression increases in C2 in response to ligand stimulation	Increased pro-tumor signal transduction	141
Palladin	Expression increases C2	Promotes pro-tumor (C2) stromal remodeling	93 and 129
Integrin signaling	C1: Inactive $\alpha_v\beta_5$ , activated $\alpha_5\beta_1$ at plasma membrane; localized at 3D adhesions C2: $\alpha_v\beta_5$ is active, activated $\alpha_5\beta_1$ conformation is detected at both elongated 3D adhesions and intracellular vesicles ( <i>i.e.</i> , at multi-vesicular bodies and others).	Modifies cell–ECM interactions to render CAFs with C1 or C2 ( <i>i.e.</i> anti- or pro-tumor, respectively) functions	17, 51, 60, 93, 119, 142–151

on the effective engagement of integrins.<sup>119,142–151</sup> Thus, CDMs constitute an excellent model system to study CAF heterogeneity, TGFβ1 modulation and signaling, and more.<sup>60</sup>

Because CDMs were developed with the intent to culture cells that replicate *in vivo* cellular behaviors and signal transduction,<sup>54</sup> CDMs have also served in studies of cell migration mechanisms that would otherwise be impossible to detect in cells cultured in classic 2D systems.<sup>18,19,89,91,152</sup> These types of studies have demonstrated that the architecture and composition of the ECM dictates cell migration. To this end, it is now clear that cells traveling through fibrillar matrices require a complex and coordinated locomotion program, involving actomyosin, Rho family

guanosine triphosphatase (GTPase)-regulated contraction, and the regulation of cell-matrix and/or cell-cell adhesions. Additionally, as cell migration is constrained by the rigidity and positioning of the cell nuclei with respect to ECM fiber arrangement, porosity or restriction, and plasticity, cells also require an ability to dynamically alter ECM fibers (*i.e.*, via matrix crosslinking or matrix digesting enzymes such as metalloproteases).<sup>89,91,92,153–158</sup> Cell-matrix interactions are also responsible for regulating cell morphology and proliferation among others.<sup>9,159–164</sup> This type of cell-matrix crosstalk is mediated by integrins, which transmit extracellular or environmental cues through cytoskeletal rearrangements that can result in profound cellular changes; including physical and biochemical alterations to both the cell and its nucleus, often resulting in changes in gene expression.<sup>8,9,165–174</sup>

Researchers have also improved upon the use of CDMs by providing an underlying gel (*i.e.* acrylamide pre-coated with collagen) to the culture surface before seeding cells on top to produce CDMs.<sup>20</sup> Doing so not only removes the initial stress imposed on cells by the rigid plastic or glass culturing dish, but also adds the possibility of fine-tuning culturing substrates to match the stiffness of physiological or pathological environments.<sup>20</sup> As such, CDMs are being used in a wide range of applications and their utility is only expected to grow.<sup>55,152</sup>

## 16.4 Clinically Significant Insights Gained from Studying CDMs

One of the most effective ways to decrease the mortality rates for any cancer is to detect and diagnose it as early as possible. As such, there is high importance placed on developing methods for early detection, predicting prognosis, elucidating effective therapies, and foreseeing the likelihood of recurrence. Thus, patient biopsies are not only characterized by tumor stage and grade, but also by individual phenotypic traits and molecular characteristics of the tumor (*e.g.* mutations and genetic predisposition). For example, in invasive breast carcinoma, Keely and colleagues defined a highly aligned collagen signature known as tumor-associated collagen signature-3 (TACs 3) to be correlative with poor patient survival.<sup>175</sup> The same team was able to further adapt their collagen assessment technique to a semi-automatic screening protocol to analyze the collagen signatures in breast cancer samples and propose their use as prognostic biomarkers.<sup>176</sup> Similarly, using tissue biopsies from pancreatic cancer patients, the ratios between stromal  $\alpha$ SMA and collagen type I expression in the TME were found to serve as independent predictors of patient outcomes<sup>177</sup> (see Table 16.2 for a selected list of predictive stromal signatures). Similarly, in renal cell carcinoma, data gained from CDMs *in vitro* were used to analyze a cohort of tissue samples. In doing so, researchers not only identified those who underwent surgery at early stages of their disease (*i.e.*, non-metastatic patients) but also identified increased

stromal palladin as an indicator of recurrence risk.<sup>93</sup> From these studies, CDMs have also been useful in the identification of C1 vs. C2 traits (Figure 16.1 and Table 16.1), which were reported as stromal signatures predicting recurrence rates as well as survival in renal and pancreatic cancer patients.<sup>60</sup> Similar signaling axes, coupled with novel imaging approaches, have enabled investigators to assess levels of fibrosis, marked by significant ECM remodeling (*i.e.*, common source of TACs 3), to provide prognostic and/or diagnostic insights.<sup>51,178</sup>

Furthermore, TME phenotypic and molecular signatures have been greatly informative for the selection of personalized therapies for cancer patients.<sup>17,60,93,177,179–183</sup> For example, relevant traits of the TME-associated ECM, such as the “matrisome” profile (*i.e.* ECM protein content), its topographical features, stromal gene signatures, as well as the percentage of stroma coverage, have all been considered clinically significant traits.<sup>2,3,46,175,176,184–186</sup> Additionally, by analyzing CDM production *in vitro*, it is possible to identify differences in the biochemical composition of the ECM and the architectural reorganization of its fibers.<sup>42</sup> In fact, Brisson and colleagues demonstrated that altering the ratio between collagen type I and III in fibroblasts, specifically reducing collagen III in favor of collagen I, resulted in the production of highly organized pro-tumor ECMs<sup>187</sup> (Table 16.2).

As such, some of these pathological ECM characteristics are being used in clinical settings to gauge therapeutic effectiveness.<sup>188,189</sup> An interesting example of pro-tumor TME modifications is the observed increased hyaluronic acid (HA) levels in pancreatic cancer-associated desmoplasia. It was suggested that the natural ability of HA to accumulate water underlies the “swollen” desmoplasia that is characteristic of the pancreatic cancer stroma. This desmoplastic reaction increases interstitial pressure which collapses local blood vessels; and when combined with the absence of lymph drainage, creates a physical and biochemical challenge for delivering chemotherapeutic agents into the tumor mass.<sup>95</sup> This is further substantiated by the fact that stromal targeting can sometimes improve drug delivery.<sup>190</sup>

Currently, a phase III clinical trial using an enzyme that degrades HA is being performed in combination with front line therapies in advanced pancreatic cancer patients presenting high desmoplastic HA levels.<sup>191</sup> Additional CAF-secreted ECM proteins and cytokines, which are capable of regulating the TME, have also been targeted for therapeutic purposes.<sup>33,36,37,41,192–197</sup> Select literature reviews are available that list potential matrisomal factors, as well as their associated functions for altering physiological and pathological ECMs, proposed to advance cancer treatments when targeted.<sup>198</sup>

Collectively, studies using CDMs *in vitro* provide us with an invaluable tool for understanding the characteristics and behavior of a tumor cell in an *in vivo*-like context.<sup>20,180,199</sup> Moreover, the use of CDMs together with additional approaches has allowed researchers to investigate how the ECM is able to

**Table 16.2** Clinically applicable characteristics of cancer-associated desmoplasia. A compiled list of protein signatures reported *in vivo* that can be used to inform clinical outcomes or serve as targets for adjunctive therapies.

Desmoplastic stromal component	Cancer-associated signature	Effect	Application	Reference
Tumor Associated Collagen Signature (TACS) 3	Highly aligned ( <i>i.e.</i> anisotropic)	Correlated with poor survival	Prognostic signature	175 and 176
Ratio of Collagen I:III	Collagen III Decreases	Correlated with pro-tumor ECM	Prognostic signature	187
Ratio of $\alpha$ SMA: Collagen I	$\alpha$ SMA is high, collagen I is low	Predicts poor patient outcome <sup>a</sup>	Prognostic signature	177
C1 vs. C2 Phenotypes	Detection of C2 CAF traits (as depicted in Figure 16.1)	Enhances tumor growth, proliferation, survival, and metastasis	Prognostic signature and potential therapeutic target(s)	33, 36, 37, 41, 42, 51, 93, 178–181, 192, 194–197 and 199
Cytokine Production	Increased production favoring pro-migratory, immunosuppressive phenotype	Tumor and immune regulation	Therapeutic target(s)	1, 3, 17, 46, 140, 182, 184–186 and 189
Hyaluronic Acid	Increased in TME-associated ECM	Increases stromal tension and pressure; Inhibition improves drug delivery	Therapeutic target	95 and 191
TGFB1	Evidence of increased signaling	Inhibition shifts ECM from C2 to C1; improves chemotherapy efficacy	Therapeutic target	41, 114–119, 122–130 and 190
FAK	Increase of tyrosine phosphorylated 397 in elongated 3D adhesion structures	Inhibition slows metastasis and tumor proliferation	Therapeutic target	54, 60, 61, 109–113 and 200

<sup>a</sup>Reciprocal signatures were also reported (*e.g.* low  $\alpha$ SMA with high collagen I predicts better patient outcome).

regulate certain hallmarks of cancer.<sup>192</sup> For example, properties of tumor ECMs can modify the efficacy of therapeutic agents in both breast and pancreatic cancers. TGF $\beta$ 1-signaling inhibition has been shown in breast cancer models to improve chemotherapy efficacy by reverting the ECM from a cancer-associated profile (C2) to one representative of a normal state (C1).<sup>41</sup> Additionally, targeting the ECM to disrupt cell adhesion signaling pathways *via* the use of FAK inhibitors has been attempted in preclinical settings; showing promising results in mitigating metastatic behavior and slowing tumor proliferation.<sup>200</sup> Thus, unique stromal signatures gained from *in vitro* CDM analyses can provide additional prognostic information about the TME in a clinical setting and putative biomarkers to inform therapeutic options (Table 16.1).

## 16.5 Effects of the Distinct ECM Biochemistry and Topography

An added challenge of targeting the TMEs is the unpredictability of how the drug will physically interact with the ECM. In addition to the physical pliability of the ECM, the many polar glycoproteins interwoven throughout the ECM give it an ionic charge. For example, collagen (being positively charged) attracts negatively charged molecules and enables the formation of drug aggregates.<sup>201</sup> The size of molecules being delivered can also be an important factor to consider given the density of the ECM.<sup>202</sup> Even very small liposomes (~90 nm) encompassing oncolytic viruses injected into tumors failed to diffuse far from the injection site.<sup>203</sup> Similarly, extracellular collagen was observed to restrict 150 nm viral particles' distribution throughout the tissue, while matrix degradation using collagenase was able to dramatically increase particle distribution.<sup>96</sup>

Taken together, there is a significant body of evidence supporting the manipulation of the ECM's physical structure and biochemical composition to improve drug delivery to tumor cells. Encouragingly, technological advances, such as tunable collagen cell substrates and high-throughput systems, are allowing researchers to address nuanced questions that could not be asked before, such as how to overcome ECM-induced drug resistance.<sup>20,49,199,204–206</sup> Therefore, future developments and use of pathologically accurate CDMs are expected to result in considerable advancements in the understanding of cancer biology in general and particularly of the TME field.

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