Collagen Linearization within Tumors

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It is now well appreciated that the tumor microenvironment (TME) surrounding primary tumors impacts tumor growth, progression (invasion and migration), and response to therapy. Broadly speaking, the TME is composed of cells (immune cells, activated fibroblasts, adipocytes, endothelial cells), acellular extracellular matrix (ECM), and cytokines or growth factors, some of which are bound or tethered to the ECM proteins. All these compartments undergo significant changes during tumor development and progression. Changes to the ECM, in particular, can dramatically influence cancer biology. This has stimulated the development of therapies that directly reverse or prevent the structural changes in the TME ECM that facilitate cancer progression. But to do so, in a rational manner, we need to understand how structural changes to tumor ECM arise, are remodeled, and function to facilitate tumor cell invasion and migration that give rise to metastatic disease, which is the main cause of cancer-related deaths. In this issue of *Cancer Research*, Janjanam and colleagues show that the ratio of WISP1/WISP2 in tumors is critical for ECM collagen fiber linearization and important for metastasis. WISP2 binds ECM collagen directly and can inhibit WISP1-mediated collagen linearization. These new results offer a new approach for targeting the altered collagen ECM in tumors by preventing or reversing collagen linearization.

See related article by Janjanam et al., p. 5666

Within the extracellular matrix (ECM), the fibrillar collagens I and III are the most abundant proteins, and their abundance increases in many tumors. Second harmonic imaging microscopy of tissue and tumor slices reveals that collagen surrounding normal epithelia or early tumors is curly, smooth, and aligned parallel to the tumor surface. During tumor development, along with the increased collagen deposition in and around tumors, collagen fibers progressively thicken, linearize, and assume a perpendicular direction to the tumor surface. These observed changes have prompted the development of a prognostic tool, known as tumor-associated collagen signatures, that is a strong independent predictor of clinical outcome for many tumors (1).

Progressive collagen fiber thickening and linearity both contribute to increases in tumor stromal stiffness. Tissue stiffness in and of itself can facilitate tumor cell invasion and migration (durotaxis) during metastatic dissemination. In addition to conferring altered mechanical properties to tumors, tumor collagen matrices also contain an abundance of embedded growth factors and cytokines, which can be released upon fiber contraction, stretching, or cleavage. Collagen fiber cleavage also releases cryptic collagen fragments that can be biologically active.

In tumors, excess production of secreted lysyl oxidases enzymes (LOX) cross-link collagen fibers in the ECM and are thought to be a key contributor to the generation of thick collagen fibers (2). Long, straight, and thick collagen fibers are also posited to provide "tracks" for tumor cells to migrate over or through (3). Precisely how tumor collagen fibers are remodeled to extended linear tracks is not fully appreciated. Cancer-associated fibroblast (CAF) embedded within the collagen matrix generate mechanical tension within the ECM and this function can linearize collagen fibers (4). CAFs can also adhere to tumor cells at the leading edge through the formation of heterotypic E-cadherin/N-cadherin associations, which can lead tumor cells away from primary tumors (5). In recent work, the Labelle laboratory found that TGFβ1-stimulated breast tumor cells secrete a cellular communication network (CCN) family member, CCN4 (WISP1), that can directly bind collagen and linearize collagen fibers in the absence of cell-generated mechanical force (6). *In vivo*, experimental overexpression of WISP1 in tumor cells results in increased collagen fiber linearization and increased metastases, and genetic inhibition of *Ccn4* alone can block TGFβ1-induced tumor cell migration through collagen. This appears to be the result of CCN4 overproduction alone as, at least in cell lines *ex vivo*, overexpression of CCN4 does not significantly alter gene expression. Indeed, high levels of CCN4 production by human tumors is a negative prognostic variable. This observation provides yet another approach toward normalizing collagen fiber remodeling in tumors, which could provide therapeutic benefit. Now, Janjanam and colleagues in the Labelle group have shown that the related CCN protein CCN5 (WISP2) also binds collagen directly and inhibits CCN4-mediated collagen linearization, most likely by interacting with CCN4 and preventing its binding to collagen (7). While aggressive tumors have high levels of CCN4 compared with normal tissues, CCN5 levels decrease in breast tumors. Through multiple experimental approaches they show that the ratio of CCN4/CCN5 (WISP1/WISP2) in tumors is critical for collagen fiber linearization *in vitro* and *in vivo*, and critically important for metastases. These compelling results suggest that the inhibition of collagen linearization in tumors, by WISP2 for example, could be a novel therapeutic approach to the prevention of tumor metastasis.

The CCN family of secreted matricellular proteins are a heretofore underappreciated component of the ECM (8). This matricellular family of proteins are secreted into the extracellular environment and their production is dysregulated in pathologic conditions in which fibroblasts are activated, such as in fibrotic disease and cancers. There are six CCN proteins that share a common four-module domain structure, except CCN5, which lacks a C-terminal heparin-binding domain. It appears that this C-terminal domain may be critical for controlling collagen linearization, but not collagen binding, but further

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Cancer Res 2021;81:5611–2

doi: 10.1158/0008-5472.CAN-21-2939

research would be required to determine whether this could be utilized therapeutically. One can speculate that binding of CCN4 could stabilize linear collagen fibers, preventing their folding or curving. Open questions in this field include whether there is any interplay between CCN4-mediated collagen linearization and LOX-mediated collagen fiber cross-linking, as well as whether the balance of all CCN family members is critical for collagen linearization. In various mouse models of disease, the described effects of CCN proteins are pleiotropic and thought to be context dependent. Functionally, many studies support their interaction with and affecting integrin functions such as adhesion. Similar domains to the C-terminal domain of CCN4 are present in other proteins such as NGF, VEGF, PDGF, TGFβ, and vWF, and for these proteins, it appears to mediate their dimerization.

In general, current approaches to repairing the altered collagen ECM in tumors have focused on five approaches, with mixed clinical results in human cancers (9). These include: (i) inhibiting collagen synthesis by tumor and tumor-associated stromal cells; (ii) inhibiting CAF contractility; (iii) promoting degradation of excess collagen; (iv) inhibiting collagen fiber cross-linking; and (v) blocking collagen interactions with cells. These new results offer another new approach—to prevent or reverse collagen linearization and its pathologic sequelae. Persistent challenges with all these approaches include whether an already formed tumor matrix (established fibrosis) is reversible, a lack of specificity or selectivity of any one approach, and the possibility that excessive removal of tumor ECM components may result in tumor "collapse," which decreases drug penetration, in addition to release of embedded cytokines and growth factors as well as the generation of biologically active cryptic collagen fragments. As a result, whatever approach is utilized should perhaps attempt to normalize the ECM, or better yet prevent the formation of the tumor permissive ECM, rather than aim to remove the tumor-generated ECM entirely.

Authors' Disclosures

C.E. Barcus reports grants from American Cancer Society during the conduct of the study. G.D. Longmore reports grants from NIH and American Cancer Society during the conduct of the study; grants from Pfizer-CTI and Centene Corporation outside the submitted work. No other disclosures were reported.

Acknowledgments

This work was supported by NIH grants CA196205, CA223758, and CA210173 (to G.D. Longmore) and by the American Cancer Society ACS 133574-PF-19-204-01-CSM (to C.E. Barcus).

Received August 31, 2021; accepted August 31, 2021; published first November 15, 2021.

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