Immune Modulatory Properties of Collagen in Cancer

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During tumor growth the extracellular matrix (ECM) undergoes dramatic remodeling. The normal ECM is degraded and substituted with a tumor-specific ECM, which is often of higher collagen density and increased stiffness. The structure and collagen density of the tumor-specific ECM has been associated with poor prognosis in several types of cancer. However, the reason for this association is still largely unknown. Collagen can promote cancer cell growth and migration, but recent studies have shown that collagens can also affect the function and phenotype of various types of tumor-infiltrating immune cells such as tumor-associated macrophages (TAMs) and T cells. This suggests that tumor-associated collagen could have important immune modulatory functions within the tumor microenvironment, affecting cancer progression as well as the efficacy of cancer immunotherapy. The effects of tumor-associated collagen on immune cells could help explain why a high collagen density in tumors is often correlated with a poor prognosis. Knowledge about immune modulatory functions of collagen could potentially identify targets for improving current cancer therapies or for development of new treatments. In this review, the current knowledge about the ability of collagen to influence T cell activity will be summarized. This includes direct interactions with T cells as well as induction of immune suppressive activity in other immune cells such as macrophages. Additionally, the potential effects of collagen on the efficacy of cancer immunotherapy will be discussed.

Keywords: cancer immunology, collagen, extracellular matrix, tumor microenvironment, immunotherapy, T cells, macrophages, matrix immunology

INTRODUCTION

With a constantly growing knowledge about the extracellular matrix (ECM), it has become clear that the ECM is by no means an inert scaffold, but rather a dynamic structure that can regulate the function of cells in contact with it (1). This ability of the ECM to influence cellular responses has been a major focus area in the matrix biology research field within the last decades. In connection to cancer, the ECM has been studied extensively in order to elucidate exactly how it influences tumor progression and metastasis. The majority of these studies have focused on the ability of the ECM to modulate the behavior of cancer cells or to stimulate the malignant transformation of epithelial cells.
In the field of cancer immunology, much less attention has been given to the ECM and its potential role in modulating immune cell activity. However, recent reports have shown that the ECM can indeed influence the behavior of immune cells, thereby launching a potentially new research field: matrix immunology.

**EXTRACELLULAR MATRIX REMODELING IN CANCER**

**Collagen Organization**

The ECM is a complex network of various macromolecules surrounding the cells within the body. It is composed of two classes of macromolecules, the fibrous proteins and the proteoglycans consisting of core proteins with one or more glycosaminoglycans (GAGs) covalently attached (2, 3). The main types of fibrous proteins are elastin, fibronectin, laminins and collagens (3, 4). The ECM can be classified into two categories based on function; the basement membrane and the interstitial matrix. The basement membrane forms an anchoring sheet-like layer between the parenchyma and the connective tissue. It is mainly composed of collagen type IV, laminins, nidogen 1 and 2, and various proteoglycans such as perlecan (5). The interstitial matrix is the matrix surrounding the cells. It is composed of proteoglycans and various fibrous ECM proteins secreted mainly by fibroblasts within the stroma (3, 6). In cancer, fibroblasts are the main producers of the tumor-specific ECM, but other cells can also contribute. These cells include endothelial cells (7), cancer cells (8, 9), and immune cells such as macrophages (3, 5, 10). Besides secreting ECM components, fibroblasts can also exert tension on the matrix, organize collagens into sheets and fibers, and influence the alignment of the collagen (3).

Collagens are the main constituents of the ECM comprising around 30% of the whole protein mass in the body (3, 11). 28 types of collagens have been identified (12), which based on their structure and function can be divided into subtypes including fibril-forming, fibril-associated collagens with interrupted triple helices (FACITs), network-forming, transmembrane, endostatin-producing, anchoring fibrils and beaded-filament-forming collagen (13, 14). The fibrillar collagen type I is the most abundant type of collagen and the primary constituent of the interstitial matrix (3, 15).

Collagen is composed of three left-handed polypeptide chains called α-chains held together by inter-chain hydrogen bonds. These intertwine to form a right-handed triple helical structure. The α-chains are composed of an amino- and a carboxy terminus flanking Gly-X-Y repeats. Of these, X is often a proline and Y a hydroxyproline. Each type of collagen contains a unique combination of α-chains (16, 17). The main type of collagen, collagen type I, consists of two α1-chains and one α2-chain (16, 18). Collagens are initially transcribed and translated into pre-pro-polypeptides. These undergo multiple post-translational modifications in the endoplasmic reticulum (ER) and in the Golgi apparatus. Following this, the resulting procollagen is packed into secretory vessels and transported to the extracellular space (18, 19). In the extracellular space the procollagen is modified by proteases to remove the N- and C-propeptide domains, generating mature collagen units that polymerize to form insoluble collagen fibers (18). Lastly, cross-linking can be introduced by lysyl-oxidase (LOX) in order to generate larger collagen fibers (18, 20, 21), and increase the stiffness of the ECM (22).

**Collagen Degradation**

The tight triple helical structure of collagen renders it very resistant to proteolytic cleavage. In fact, only a few proteases have been shown to be able to cleave native collagen type I under physiological conditions. These include members of the matrix metalloproteinase (MMP) family and the cysteine protease family. The MMPs capable of cleaving collagen are referred to as collagenases and include MMP-1, -2, -8, -13 and -14 (23). In collagen type I, the cleavage happens primarily at Gly775-Ile776 and Gly775-Leu776 in the α-1 and α-2 chain, respectively. This cleavage generates well-defined fragments ¼ and ¾ of the length of the full molecule (24). The exact molecular mechanism of MMP-mediated collagen cleavage has been excellently reviewed in (25). As opposed to the intact collagen, these fragments are unstable under physiological conditions and prone to degradation by additional proteases including MMP-2 and -9, the so-called gelatinases, as well as the serine protease fibroblast activation protein (FAP) (26–28). The proteolytically generated collagen fragments can also be internalized by receptor-mediated uptake and routed to the lysosomes for complete degradation by cysteine proteases (29–31). This process has been shown to be mediated primarily by two endocytic collagen receptors, the mannose receptor (MR)/CD206 and the urokinase plasminogen activator receptor-associated protein (uPARAP)/Endo180/CD280, which are expressed mainly by macrophages and fibroblasts, respectively (32, 33).

**Collagen Remodeling During Cancer Development**

During tumorigenesis the ECM is extensively remodeled. The existing ECM is degraded and substituted with a tumor-specific ECM, which is often more linearized, of increased stiffness, and has a high collagen content. Degradation of the ECM involves the concerted action of multiple proteolytic systems and several different cell types of the tumor microenvironment (34). The degradation of collagen type I alone involves cleavage of collagen fibers by the collagenolytic MMPs MMP-1, MMP-8, MMP13, and MMP14 (MT1-MMP) which can be expressed by cancer cells or stromal cells (34). Complete collagen turnover involves subsequent lysosomal degradation upon cellular uptake of collagen fragments mediated by the MR or uPARAP (33, 35). In tumors, collagen internalization is primarily mediated by TAMs and CAFs, with M2-like TAMs being the dominant collagen-internalizing cell type (36, 37). CAFs are the central cell type responsible for production of collagen in the tumor microenvironment, although studies have shown that macrophages and cancer cells can also contribute to the production of collagen (36, 38). CAFs have recently been
recognized as a group of several CAF subsets and especially one of these CAF subsets seems to be the main producer of collagen in the tumor microenvironment (39, 40). The newly synthesized ECM is characterized by being of high stiffness and density, and very rich in collagen. A high collagen-density and degree of collagen fiber alignment have been linked to a poor prognosis of several cancers. This includes breast cancer, pancreatic cancer, gastric cancer, and oral squamous cell carcinomas (41–45). The reason for this correlation is still not clear. The effects of collagen density and tumor stiffness on cancer cells have been investigated for more than a decade, and recently, studies have also investigated the effect of collagen on immune cells such as tumor-infiltrating lymphocytes (TILs) and tumor-associated macrophages (TAMs).

**EFFECTS OF COLLAGEN DENSITY ON CANCER CELLS**

Increased stiffness compared to healthy tissue is a characteristic of most solid tumors that render them detectable by palpation. The high stiffness of tumor tissue has been shown to correlate with increased deposition of collagen as well as increased crosslinking of collagen fibers (46–49). The high ECM stiffness is not only a passive bystander of cancer, but can also affect and drive many stages of tumor progression; from malignant transformation and increased metabolic adaptability to enhanced invasation, facilitating metastasis (50). In healthy tissue, matrix stiffness also controls many important cellular functions, such as development and homeostasis (51). In vitro, substrate stiffness has been shown to affect naïve mesenchymal stem cells, which when cultured on soft matrices, mimicking brain tissue, commit to a neuron-like lineage while when cultured on rigid matrices, mimicking collagenous bone, commit to an osteogenic lineage (52).

In connection to cancer, the link between matrix stiffness and malignant transformation of epithelial cells has been investigated. Using mammary epithelial cells (MECs), Paszek et al. showed in a seminal study that increased collagen density led to perturbed morphology and disrupted basal polarity in vitro (53). They demonstrated that increased matrix stiffness drives a mechanoregulatory feedback loop in which focal adhesions (FA) are promoted through integrin aggregation. This in turn activates signaling pathways, increases cytoskeletal tension and further FA formation, promoting malignant transformation (53). This signaling loop was also demonstrated in a study by Provenzano et al. (54). In addition, they found that increased collagen density in vitro promoted an invasive phenotype of MECs and caused altered gene expression including upregulation of genes associated with proliferation (54). Increased matrix stiffness through collagen crosslinking was in another study showed to promote FA and induce invasion through enhanced signaling of PI3K (48). PI3K signaling has also been shown to be important for epithelial-mesenchymal transition (EMT) (55). When normal murine mammary gland epithelial cells were treated with transforming growth factor β (TGF-β), culture on soft matrices induced apoptosis while culture on stiff matrices led to EMT through the PI3K/Akt pathway (55). Increased matrix stiffness has also been suggested to modulate the metabolism of cancer cells. Specifically, culture of the metastatic breast cancer cell line 4T1 on a high-density collagen matrix resulted in an increased capacity to use glutamine as fuel source for mitochondrial respiration. This was not observed to the same degree for the non-metastatic breast cancer cell line 4T07 (56).

Metastatic disease requires the ability of a malignant cell to escape the primary tumor site by binding to vasculature and intravasate to reach a secondary location. Matrix stiffness has been shown to be of importance for this process. In response to increased stiffness, endothelial cells were shown to upregulate the protein cellular communication network factor 1 (CCN1), which activated β-catenin causing upregulation of N-cadherin on the surface of the endothelium. This was in turn shown to facilitate cancer cell-endothelium binding (57, 58). A high matrix stiffness can also induce epithelial-to-mesenchymal transition (EMT) in cancer cells, leading to increased metastasis (59). This process is dependent on stiffness-induced nuclear translocation of the transcription factor TWIST1 (59). LOX-induced collagen cross-linking is an important mediator of increased matrix stiffness in tumors and a driver of metastatic tumor growth (60, 61). Consequently, inhibition of LOX has been shown to reduce metastasis (61, 62).

Indirectly, a stiff matrix is also capable of supporting tumor progression by favoring growth of endothelial cells and thereby stimulating angiogenesis (63). Additionally, matrix stiffening promotes the activity of the transcriptional co-activators Yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ), which in turn are required for CAF-induced matrix stiffening, creating a positive feedback loop further driving the cancer-promoting effects described above (64).

**COLLAGEN CAN AFFECT THE IMMUNE ENVIRONMENT IN TUMORS**

In addition to the effects on cancer cells, collagen has also been shown to affect tumor infiltrating immune cells. Of these, TILs and TAMs are of special interest due to their cytotoxic and anti-inflammatory activities, respectively.

**Collagen-Mediated Modulation of T Cell Activity**

T cells are lymphoid cells that can be divided into several subsets depending on their T cell receptor (TCR) and expression of coreceptors, in particular CD4 and CD8. In the tumor microenvironment, especially the CD8+ T cells are of interest due to their cytotoxic activity. CD4+ T cells mainly acts to orchestrate the activity of other immune cells, but they can also have direct cytotoxic activity (65).

However, the ability of T cells to kill cancer cells is often suppressed in the tumor due to the existence of a highly immune
suppressing tumor microenvironment. This tumor microenvironment is characterized by containing cells with an immunosuppressive phenotype including myeloid-derived suppressor cells (MDSCs), M2-polarized macrophages, and regulatory T cells (Tregs), and by the upregulation of several immune inhibitory molecules such as programmed death ligand 1 (PD-L1) and -2 (PD-L2), TGF-β, indoleamine 2,3-dioxygenase (IDO), and arginase 1 (ARG1) (66–69). Several studies have investigated the T cell inhibitory effects of these cellular components of the tumor microenvironment, but recently the effect of the ECM on T cell activity has also gained attention. ECM components, such as collagen, have been reported to directly or indirectly influence the migration, phenotype, and function of T cells.

Collagen Can Control the Migration of T Cells
Collagen in the tumor microenvironment can affect the ability of T cells to kill cancer cells by regulating the migration of T cells into the tumor. The organization of collagen in tumors is highly heterogeneous but generally found to be more closely packed in the tumor periphery and more loosely packed within the center of the tumor. In addition, the collagen fibers are often aligned perpendicularly to the tumor boundary (42, 45). The density of collagen and the degree of collagen alignment are strong negative prognostic factors.

T cells can efficiently migrate in different environments including collagen matrices using an amoeboid migration mode (70). In 3D culture assays, T cells do however migrate slower through collagen gels of high density compared to low density (71, 72). This reduced migration speed was suggested to be a consequence of decreased pore size of the matrix (72). The increased stiffness associated with a higher collagen density could, however, also contribute to the reduced migration speed since matrix stiffness has also been shown to affect T cell migration (73, 74). In a study using optically tunable hydrogels, it was elegantly demonstrated that increased matrix stiffness in this assay system led to reduced T cell migration independently of the pore size (75). In contrast to cancer cells, which depend on proteolytic remodeling of the collagen fibers, T cell migration in collagen is independent of proteolytic remodeling of the collagen fibers (72, 76). In vitro studies have also demonstrated that T cells preferentially migrate along the collagen fibers, indicating that the collagen orientation could control the migration of T cells (77).

The decreased T cell migration speed in a high-density collagen matrix as well as the ability of collagen fibers to guide T cell migration suggest that collagen in the tumor microenvironment could limit T cell infiltration. In agreement with these in vitro studies, migration along the collagen fibers has also been elegantly demonstrated using ex vivo culture of tissue slices from lung tumors and ovarian tumors (78–80). These studies confirm that the alignment of collagen fibers can limit T cell migration into the tumor core. Similar indications of collagen-mediated restriction of T cell infiltration were observed in a murine prostate cancer model as well as in human pancreatic cancer samples (77, 81).

Collagen Density Can Regulate T Cell Activity
T cell activation involves the formation of an immunological synapse between a T cell and an antigen-presenting cell (82). Older studies have shown that a collagen-dense environment can affect this interaction and reduce T cell activation (83, 84). Recently, we have identified that collagen density can also profoundly affect the activity of T cells after the initial activation phase (85). Specifically, cultivation of pre-activated T cells in a 3D high-density collagen matrix, mimicking tumor ECM, led to decreased proliferation compared to T cells cultured in a low-density collagen matrix. In addition, the T cells cultured in high collagen density upregulated Treg markers and downregulated cytotoxic T cell activity markers (Figures 1A, B). In alignment with these changes in the cells’ gene expression profile, we found that TILs cultured in 3D matrices of high collagen density compared to low collagen density were subsequently less capable of killing autologous melanoma cells, showing that collagen can directly affect the function of TILs (Figure 1C). Whole-transcriptome analyses indicated that the underlying mechanism of collagen-mediated modulation of T cell activity might involve autocrine TGF-β signaling (85). We did not investigate if the increased stiffness associated with a higher collagen density could be involved in the effects on T cell activity. The study of mechanosensing in T cells is still in its infancy, but an interesting study by O’Connor et al. showed that T cell activation was mitigated when cells were cultured on substrates of increasing stiffness (87). The effect included reduced proliferation and expression of cytokines associated with T cell activity. However, other studies of the effects of substrate stiffness on T cell biology, have not demonstrated a similar modulation of T cell activity (88, 89). The exact reason for these discrepancies is still unclear but could be a consequence of differences in substrates, T cell origin, and T cell stimulation between the different studies.

Collagen has also been shown to affect T cells in vivo. During tissue regeneration, implantation of collagen scaffolds into wounded muscles of mice promotes the formation of an immunosuppressive microenvironment (90). Specifically, collagen led to an increase in the CD4:CD8 ratio among the infiltrating T cells and the CD4+ T cells were skewed toward a Th2 phenotype. Additionally, the T cells in the collagen-implanted wounds expressed higher levels of anti-inflammatory cytokines (90).

Collagen-Receptors Expressed by T Cells
A possible way collagen can exert its effect on T cells is through the interaction with receptors on the surface of the cells. Several collagen binding receptors can be expressed by T cells. These include leukocyte-associated Ig-like receptor-1 (LAIR-1), discoidin domain receptor 1 (DDR1), and several integrins.

LAIR-1 is an immune-inhibitory transmembrane receptor with an Ig-like extracellular domain (91). It is a member of the Ig superfamily, and has been found to be expressed on the majority of PBMCs and thymocytes (92, 93). LAIR-1 is expressed by CD4+ and CD8+ T cells, with expression being highest in the naïve T cells (91, 94). Collagens are high affinity ligands for LAIR-1 (95) and both transmembrane collagens and secreted
collagens, such as collagen type I, can bind to LAIR-1 on the surface of immune cells (96). For natural killer (NK) cells this interaction was shown to inhibit their cytotoxic activity (96). Using T cells isolated from LAIR-1 knockout mice, it has been demonstrated that collagen can inhibit TCR signaling in a LAIR-1 dependent manner (97). Additionally, studies have shown that cross-linking of LAIR-1 using anti-LAIR-1 antibodies directly inhibits T cell activity (91, 94). Further studies are needed to elucidate the role of LAIR-1 in the immunosuppressive tumor microenvironment, but based on current knowledge, LAIR-1 could be a promising cancer therapeutic target.

Another collagen binding receptor expressed on T cells is DDR1. The DDRs are a subfamily of receptor tyrosine kinases, consisting of DDR1 and DDR2, known to interact with a range of collagens, such as collagen type I, can bind to LAIR-1 on the surface of immune cells (96). For natural killer (NK) cells this interaction was shown to inhibit their cytotoxic activity (96). Using T cells isolated from LAIR-1 knockout mice, it has been demonstrated that collagen can inhibit TCR signaling in a LAIR-1 dependent manner (97). Additionally, studies have shown that cross-linking of LAIR-1 using anti-LAIR-1 antibodies directly inhibits T cell activity (91, 94). Further studies are needed to elucidate the role of LAIR-1 in the immunosuppressive tumor microenvironment, but based on current knowledge, LAIR-1 could be a promising cancer therapeutic target.

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collagens (98). DDR1 consists of six isoforms and is primarily expressed by epithelial and carcinoma cells (99, 100). DDR2 is mainly expressed by cells of mesenchymal origin like fibroblasts and smooth muscle cells (101). A few studies have, however, shown that DDR1 expression is induced by TCR activation of T cells and plays a role in the migration of T cells through collagen matrices (101, 102). It is therefore speculated that DDR1 could be an interesting therapeutic target for improving T cell migration to the tumor (101).

Lastly, several integrins found on T cells are known to bind collagen. These include α1β1 integrin and α5β1 integrin (5, 103). These integrins are expressed on activated T cells and upon TCR stimulation they promote adhesion to the ECM (104). β1 integrins can also have co-stimulatory effects on antigen-stimulated T cells, rendering them more proliferative when cultured on surfaces coated with collagen type I compared to other ECM components such as fibronectin (105). This co-stimulatory effect was shown to be particularly potent in the case of collagen type I mediated activation of α5β1 integrin (106). Also, α1β1 and α5β1 integrins expressed by activated T cells have been found to be important for the generation of an inflammatory response in a mouse model of delayed type hypersensitivity (107). Th17 cells express α1β1 integrin, and the binding of collagen to this integrin leads to increased interleukin (IL)-17 synthesis (108). In the same study, it was shown that blockade of α5β1 integrin decreases the severity of collagen-induced arthritis in mice (108).

Collagen-Mediated Regulation of the Immune Suppressive Activity of Macrophages

In the tumor microenvironment, TAMs are often detected in close contact with collagen (119), and it is therefore an appealing hypothesis that the interaction with collagen could modulate the activity of the cells. This could happen during the differentiation of monocytes to macrophages or during the polarization towards an M2-like phenotype.

Cultivation of primary monocytes on a collagen type I coated surfaces has been shown to stimulate the differentiation to macrophages (120, 121), and during PMA-induced differentiation of the monocytic cell line U937, collagen type I causes reduced production of pro-inflammatory cytokines (122).

We have recently shown that 3D-cultured macrophages are directly affected by high collagen densities mimicking the ones found in tumors (86). Macrophages cultured in collagen matrices of high density compared to low density acquired a distinct expression profile, which included the differential expression of immune-regulatory genes and genes encoding chemokines. Co-culture assays revealed that macrophages cultured in high-density collagen inhibited the proliferation of T cells more than macrophages cultured in low-density collagen (Figures 1D, E). Additionally, the gene-expression changes were associated with a decreased ability to attract CD8+ T cells (Figures 1F–H). Altogether these findings illustrate that the surrounding collagen density can instruct macrophages to become more anti-inflammatory (86).

In alignment with these findings, culture of monocytes on decellularized matrices from colorectal tumor tissue or from normal colorectal tissue strikingly showed that tumor matrices drive monocytes towards M2-polarization (123). This could be a consequence of the higher collagen density of tumor matrices compared to the matrices from normal tissue (123). Similarly, decellularized matrices from obese and lean breast tissue were used to examine the effects of these matrices on macrophage function (124). Obesity was associated with increased amounts of interstitial collagen, and when bone-marrow derived macrophages (BMDMs) were cultured on the matrices derived from obese tissue, they acquired both morphological and genetic characteristics similar to those of M2-like TAMs (124).

In addition to these studies of primary monocytes/macrophages, the human monocytic cell line THP-1 has also been shown to acquire an M2-like phenotype when cultured in a gelatin-based hydrogel compared to regular tissue-culture conditions (125). In another study using PMA-stimulated THP-1 cells cultured in collagen gels of low or high density, it was, however, unclear if an increase in the surrounding collagen density stimulated M2-polarization of these THP-1 derived macrophages (126).

The exact reason why macrophages cultured in high-density collagen seem to acquire an M2-like phenotype still needs to be elucidated. One of the underlying mechanisms could involve the increased stiffness associated with an increased collagen density.

Studies have shown that cultivation of primary macrophages on stiff ECM surfaces can affect their migration speed, morphology, proliferation, and phagocytic activity (127, 128).
and make them acquire a more M2-like phenotype (128, 129). Additionally, it has been shown that cultivation of THP-1 derived macrophages on stiff surfaces increases the expression of M2-markers compared to culture on softer surfaces (130). However, other studies have shown that macrophages can also upregulate the expression of M1-markers when cultured on stiff surfaces (131–133). The opposing results could be due to the different range of stiffness, type of coating, cell types, and stimulation used. For example, one study found that cultivation of unstimulated BMDMs on hydrogels of increased stiffness led to upregulation of M2-markers, whereas the same cell type stimulated with lipopolysaccharide (LPS) downregulated M2-markers and upregulated M1-markers on stiffer surfaces (133). Another example of how macrophages of different origin and activation state respond differently to ECM changes was shown by Stahl and colleagues (134). They found that pulmonary macrophages isolated from patients with idiopathic pulmonary fibrosis acquired a more M2-like phenotype when cultured on various types of collagen compared to pulmonary macrophages isolated from healthy donors (134). The mechanosensing ability of macrophages has been suggested to involve the ion channel transient receptor potential cation channel subfamily V member 4 (TRPV4) (128, 132) and the transcriptional coactivator YAP (135).

Several studies have indicated that collagen can stimulate M2-polarization of macrophages in vivo. For instance, implantation of collagen gels into injured muscles of mice resulted in an increased amount of M2-like macrophages compared to saline injected control mice (90). This effect was, however, shown to be dependent on collagen-induced Th2-polarization of CD4⁺ T cells (90). During skin wound healing in mice, it was also demonstrated that collagen injected into wounds led to M2-polarization of macrophages. This effect appeared to be mediated by the acid-sensing pathway-associated lysosomal adaptor protein, Lamtor1, indicating that phagocytosis of collagen and subsequent lysosomal signaling could be critical for the observed M2-polarization (136). In rats, implantation of crosslinked collagen disks likewise resulted in increased accumulation of M2-like CD206⁺ macrophages (137).

Collagen-Receptors Expressed by Macrophages

Several collagen-binding receptors are expressed by macrophages and could be involved in the cellular response to the surrounding collagen.

DDR1 can be expressed by macrophages and affect their cellular functions. DDR1 mRNA has been detected in human monocytic cells and the expression of DDR1 increases upon activation with IL-1β, granulocyte-macrophage colony-stimulating factor (GM-CSF), LPS, or phytohemagglutinin (PHA) (138). The role of DDR1 for macrophage function has been addressed in studies using DDR1-overexpressing THP-1 cells (138, 139). Here it was reported that the DDR1 isoforms DDR1a and DDR1b increased the adherence to collagen coated plates in a β1-integrin independent manner (138). Additionally, DDR1a promoted the ability of THP-1 cells to migrate through 3D collagen matrices (138). Cultivation of DDR1b-overexpressing THP-1 monocytes on collagen-coated surfaces increased the expression of inflammatory markers such as IL-1β, IL-8, MIP-1a, and MCP-1 compared to mock-transfected control cells (139). In the murine macrophage cell line J774A.1, collagen induced iNOS expression and consequently nitric oxide production in a DDR1-dependent manner (140). Murine Kupffer cells have also been shown to express DDR1, and pretreatment of these cells with collagen led to an increased ability to attract cancer cells in a DDR1-dependent manner (141).

Several types of integrins are expressed on macrophages, with the β2-integrin family being the most common (142). However, macrophages can also express the collagen-binding α2β1-integrin (142). This has been demonstrated for primary macrophages isolated from the peritoneum of mice, and for these cells α2β1-integrin is essential for adhesion to collagen (143). In a recent study, it was furthermore shown that α2β1-integrin mediated the migration and mechanosensing of macrophages cultured on 3D collagen matrices upon deformation by contracting fibroblasts (144). α2β1-integrin is also expressed by the monocytic cell line, THP-1, where it is involved in M2-polarization induced by 3D culture in a gelatin-based hydrogel (125).

LAIR-1 is expressed by the majority of myeloid cells, including monocytes and macrophages, and it has been reported to have inhibitory effects on their cellular functions (93). In monocytes, LAIR-1 ligation with an agonistic antibody inhibited TLR-mediated activation (145). Additionally, cultivation of M1-stimulated murine or human macrophages on surfaces coated with LAIR-1 ligand peptide has been shown to result in decreased secretion of the pro-inflammatory cytokine TNFα as well as T cell attracting chemokines (146). Recently, it was demonstrated that LAIR-1 also promotes the differentiation from classical to non-classical monocytes in the bone-marrow (147). Surprisingly, this study also showed that global or myeloid-specific deletion of LAIR-1 in mice increased experimental lung metastasis of B16-F10 melanoma (147).

Further studies are needed to elucidate the role of LAIR-1 in other cancer models and its role in modulating anti-tumor immunity.

Osteoclast-associated receptor (OSCAR) is another collagen-binding receptor, which is expressed in osteoclasts in mice, and in monocytes, macrophages, dendritic cells, and osteoclasts in humans (148–150). It is a member of the leucocyte receptor complex that associates with FcRγ, and through FcRγ-signaling OSCAR is critical for osteoclastogenesis (150, 151). In contrast to LAIR-1, OSCAR-signaling has mainly been associated with immune activation (35). In dendritic cells derived from human blood monocytes, OSCAR was shown to trigger cellular activation events (152), and in monocytes it potentiates the pro-inflammatory response of Toll-like receptor (TLR) ligands (153). This effect was confirmed in another study, where collagen stimulated the release of pro-inflammatory cytokines from monocytes in an OSCAR-dependent manner (154).

Macrophages also express the MR, which is an endocytic receptor with multiple ligands including collagen (155, 156). The receptor binds collagen and proteolytically generated collagen-
fragments and promotes cellular internalization of the collagen for lysosomal degradation (32, 157, 158). In vivo, this process of MR-mediated collagen internalization has been demonstrated by macrophages in the skin (37, 159) and in tumors (36, 160), and genetic deletion of MR leads to an accumulation of collagen in murine tumors (36). It is not yet known if the MR-collagen interaction can lead to changes in macrophage activity and function.

THE ROLE OF COLLAGEN FOR IMMUNE ACTIVITY IN TUMORS IN VIVO

Several studies have attempted to elucidate the role of collagen for cancer progression and metastasis in vivo. However, only relatively few of these studies specifically investigated the effects of collagen on immune cell infiltration and activity.

The transgenic Col1a1tm1jae (ColR) mice have a mutation in the collagenase cleavage site of the α1 chain of collagen type I, and as a consequence of the reduced collagen turnover they accumulate collagen in various tissues. These mice were interbred with MMTV-PymT mice, which spontaneously develop mammary cancer, and used by Keely and colleagues to investigate the effect of increased collagen density on mammary cancer progression and metastasis. The large collagen accumulations observed in the tumors of these mice were accompanied by increased tumor growth and metastasis (161). In another study by the same group, it was demonstrated that the increased breast tumor growth in the collagen accumulating transgenic mice was associated with increased infiltration of macrophages, neutrophils and α-smooth muscle actin (αSMA)-positive fibroblasts (162). The collagen-dense tumors were additionally characterized by an increased expression of cyclooxygenase-2 (COX-2) and prostaglandin E2 (PGE2). Blocking of COX-2 with Cecelecoxib decreased collagen deposition and infiltration of macrophages and neutrophils, suggesting that COX-2 modulates tumor progression in collagen dense tumors (162). In a follow-up study, the cell composition of the tumor microenvironment was examined by flow cytometry (163). No significant changes in cell composition were observed although a trend towards increased neutrophil recruitment was seen. Additionally, the cytokine profiling indicated a change in neutrophil activation. A potential role of neutrophils was confirmed by blocking the recruitment of these cells, which limited the increased tumor growth in the collagen-accumulating mice (163). Another study using orthotopically injected breast cancer cells, showed no difference in primary tumor growth between Col1a1tm1jae and wildtype (wt) mice, but significantly more metastasis to liver and lung in the collagen accumulating mice (164). The tumor-promoting effects of collagen observed in Col1a1tm1jae mice was also supported by a study from Northey et al. that showed increased proliferation of MECs indicative of a higher risk of breast cancer development (165). These seminal studies were proceeded by another well-performed study, which used Col1a1tm1jae mice combined with a chemically induced or transplanted model of hepatocellular cancer (166). In this study the Col1a1tm1jae mice were, however, found to develop significantly fewer and smaller tumors compared to wt mice (166). The observed tumor growth reduction in Col1a1tm1jae mice was suggested to be a consequence of the lack of proteolytically generated collagen fragments that stimulate integrin-signaling (166).

Recently, three studies have investigated the role of collagen for tumor progression, using conditional collagen type I knockout (KO) mice. Chen et al. inactivated the Col1a1 gene in αSMA+ fibroblasts and combined this with a genetically induced model of pancreatic ductal carcinoma (PDAC) (167). In this model system, they observed a 50% reduction in stromal collagen type I and, interestingly, an accelerated tumor growth and decreased overall survival compared to control mice. The tumors in conditional Col1a1 KO mice had increased infiltration of CD206+;F4/80+;Arg1+ myeloid cells and lower levels of B- and T cells (167). These changes in the immune microenvironment were suggested to be part of the reason for the observed effects on tumor growth. Two other recent studies used a similar approach to study the effects on Col1a1 inactivation in CAFs or in the entire liver of mice with intrahepatic cholangiocarcinoma or liver metastases (168, 169). The conditional collagen type I KO mice did not show altered primary tumor growth, but did show increased growth of liver metastases (168, 169). In the liver metastases, no differences in immune infiltration and inflammatory markers were found, apart from a decrease in Cd4 and Foxp3 mRNA levels (169). Instead the authors proposed that the anti-tumorigenic effect was due to collagens ability to physically restrict tumor expansion (169).

The results are in line with a previous study where SPARC-/- mice, which display reduced deposition of fibrillar collagen, were used to study the effects of collagen on pancreatic cancer growth and liver metastasis. Tumors in SPARC-/- mice had decreased levels of collagen type I and III and higher levels of TAM-infiltration, which were associated with reduced survival and increased metastasis (170). Another study used mice with impaired pro-collagen processing to show that collagens produced selectively by the cancer cells had anti-tumorigenic effects (171).

In these in vivo studies of the effects of collagen on tumorogenesis and metastasis, it was not investigated how collagen deposition in the tumor affects the efficacy of cancer immunotherapy. In one study it was, however, shown that depletion of αSMA+ fibroblasts reduced the amount of collagen, and that this was accompanied by increased efficacy of anti-CTLA-4 therapy (172).

Lastly, studies have directly or indirectly investigated the effects of LOX-mediated collagen cross-linking on tumor growth in mice. Treating early stage PDAC with a LOX-inhibitor together with the chemotherapeutic drug gemcitabine, increased the overall survival of the mice by reducing metastasis. Interestingly, the combination therapy led to an increased number of macrophages and neutrophils in the primary tumors (173). However, another study reported that LOX knock-down using shRNA reduced the number of CD11b+ cells in breast cancer lung metastases (174).
In late stage murine PDAC, a LOX-inhibitor did not have any effect on survival of the mice but, interestingly, tumors were found to have an increased number of T cells and decreased number of neutrophils (173). It could thus be of interest to further combine this treatment with immunotherapy.

Based on in vitro and in vivo studies, collagen is likely influencing anti-tumor immune responses by directly modulating T cell activity as well as through the regulation of macrophage activity (Figure 2).

THE EFFECTS OF TUMOR COLLAGEN ON IMMUNOTHERAPY

Immunotherapy is a promising new type of cancer therapies using the ability of the body’s own T cells to kill cancer cells. However, a large number of patients do not respond to these treatments. As outlined in this review, collagen could be a novel target for improving the efficacy of immunotherapies such as checkpoint inhibitors (74, 175–180), TIL-based therapy (181), and cancer vaccines (182). The hypothesis that collagen in the tumor microenvironment can affect the outcome of cancer immunotherapy is supported by studies that have identified the serum level of the N-terminal pro-peptide of collagen type III (PRO-C3) as a marker of poor prognosis following anti-CTLA-4 and anti-PD-1 checkpoint inhibitor therapy in metastatic melanoma patients (183, 184). This serum marker is believed to reflect the presence of a highly collagen-dense tumor microenvironment (185).

In lung cancer, a high collagen level has also been shown to correlate with reduced efficacy of anti-PD-1/PD-L1 therapies (175). In mouse models of lung cancer, anti-PD-L1 resistance was shown to be associated with enhanced deposition of collagen, as well as fewer and more exhausted tumor-infiltrating CD8+ T cells. The effect of collagen on CD8+ T cells was mediated by LAIR-1, and combining anti-PD-1 with blockade of LAIR-1 significantly increased the therapeutic efficacy (175). Blockade of LAIR-1 has also been shown to increase the number of tumor-infiltrating CD4+ and CD8+ T cells and enhance the efficacy of anti-PD-L1 treatment in humanized murine xenograft models of several cancers, including colon- and pancreatic cancer (176, 177). In another study, it was shown that reduction of tumor stiffness in mice using a LOX-inhibitor increased the number of tumor-infiltrating T cells and improved the response to anti-PD-1 therapy (74). Reduction of collagen deposition in tumors, has also been obtained through inhibition of focal adhesion kinase (FAK) in murine models of pancreatic cancer (181). In addition to reducing collagen-density in tumors, inhibition of FAK decreased the number of infiltrating anti-inflammatory immune cells such as TAMs and Tregs, and increased the number of CD8+ T cells (181). These changes were associated with significantly improved efficacy of both TIL-based therapy and checkpoint inhibitor therapy (181). Recently, the use of a bacterial-based agent for delivery of collagenase to murine pancreatic tumors was demonstrated (186). This approach led to reduced collagen levels in the tumors and, importantly, also to enhanced efficacy of checkpoint inhibitor treatment (186). The ability of collagen to influence immunotherapy could involve DDR2-signaling since treatment of several murine cancer models with a combination of anti-PD-1 antibody and a DDR2-inhibitor led to an increase in CD8+ T cells and a reduced tumor burden (187). Inhibition of TGF-β has also been shown to reduce collagen levels in tumors (188–190) and to improve checkpoint inhibitor therapy (180, 190). The exact mechanism of action of
this blockade is, however, difficult to delineate because of the multiple roles of TGF-β including direct effects on immune-suppressive cells in the tumor microenvironment (191).

The negative influence of collagen on cancer immunotherapy, was not observed in a study by Elisseff and colleagues where they co-injected B16-F10 melanoma cells and urinary bladder matrix (UBM) scaffolds into mice (192). These scaffolds, which are characterized by high levels of collagen, decreased tumor growth and improved the response to anti-PD-L1 or anti-PD-1 treatment (192). The tumor microenvironment of these UBM-associated tumors was characterized by increased number of CD4+ T cells and NK cells, and fewer Tregs compared to control tumors (192). It should, however, be noted that these UBM-scaffolds do not only consist of collagen but also contain other ECM-components, which could have multiple biological roles.

Collagen can interact with a number of ECM proteins, and consequently the level of collagen in tumors could correlate with the levels of other ECM proteins as well. This situation has been observed for hyaluronan, for which an increasing intratumoral content was accompanied by an increasing collagen level (193). Consequently, it cannot be excluded that immune modulation associated with high collagen levels could also be a consequence of altered levels of other ECM components.

CONCLUSION

The ability of the ECM to influence immune cell behavior constitutes a novel research field, which could be termed matrix immunology. Here we have reviewed the current knowledge about the ability of collagen to directly or indirectly affect T cell activity. The majority of the reviewed studies focus on collagen type I, which is the most abundant of the collagens. It should, however, be noted that other less abundant collagen types could have different effects on the activity of immune cells.

In addition to collagen, the ECM also contains many other components that all potentially could influence the cells in contact with it. Some of these components, such as versican, extracellular matrix protein-1 (ECM1) and hyaluronan, have already been suggested to have direct immune modulatory function (194–196). The current knowledge about the ability of different ECM components including hyaluronan to modulate immune activity has been excellently reviewed by (197). However, for most ECM components it is still unknown if they can influence immune cell activity. The immune modulatory functions of the ECM could influence the development and progression of cancer as well as the outcome of cancer therapies. Consequently, future studies within this field could reveal targets for new cancer therapies. Finally, it should be noted that the importance of the ECM in regulating immune activity extends beyond the cancer research field, since the dysregulation of immune activity is a key feature of multiple other pathological conditions.

AUTHOR CONTRIBUTIONS

AR, M-LT, and DM wrote, edited, and approved the final version of the manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Bonnans C, Chou J, Werb Z. Remodelling the Extracellular Matrix in Development and Disease. Nat Rev Mol Cell Biol (2014) 15:786–801. doi: 10.1038/nrm3904
2. Walimbe T, Panitch A. Proteoglycans in Biomedicine: Resurgence of an Underrated Class of ECM Molecules. Front Pharmacol (2020) 10:1661. doi: 10.3389/fphar.2019.01661
3. Frantz C, Stewart KM, Weaver VM. The Extracellular Matrix at a Glance. J Cell Sci (2010) 123:4195–200. doi: 10.1242/jcs.0283820
4. Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. Molecular Biology of the Cell. 6th Ed. New York: Garland Science (2015).
5. Theocaris AD, Skandalis SS, Gialeli C, Karamanos NK. Extracellular Matrix Structure. Adv Drug Deliv Rev (2016) 97:4–27. doi: 10.1016/j.addr.2015.11.001
6. De Veer O, Demetter P, Mareel M, Bracke M. Stromal Myofibroblasts are Drivers of Invasive Cancer Growth. Int J Cancer (2008) 123:2229–38. doi: 10.1002/ijc.23925
7. Sage H. “Collagen Synthesis by Endothelial Cells in Culture.” In: EA Jaffe, editor. Biology of Endothelial Cells. Boston, MA: Springer US (1984) 161–77. doi: 10.1007/978-1-4613-2825-4_16
8. Tian C, Clauser KR, Øhlund D, Rickelt S, Huang Y, Gupta M, et al. Proteomic Analyses of ECM During Pancreatic Ductal Adenocarcinoma Progression Reveal Different Contributions by Tumor and Stromal Cells. Proc Natl Acad Sci USA (2019) 116:19609–18. doi: 10.1073/pnas.1908626116
9. Naba A, Clauser KR, Hoersch S, Liu H, Carr SA, Hynes RO. The Matrisome: In Silico Definition and In Vivo Characterization by Proteomics of Normal and Tumor Extracellular Matrixes. Mol Cell Proteomics (2012) 11:1–18. doi: 10.1074/mcp.M111.014647
10. Inagaki T, Fujiwara K, Shinozuka Y, Azuma M, Yamazaki R, Mashima K, et al. Perivascular Macrophages Produce Type I Collagen Around Cerebral Small Vessels Under Prolonged Hypertension in Rats. Histochem Cell Biol (2021) 155:503–12. doi: 10.1007/s00418-020-01948-9
11. McKee TJ, Perlman G, Morris M, Komarova SV. Extracellular Matrix Composition of Connective Tissues: A Systematic Review and Meta-Analysis. Sci Rep (2019) 9:1–15. doi: 10.1038/s41598-019-46896-0
12. Xu S, Xu H, Wang W, Li S, Li H, Li T, et al. The Role of Collagen in Cancer: From Bench to Bedside. J Transl Med (2019) 17:309. doi: 10.1186/s12967-019-2058-1
13. Kadler KE, Baldock C, Bella J, Boot-Handford RP. Collagens at a Glance. J Cell Sci (2007) 120:1955–8. doi: 10.1242/jcs.03453
14. Gelse K, Pöschl E, Aigner T. Collagens - Structure, Function, and Biosynthesis. Adv Drug Deliv Rev (2003) 55:1531–46. doi: 10.1016/j.addr.2003.08.022.

15. Di Lullo GA, Sweeney SM, Körkkö J, Ala-Kokko L, San Antonio JD. Mapping the Ligand-Binding Sites and Disease-Associated Mutations on the Most Abundant Protein in the Human, Type I Collagen. J Biol Chem (2002) 277:4223–31. doi: 10.1074/jbc.M110799200.

16. Brodsky B, Persikov AV. Molecular Structure of the Collagen Triple Helix. Adv Protein Chem (2005) 70:301–39. doi: 10.1016/S0065-3233(05)70009-7.

17. Fang M, Yuan J, Peng C, Li Y. Collagen as a Double-Edged Sword in Tumor Progression. Tumour Biol (2014) 35:2871–82. doi: 10.1007/s13277-013-1511-7.

18. Mouw JK, Ou G, Weaver VM. Extracellular Matrix Assembly: A Multiscale Deconstruction. Nat Rev Mol Cell Biol (2014) 15:771–85. doi: 10.1038/nrm3902.

19. Myllyharju J. “Intracellular Post-Translational Modifications of Collagens,” In: J Brinckmann, H Notbohm and PK Müller, editors. Collagens: Primer in Structure, Processing and Assembly. Berlin, Heidelberg:Springer Berlin Heidelberg (2005) 115–47. doi: 10.1007/b103821.

20. Molnar J, Glimcher MJ. Collagen Cross-Linking. Isolation of Cross-Linked Collagen IV. Exp Cell Res (2004) 293:207–10. doi: 10.1016/j.yexcr.2003.06.020.

21. Eyre DR, Glimcher MJ. Collagen Cross-Linking. Peptide Containing the Site of Cleavage of the Chick Skin Collagen (1982) 203:209. doi: 10.1042/bj2030209.

22. Baker AM, Bird D, Lang G, Cox TR, Erler JT. Lysyl Oxidase Enzymatic Function Increases Stiffness to Drive Colorectal Cancer Progression Through FAK. Oncogene (2013) 32:1863–86. doi: 10.1038/onc.2012.202.

23. Song F. Matrix Metalloproteinase Dependent and Independent Collagen Degradation. Front Biosci (2006) 11:3100. doi: 10.2741/2036.

24. Highberger HT, Corbett C, Gross J. Isolation and Characterization of a Peptide Containing the Site of Cleavage of the Chick Skin Collagen (1973) 89:202–8. doi: 10.1006/kexf.19960412.

25. Fields GB. Interstitial Collagen Catabolism. J Biol Chem (2013) 288:8785–93. doi: 10.1074/jbc.R113.451211.

26. McKleroy W, Lee T-H, Atabai K. Always Cleave Up Your Mess: Targeting the Hallmarks of Cancer. EMBO Rep (2015) 17:1. doi: 10.1002/embr.20154304.

27. Jürgensen HJ, van Putten S, Nørregaard KS, Bugge TH, Engelholm LH. Extracellular Collagenases and the Endocytic Receptor, nrm3902.

28. Fan MH, Zhu Q, Li HH, Ra HJ, Majumdar S, Gulick DL, et al. Extracellular Collagenase Activity of FAP Accelerates Collagen Degradation and Clearance Through a Mannose Receptor-Mediated Pathway. J Cell Biol (2013) 202:951–66. doi: 10.1083/jcb.201301081.

29. Wienke D, MacFadyen JR, Isacke CM. Identification and Characterization of the Most Abundant Protein in the Human, Type I Collagen. J Biol Chem (2007) 282:27037–45. doi: 10.1074/jbc.M70108300.

30. Madsen DH, Jürgensen H, Siersbæk MS, Kučzek DE, Grey Cloud L, Liu S, et al. Tumor-Associated Macrophages Derived From Circulating Inflammatory Monocytes Degrade Collagen Through Cellular Uptake. Cell Rep (2017) 21:3662–71. doi: 10.1016/j.celrep.2017.12.011.

31. McKleroy W, Lee T-H, Atabai K. Always Cleave Up Your Mess: Targeting the Hallmarks of Cancer. EMBO Rep (2014) 15:1243–53. doi: 10.1002/embr.201439246.

32. Handorf AM, Zhou Y, Halanski MA, Li WJ. Tissue Stiffness Dictates Development, Homeostasis, and Disease Progression. Organogenesis (2015) 11:1–15. doi: 10.1080/15476278.2015.1019687.

33. Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix Elasticity Directs Stem Cell Lineage Specification. Cell (2006) 126:677–89. doi: 10.1016/j.cell.2006.06.044.

34. Paszek MJ, Zahir N, Johnson KR, Lakins JN, Rozenberg GL, Gefen A, et al. Tensional Homeostasis and the Malignant Phenotype. Cancer Cell (2005) 8:241–54. doi: 10.1016/j.ccr.2005.08.010.

35. Jürgensen HJ, van Putten S, Nørregaard KS, Bugge TH, Engelholm LH, Behrendt N, et al. Cellular Uptake of Collagens and Implications for Cell Migration in Disease. Cell Mol Life Sci (2020) 77:3161–76. doi: 10.1007/s00018-020-03481-3.
54. Provenzano PP, Inman DR, Elceirki KW, Keely PJ. Matrix Density-Induced Mechanoregulation of Breast Cell Phenotype, Signaling and Gene Expression Through a FAK-ERK Linkage. Oncogene (2009) 28:4326–43. doi: 10.1038/onc.2009.299

55. Leight JL, Wozniak MA, Chen S, Lynch ML, Chen CS. Matrix Rigidity Regulates a Switch Between TGF-β1-Induced Apoptosis and Epithelial-Mesenchymal Transition. *Mol Biol Cell* (2012) 23:781–91. doi: 10.1091/mbc.E11-06-0537

56. Morris BA, Burkel B, Ponik SM, Fan J, Condeelis JS, Aguero-Ghiso JA, et al. Collagen Matrix Density Drives the Metabolic Shift in Breast Cancer Cells. *EBioMedicine* (2016) 13:146–56. doi: 10.1016/j.ebiom.2016.10.012

57. Reid SE, Kay EJ, Neilson LJ, Henze A, Serneels J, McGhee EJ, et al. Tumor Matrix Stiffness Promotes Metastatic Tumor Cell Interaction With the Endothelium. *EMBO J* (2017) 36:2373–89. doi: 10.15252/embj.201694912

58. Leu SJ, Lam SCT, Lau LF. Pro-Angiogenic Activities of CYR61 (CCN1) Mediated Through Integrins αvβ3 and αvβ1 in Human Umbilical Vein Endothelial Cells. *J Biol Chem* (2002) 277:46248–55. doi: 10.1074/jbc.M209288200

59. Wei SC, Fattet L, Tsai JH, Guo Y, Pai VH, Majeski HE, et al. Matrix Stiffness Expression Through a FAK-Mediated Collagen Crosslinking Is Responsible for Fibrosis-Enhanced Metastasis. *J Immunol* (2013) 86. doi: 10.1182/ncb3157

60. Shanbhag V, Jasmer-McDonald K, Zhu S, Martin AL, Gudekar N, Khan A, et al. ATP7A Delivers Copper to the Lysyl Oxidase Family of Enzymes and Promotes Tumorigenesis and Metastasis. *Proc Natl Acad Sci USA* (2019) 116:6836–41. doi: 10.1073/pnas.1817473116

61. Cox TR, Bird D, Baker AM, Barker HE, Ho MWY, Lang G, et al. LOX-Mediated Collagen Crosslinking Is Responsible for Fibrosa-Enhanced Metastasis. *Cancer Res* (2013) 73:1721–32. doi: 10.1158/0008-5472.CAN-12-2233

62. Pickup MW, Lakhi H, Acrebi I, Owens P, Gorska AE, Chytli A, et al. Stromally Derived Lysyl Oxidase Promotes Metastasis of Transforming Growth Factor-β-Deficient Mouse Mammary Carcinomas. *Cancer Res* (2013) 73:5356–46. doi: 10.1158/0008-5472.CAN-13-0012

63. Mammino A, Connor KM, Mammino T, Yang CW, Hu D, Aderman CM, et al. A Mechanosensitive Transcriptional Mechanism That Controls Angiogenesis. *Nature* (2009) 457:1103–8. doi: 10.1038/nature07765

64. Calvo F, Ege N, Grande-Garcia A, Hooper S, Jenkins RP, Chaudhry SI, et al. A Mechanosensitive Transcriptional Mechanism That Controls the T Cell Immunological Synapse. *J Exp Med* (2013) 73:3422–32. doi: 10.1016/S1074-7613(00)00327-7

65. Nicolas-Boluda A, Vaquero J, Vimeux L, Guilbert T, Barrin S, Quek L, McEwen-Smith RM, et al. Tumor Stiffening Reversion Through CollagenCrosslinking Inhibition Improves T Cell Migration and Anti-Pd-1 Treatment. *Elife* (2021) 10:1–29. doi: 10.7554/eLife.58688

66. Pentcheva-Hoang T, Corse E, Allison JP. Negative Regulators of T-Cell Motility: From Matrix to Membrane. *Adv Mater* (2019) 31:1–11. doi: 10.1002/adma.201806757

67. Wolf K, Müller B, Bormgarr B, Bröcker EB, Friedl P. Amoeboid Shape Change and Contact Guidance: T-Lymphocyte Crawling Through Fibilar Collagen Is Independent of Matrix Remodeling by MMPs and Other Proteases. *Biosci* (2003) 52:3262–9. doi: 10.1182/blood-2002-12-3791

68. Judokusumo E, Tabdanov E, Kumari S, Dustin ML, Kam LC. Prevailing Role of Contact Guidance in Intrastromal T-Cell Trapping in Human Pancreatic Cancer. *Cancer Cell* (2014) 20:348–3. doi: 10.1016/j.ccr.2013.12.003

69. Peranzoni E, Rivas-Calcedo A, Bougherara H, Salmon H, Donnadieu E. Positive and Negative Influence of the Matrix Architecture on Antitumor Immune Surveillance. *Cell Mol Life Sci* (2013) 70:4431–48. doi: 10.1007/s00018-013-1339-8

70. Hartmann N, Giese NA, Giese T, Poschke I, Wernert J, et al. Prevailing Role of Contact Guidance in Intrastromal T-Cell Trapping in Human Lung and Ovarian Carcinomas Reveals How Different Tumor Microenvironments Control T Lymphocyte Migration. *Front Immunol* (2015) 6:500. doi: 10.3389/fimmu.2015.00500

71. Peran Zhou, Rivas-Calcedo A, Bougherara H, Salmon H, Donnadieu E. Positive and Negative Influence of the Matrix Architecture on Antitumor Immune Surveillance. *Cell Mol Life Sci* (2013) 70:4431–48. doi: 10.1007/s00018-013-1339-8

72. Dustin ML, Choudhari K. Signaling and Polarized Communication Across the T Cell Immunological Synapse. *Annu Rev Cell Dev Biol* (2016) 32:303–25. doi: 10.1146/annurev-cellbio-100814-125330

73. Larsen AMH, Kuczek DE, Kalvisa A, Siersbæk MS, Thorseth M-L, Johansen AZ, et al. Developing Antigen Presentation in Extracellular Matrix: Interactions of T Cells With Dendritic Cells Are Dynamic, Short Lived, and Sequential. *Immunity* (2000) 13:323–32. doi: 10.1016/S1074-7613(00)00327-7

74. Hartmann N, Giese NA, Giese T, Poschke I, Wernert J, et al. Prevailing Role of Contact Guidance in Intrastromal T-Cell Trapping in Human Pancreatic Cancer. *Cancer Cell* (2014) 20:348–3. doi: 10.1016/j.ccr.2013.12.003

75. Hörner M, Raute K, Hummel B, Madl J, Creusen G, Thomas OS, et al. Tumor Stiffening Reversion Through Collagen Crosslinking Inhibition Improves T Cell Migration and Anti-Pd-1 Treatment. *Elife* (2021) 10:1–29. doi: 10.7554/eLife.58688

76. Tabdanov ED, Rodriguez-Mercado NJ, Cartagena-Rivera AX, Puram VV, Callaway MK, Ensinger EA, et al. Engineering T Cells to Enhance 3D Migration Through Structurally and Mechanically Complex Tumor Microenvironments. *Nat Commun* (2021) 12:1–17. doi: 10.1038/s41467-021-21985-5

77. Rømer et al. Collagen Modulates Immune Activity

78. Sadler K, Estrellas K, Allen BW, Wolf MT, Fan H, Tam AJ, et al. Developing a Pro-Regenerative Biomaterial Scaffold Microenvironment Requires T Helper 2 Cells. *Science* (2016) 352:2366–70. doi: 10.1126/science.aad9272

79. Maasho K, Masilamani M, Valas R, Basu S, Coligan JE, Borrego F. The Inhibitory Lymphocyte-Associated Ig-Like Receptor-1 (LAIR-1) Is Expressed in Initial Antigen Presentation.
at High Levels by Human Naive T Cells and Inhibits TCR Mediated Activation. *Mol Immunol* (2005) 42:1521–30. doi: 10.1016/j.molimm.2005.01.004.

92. Meyaard L, Adema GJ, Chang C, Woollatt E, Sutherland GR, Lanier LL, et al. LAIR-1: a Novel Inhibitory Receptor Expressed on Human Mononuclear Leucocytes. *Immunity* (1997) 7:283–90. doi: 10.1016/S1074-7613(00)80530-0.

93. Meyaard L. The Inhibitory Collagen Receptor LAIR-1 (CD305). *J Leukoc Biol* (2008) 83:799–803. doi: 10.1189/jbl.0b0133e318076069.

94. Jansen CA, Cruisen CWA, de Ruiter T, Nanlohy N, Willems N, Janssens-Korpela PL, et al. Regulated Expression of the Inhibitory Receptor LAIR-1 on Human Peripheral T Cells During T Cell Activation and Differentiation. *Eur J Immunol* (2007) 37:914–24. doi: 10.1002/eji.200636678.

95. Lebbink RJ, de Ruiter T, Adelmeijer J, Brenkman AB, Van Helvoort JM, Koch M, et al. Collagens Are Functional, High Affinity Ligands for the Inhibitory Immune Receptor LAIR-1. *J Exp Med* (2006) 203:1419–25. doi: 10.1084/jem.20052554.

96. Rygiel TP, Stolte EH, de Ruiter T, van de Weijer ML, Meyaard L. Tumor-Expressed Collagens Can Modulate Dendritic Cell Function Through the Inhibitory Collagen Receptor LAIR-1. *Mol Immunol* (2011) 49:402–6. doi: 10.1016/j.molimm.2011.09.006.

97. Park JE, Brand DD, Rosloniec EF, Yi AK, Stuart JM, Kang AH, et al. Leukocyte-Associated Immunoglobulin-like Receptor 1 Inhibits T-Cell Signaling by Decreasing Protein Phosphorylation in the T-Cell Signaling Pathway. *J Biol Chem* (2020) 295:2329–47. doi: 10.1074/jbc.RA119.011350.

98. Vogel W, Ghis GD, Alves F, Pawson T. The Discoidin Domain Receptor 1 Inhibits T Cell Migration in Three-Dimensional Collagen. *FASEB J* (2001) 15:1321. doi: 10.1096/fj.00-6626fej.

99. Yeh YC, Lin HH, Tang MJ. Dichotomy of the Function of DDR1 in Cells and Disease Progression. *Biochim Biophys Acta - Mol Cell Res* (2019) 1866:11873. doi: 10.1016/j.bbamar.2019.04.003.

100. Chetoui N, El Azreq MA, Boisvert M, Bergeron MÈ, Aoudjit F. Discoidin Domain Receptor 1 Expression in Activated T Cells Is Regulated by the ERK MAP Kinase Signaling Pathway. *J Cell Biochem* (2011) 112:6676–74. doi: 10.1002/jcb.23300.

101. Alves F, Saupé S, Ledwon M, Schaub F, Hiddemann W, Vogel W. Identification of Two Novel, Kinase-Deficient Variants of Discoidin Receptor 1: Differential Expression in Human Colon Cancer Cell Lines. *FASEB J* (2001) 15:1321–3. doi: 10.1096/fj.00-6626fej.

102. Plcé P, Crisafulli A, Laursen TB, Mecks TD, Vetter P, Samoflack T, et al. Collagen-Binding Integrins, AG, de Fougerolles AR, et al. Expression and Functional Importance of Collagen-Binding Integrins, α1β1 and α2β1, on Virus-Activated T Cells. *J Immunol* (2003) 171:2804. doi: 10.4049/jimmunol.1501688.
129. Chen M, Zhang Y, Zhou P, Liu X, Zhao H, Zhou X, et al. Substrate Stiffness Modulates Bone Marrow-Derived Macrophage Polarization Through NF-kB Signaling Pathway. Bioact Mater (2020) 5:880–90. doi: 10.1016/j.bioactmat.2020.05.004

130. Xing X, Wang Y, Zhang X, Gao X, Li M, Wu S, et al. Matrix Stiffness-Mediated Effects on Macrophages Polarization and Their LOX-L2 Expression. FEBS J (2021) 288:3465–77. doi: 10.1111/febs.15566

131. Liu XQ, Chen XT, Liu ZZ, Gu SS, He LJ, Wang KP, et al. Biomimetic Matrix Stiffness Modulates Hepatocellular Carcinoma Malignant Phenotypes and Macrophage Polarization Through Multiple Modes of Mechanical Feedbacks. ACS Biomater Sci Eng (2020) 6:3994–4004. doi: 10.1021/acsbiomaterials.0c00669

132. Dutta B, Goswami R, Rahaman SO. TRPV4 Plays a Role in Matrix Stiffness-Induced Macrophage Polarization. Front Immunol (2020) 11:5701195. doi: 10.3389/fimmu.2020.5701195

133. Blakney AK, Swartzlander MD, Bryant SJ. The Effects of Substrate Stiffness on the In Vitro Activation of Macrophages and In Vivo Host Response to Poly(Ethylene Glycol)-Based Hydrogels. J BioMed Mater Res A (2012) 100:1375–86. doi: 10.1002/jbm.a.34104

134. Stahl M, Schupp J, Iager B, Schmid M, Ziesel G, Muller-Quernheim J, et al. Lung Collagens Perpetuate Pulmonary Fibrosis via CD204 and M2 Macrophage Activation. PloS One (2013) 8:1–10. doi: 10.1371/journal.pone.0081382

135. Meli VS, Atcha H, Veerasubramanian PK, Nagalla RR, Luu TU, Chen EY, et al. YAP-Mediated Mechanotransduction Tunes the Macrophage Inflammatory Response. Sci Adv (2020) 6:1–14. doi: 10.1126/sciadv.abb8471

136. He C, Yang Z, Jin Y, Qi X, Chu J, Deng X. ADF Scaffolds Generate a Pro-Regenerative Microenvironment During Full-Thickness Cutaneous Wound Healing Through M2 Macrophage Polarization via Lamtor1. Front Physiol (2018) 9:657. doi: 10.3389/fphys.2018.00657

137. van Putten SM, Ploeger DTA, Popa ER, Bank RA. Matrix Stiffness-Induced Macrophage Polarization Throught Multiple Modes of Mechanical Feedbacks. J Exp Med (2016) 213:4004–14. doi: 10.1084/jem.2015.24817

138. Kim YK, Chu S-H, Hsieh JY, Kamoku CM, Tenner AJ, Liu WF, et al. Matrix Stiffness-Induced Macrophage Polarization Is Involved in Antigen Presentation and Activation of Human Dendritic Cells. Blood (2004) 104:1386–95. doi: 10.1182/blood-2004-03-0850

139. Merck E, Gaillard C, Gorman DM, Montero-Julian F, Durand I, Zurawski SM, et al. OSCAR Is an Fcγ-Receptor That Is Expressed by Myeloid Cells and Is Involved in Antigen Presentation and Activation of Human Dendritic Cells. Blood (2004) 104:1386–95. doi: 10.1182/blood-2004-03-0850

140. Jürgensen HJ, Madsen DH, Ingvarsen S, Melander MC, Gärsvold H, Pathy L, et al. A Novel Functional Role of Collagen Glycosylation: Interaction With the Endocytic Collagen Receptor uPAR/Endo180. J Biol Chem (2011) 286:32736–48. doi: 10.1074/jbc.M111.266692

141. Martinez-Pomares L. The Mannose Receptor. J Leukoc Biol (2012) 92:1177–86. doi: 10.1189/jlb.0512231

142. Kuczek DE, Hubbe ML, Madsen DH. "Internalisation of Collagen: An Important Matrix Turnover Pathway in Cancer." In: RA Brekken and D Stupack, editors. Extracellular Matrix in Tumor Biology. Cham: Springer International Publishing (2012), p. 1–18. doi: 10.1007/978-3-319-60972-2_2

143. Schultz HS, Gao L, Keller P, Fleetwood AJ, Sun M, Guo W, et al. OSCAR-Collagen Signaling in Monocytes Plays a Proinflammatory Role and May Contribute to the Pathogenesis of Rheumatoid Arthritis. Eur J Immunol (2016) 46:952–63. doi: 10.1002/eji.201545896

144. van Putten SM, Ploeger DTA, Poepa ER, Bank RA. Macrophage Phenotypes in the Collagen-Induced Foreign Body Reaction in Rats. Acta Biomater (2013) 9:6902–10. doi: 10.1016/j.actbio.2013.01.022

145. Camohara H, Yamashiro S, Galligan C, Yoshimura T. Discoid Domain Receptor 1 Isosform-a (DDR1alpha) Promotes Migration of Leukocytes in Three-Dimensional Collagen Lattices. FASEB J (2001) 15:2724–6. doi: 10.1096/fj.01-0359jfe

146. Matsuyama W, Wang L, Farrar WL, Faure M, Yoshimura T. Activation of Discoid Domain Receptor 1 Isosform B With Collagen Up-Regulates Chemokine Production in Human Macrophages: Role of P38 Mitogen-Activated Protein Kinase and NF-kB. J Immunol (2004) 172:2332–40. doi: 10.4049/jimmunol.172.2.23322

147. Kim SH, Lee S, Suk K, Bakh H, Jun CD, Kim DK, et al. Discoid Domain Receptor 1 Mediates Collagen-Induced Nitric Oxide Production in J774A.1 Murine Macrophages. Free Radic Biol Med (2007) 42:3439–56. doi: 10.1016/j.frerad.2006.10.052

148. Romayor I, Badiola I, Benedicto A, Márquez J, Herrero A, Arteta B, et al. Silencing of Sinusoidal DDR1 Reduces Murine Liver Metastasis by Colon Carcinoma. Sci Rep (2020) 10:1–18. doi: 10.1038/s41598-020-75395-w

149. Hoffmann EJ, Ponik SM. Biomechanical Contributions to Macrophage Activation in the Tumor Microenvironment. Front Oncol (2020) 10:787. doi: 10.3389/fonc.2020.00787

150. Philippaux MM, Bargetzi JP, Pache JC, Robert J, Spiliopoulos A, Maudel J. Culture and Functional Studies of Mouse Macrophages on Native-Like Fibrillar Type I Collagen. Eur J Cell Biol (2009) 88:243–56. doi: 10.1016/j.ejcb.2008.11.002

151. Pakshir P, Alizadehgiashi M, Wong B, Coelho NM, Chen X, Gong Z, et al. Dynamic Fibroblast Constrictions Attract Remote Macrophages in Fibrillar Collagen Matrix. Nat Commun (2019) 10:1–7. doi: 10.1038/s41467-019-09709-6

152. Carvalheiro T, Garcia S, Pascoal Ramos MI, Giovanbone B, Radstake TRD, Marut W, et al. Leukocyte Associated Immunoglobulin Like Receptor 1 Regulation and Function on Monocytes and Dendritic Cells During Inflammation. Front Immunol (2020) 11:1793. doi: 10.3389/fimmu.2020.01793

153. Kim YK, Chu S-H, Hsieh JY, Kamoku CM, Tenner AJ, Liu WF, et al. Incorporation of a Ligand Peptide for Immune Inhibitory Receptor LAIR-1 on Biomedical Surfaces Inhibits Macrophage Inflammatory Responses. Adv Healthc Mater (2017) 6:1–18. doi: 10.1002/adhm.201700707

154. Kerrithivasan S, Šenbabaoglu Y, Martinez-Martin N, Husain B, Verschueren E, Wong A, et al. Homostatic Functions of Monocytes and Intestinal Lungs
167. Chen Y, Kim J, Yang S, Wang H, Wu C-J, Sugimoto H, et al. Type I Collagen Deletion in 

tumor-early Myofibroblasts Augments Immune Suppression and Accelerates Progression of Pancreatic Cancer. Cancer Cell (2021) 39:548–56. 
doi: 10.1016/j.ccell.2021.02.007

168. Afo S, Nair A, Brundu F, Ravichandra A, Bhattacherjee S, Matsuda M, et al. 

Promotion of Cholangiocarcinoma Growth by Diverse Cancer-Associated 

Fibroblast Subpopulations. Cancer Cell (2021) 39:883. doi: 10.1016/j.ccell.2021.05.010

169. Bhattacherjee S, Hamberger F, Ravichandra A, Miller M, Nair A, Afo S, et al. 

Tumor Restriction by Type I Collagen Opposes Tumor-Promoting Effects of Cancer-Associated 

Fibroblasts. J Clin Invest (2021) 131:e146987. 
doi: 10.1172/JCI114698

170. Arnold SA, Rivera LB, Miller AF, Carbon JG, Dineen SP, Xie Y, et al. Lack of 

Host SPARC Enhances Vascular Function and Tumor Spread in an 

Orthotopic Murine Model of Pancreatic Carcinoma. Dis Model Mech (2010) 3:57–72. 
doi: 10.1242/dmm.003228

171. Tian C, Huang Y, Clausen KR, Rickelt S, Lau AN, Carr SA, et al. Suppression of 

Pancreatic Ductal Adenocarcinoma Growth and Metastasis by Fibrillar 

Collagens Produced Selectively by Tumor Cells. Nat Commun (2021) 12:2328. 
doi: 10.1038/s41467-021-22490-9

172. Özdemir BC, Pentcheva-Hoang T, Carstens JL, Zheng X, Wu C-C, Simpson TR, 

et al. Depletion of Carcinoma-Associated Fibroblasts and Fibrosis Induces 

Immunosuppression and Accelerates Pancreas Cancer With Reduced Survival. 

Cancer Cell (2014) 25:19–34. doi: 10.1016/j.cccr.2014.04.005

173. Miller BW, Morton JP, Pines M, Saturno G, Jamieson NB, McGhee E, et al. 

Targeting the LOX / Hypoxia Axis Reverses Many of the Features That Make 

Pancreatic Cancer Deadly: Inhibition of LOX Allografts Metastasis and 

Enhances Drug Efficacy. EMBO Mol Med (2015) 7:1063–76. 
doi: 10.15252/emmm.201404827

174. Erler JT, Bennweth KL, Cox TR, Lang G, Bird D, Koong A, et al. Hyoxia- 

Induced Lysyl Oxidase Is a Critical Mediator of Bone Marrow Cell 

Recruitment to Form the Premetastatic Niche. Cancer Research (2009) 79: 35– 

44. doi: 10.1158/0008-5472

175. Peng DH, Rodriguez BL, Diao L, Chen L, Wang J, Byers LA, et al. Collagen 

Promotes Anti-PD-1/PD-L1 Resistance in Cancer Through LAIR1- 

Dependent CD8 T Cell Exhaustion. Nat Commun (2020) 11:1–18. 
doi: 10.1038/s41467-020-18298-8

176. Xu L, Wang S, Li J, Li B. Cancer Immunotherapy Based on Blocking 

Immunosuppression Mediated by an Immune Modulator LAIR-1. 

Oncoimmunology (2020) 9:1–9. doi: 10.18021/2020.201704477

177. Ramos MIP, Tian L, de Ruiter EJ, Song C, Paucarmaya A, Singh A, et al. 

Cancer Immunotherapy by NC410, a LAIR-2 Fc Protein Blocking LAIR- 

2 Receptor. Mol Cancer Ther (2013) 12:851–60. 
doi: 10.1158/1061-8427

178. Li Z, Zhang Y, Liu Z, Wu Y, Zheng Y, Tao Z, et al. ECR1 Controls T22 Cell 

Egress From Lymph Nodes Through Re-Expression of S1P1. Nat Immunol (2011) 12:178–85. 
doi: 10.1038/ni.1983

179. Siwik DA, Lord JD, Maseda S, Evanko SP, Buckner JH, Wight TN, et al. 

Cutting Edge: High Molecular Weight Hyaluronan Promotes the 

Suppressive Effects of CD4 + CD25 + Regulatory T Cells. J Immunol (2007) 179:744–7. 
doi: 10.4049/jimmunol.179.2.744

180. Gordon-Weeks A, Yuzhalin AE. Cancer Extracellular Matrix Proteins 

Regulate Tumor Immunity. Cancers (Basel) (2020) 12:1–25. 
doi: 10.3390/cancers12113331

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