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Function of cancer cell-derived extracellular matrix in tumor progression

Gao-Feng Xiong[1], Ren Xu[1,2]

[1]*Markey Cancer Center, Lexington, KY 40536, USA.*
[2]*Department of Pharmacology and Nutritional Sciences, University of Kentucky, Lexington, KY 40536, USA.*

Correspondence to: Dr. Ren Xu, Department of Pharmacology and Nutritional Sciences, University of Kentucky, BBSRB, 741 S. Limestone, Lexington, KY 40536, USA. E-mail: ren.xu2010@uky.edu

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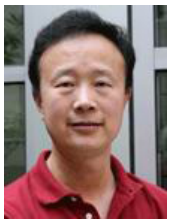

Dr. Ren Xu is an Associated Professor at the Markey Cancer Center, University of Kentucky. Research in his group focuses on the biological function and regulation of ECM microenvironment in normal tissue and cancer development. His recent findings reveal the crucial function of cancer-cell derived-ECM in breast cancer progression.

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ABSTRACT

Extracellular matrix (ECM) is an essential component of the tumor microenvironment. Cancer development and progression are associated with increased ECM deposition and crosslink. The chemical and physical signals elicited from ECM are necessary for cancer cell proliferation and invasion. It is well recognized that stromal cells are a major source of ECM proteins. However, recent studies showed that cancer cells are also an active and important component in ECM remodeling. Cancer cells deposit a significant amount of collagen, fibronectin, and tenascin C (TNC). Recent studies demonstrate that these cancer cell-derived ECM proteins enhance cancer cell survival and promote cancer cell colonization at distant sites. ECM-related enzymes and chaperone proteins, such as prolyl-4-hydroxylase, lysyl-hydroxylase, lysyl oxidase, and heat shock protein 47, are also highly expressed in cancer cells. Inhibition of these enzymes significantly reduces cancer growth, invasion, and metastasis. These factors suggest that the cancer cell-derived ECM is crucial for cancer progression and metastasis. Therefore, targeting these ECM proteins and ECM-related enzymes is a potential strategy for cancer treatment.

Quick Response Code:

INTRODUCTION

Cancer development and progression require extensive reorganization of extracellular matrix.[1,2] Extracellular matrix (ECM) is a complex mixture of structural proteins, glycoproteins, and proteoglycans, which provide not only essential physical scaffolds to maintain tissue structure but also various biochemical signals to modulate cellular function.[3-5] Altering the fine balance of ECM signal is sufficient in the long run to induce breast cancer development and progression. Increased deposition of collagen and other ECM molecules enhances the cancer tissue stiffness.[6-9]

Collagens are the most abundant protein in the ECM.[10,11] Collagen fibril has critical function for tumor cell growth, migration and metastasis.[12-14] Other ECM components, such as hyaluronan, TNC, and periostin (POSTN), are also highly expressed in metastatic tumor and play important roles in tumor metastasis niche.[8,15-18]

Fibroblasts are considered the major source for ECM in both normal and malignant tissue.[19] Surprisingly, recent studies showed that cancer cells also produce a significant quantity of ECM protein during cancer progression.[20,21] Dr. Hynes's laboratory, utilizing an elegant proteomic experiment, demonstrated that ECM molecules in cancer tissue are deposited by both cancer cells and stromal cells.[20,21] ECM proteins, such as laminin 5, hyaluronan, and TNC, are highly expressed in invasive cancer cells.[22-27] Gene expression analysis has identified that ECM protein genes are upregulated in drug-resistant cancer cells.[28] Collagen modification enzymes, including prolyl-4-hydroxylase (P4H), lysyl-hydroxylase (PLOD), and lysyl oxidase (LOX), as well as molecular chaperone heat shock protein 47 (HSP47), are highly expressed in cancer cells and are associated with tumor metastasis.[29-33]

This review summarizes recent findings about ECM microenvironment in solid tumor. The primary focus is on the role of cancer cells in ECM synthesis and the function of cancer cell-derived ECM in tumor progression.

THE EXTRACELLULAR MATRIX

ECM can be classified into two groups: the interstitial matrix and the basement membrane.[34] Basement membranes are thin layers of ECM that form the supporting structure under epithelial and endothelial cells.[35] Basement membrane has a distinctive composition containing type IV collagen, laminins, entactins, and proteoglycans.[7,36] The interstitial matrix, which is primarily produced by stromal cells, fills in the interstitial space between cells. The interstitial matrix is rich in types I, III, V, VI, VII, and XII collagens, as well as proteoglycans and various glycoproteins such as TNC and fibronectin.[37]

Collagen is the most abundant protein *in vivo*. Forty-four collagen genes have been identified in the human genome; they generate at least 28 different types of collagen. From precursor procollagen to final collagen fibril, collagen synthesis process involves several important modification enzymes.[10,38] Proline and lysine hydroxylation are well characterized modifications on procollagen, which are catalyzed by two different enzymes: P4H and PLOD. Collagen P4H catalyzes the formation of 4-hydroxyproline, which is essential to the proper folding of newly synthesized procollagen chains.[39,40] PLOD catalyzes the hydroxylation of lysyl residues in collagen-like peptides, which is critical for the formation of intermolecular crosslinks.[41,42] LOX is enzyme-catalyzing formation of aldehydes from lysine residues in collagen after collagen secretion, which is required for collagen fibril formation.[43,44] HSP47 is a molecular chaperone that promotes maturation of collagen molecules by inhibiting the aggregation of collagen in endoplasmic reticulum (ER).[45-47] The expression of collagen-modification enzymes and molecular chaperone is often associated with increased collagen deposition in cancer tissue.[30-33,48-51] Enhanced enzyme activities are often associated with increased collagen deposition in cancer tissue.

ECM PLAYS IMPORTANT ROLES IN TUMOR PROGRESSION

ECM is a major component of tumor microenvironment and plays critical roles in cancer development and progression. Increased ECM proteins deposition and crosslink provide necessary biochemical and biophysical cues to promote cancer cell proliferation, migration, and invasion.[12,52-54] Laminin-322 is specifically localized in the dense fibrotic zone around invasive ductal carcinoma, providing a specialized microenvironment for guiding tumor invasion.[52] Gamma 2 chain of laminin 5 (laminin 5 γ2) is highly expressed in invasive mammary, colon, melanoma, and sarcoma cancer cells. Laminin 5 plays a role in establishing focal adhesions of cancer cells and contributes to cancer dissemination.[24-26]

ECM molecules, such as POSTN, fibronectin, and hyaluronan, are important components of the metastatic niche.[7] POSTN is a secreted extracellular matrix protein originally identified from mesenchymal cells.[8,16,17] Deletion of POSTN has little effect on normal

Table 1: Stroma cells and cancer cells-derived ECM proteins and ECM regulators

	Stroma cells	References	Cancer cells	References
Collagens	Collagen I	[20,21,66]	Collagen I	[20,21,53,65]
	Collagen II	[20,21]	Collagen II	[20,21]
	Collagen III	[20,21,66,67]	Collagen III	[20,21,53]
	Collagen IV	[20,21]	Collagen IV	[20,21,28,65,68]
	Collagen V	[20,21,53,66,67]	Collagen V	[20,21,53,63]
	Collagen VI	[20,21,66]	Collagen VI	[20,21,28,53,68]
	Collagen VII	[21]	Collagen VII	[20,21,68]
	Collagen X	[20,21,66]	Collagen VIII	[20,53,63]
	Collagen XI	[20,21,66]	Collagen IX	[20,68]
	Collagen XII	[20]	Collagen X	[20,21,53,63]
	Collagen XIV	[20,21,66]	Collagen XI	[20,21,53,63,68]
	Collagen XV	[20,21]	Collagen XII	[20,21,31,63,65]
	Collagen XVI	[20]	Collagen XV	[20,21,65,68]
	Collagen XVIII	[20,21]	Collagen XVI	[20,21,28,65]
	Collagen XVIIII	[21]	Collagen XVIII	[20,21,65]
	Collagen XXIV	[20,21]	Collagen XIX	[20,21]
	Collagen XXVIII	[20]	Collagen XXII	[20,21,63]
			Collagen XXIV	[20,21,68]
Other ECM glycoproteins	Fibrinogen	[20,21]	Laminin α4	[20,21,28,65]
	Dermatopontin	[20,21]	Laminin β1	[20,21,28,65,68]
	Elastin	[20,21]	Laminin β2	[20,68]
	Fibronectin1	[20,21,66]	Laminin γ2	[20,21,66,68]
	Laminin α2	[20,67]	Fibronectin1	[20,21,28,65,68]
	Laminin β2	[20,21]	Elastin	[20,21]
	Nidogen-1	[20,67]	LTBP1	[20,21,68]
	Nidogen-2	[21,66]	LTBP4	[20,21]
	ECM 1	[21]	Nidogen-1	[20,21]
	Fibulin 2	[20,21]	Nidogen-2	[20,21]
	LTBP2	[20,21]	ECM 1	[20,21,28,68]
	Tenascin N	[20]	Peroxidasin	[20,21]
	EMILIN2	[20,21,66]	TINAGL1	[20,21]
	TNC	[20,66,67]	TNC	[20,21,66]
	POSTN	[21,66]	Hyaluronan	[20]
	Hyaluronan	[21]	Thrombospondin-1	[20,21]
	Thrombospondin-1	[20]	SPARC	[20,53,65,68]
	SPARC	[21,66,68]		
	Vitronectin	[20,21]		
Proteoglycan	Asporin	[20,21]	Biglycan	[20,21,28]
	Biglycan	[20,66]	HAPLN1	[20,65]
	Decorin	[20,21,67]	Decorin	[20,21,53,65,68]

Continued...

	Stroma cells	References	Cancer cells	References
ECM regulators	Cathepsin B	[20,21]	Cathepsin B	[20]
	ITIH1	[20,21]	Osteonectin	[20,68]
	ITIH2	[20,21]	P4HA1	[20,21,31,32]
	Plasminogen	[20,21]	PLOD1	[20,21]
	P4HA1	[50]	PLOD2	[20,21,30]
	P4HA2	[50]	PLOD3	[20,21]
	PLOD2	[50]	LOX	[20,21,65]
	PLOD3	[20,21]	LOXL2	[20,21]
	HSP50	[20,21]	LOXL4	[20]
	LOXL1	[21]	HSP50	[20,21,33]
Secret factors	TGFβ1	[20,21,66]	S100-A13	[20]
	S100-A9	[21]	S100-A4	[20,21]
			S100-A6	[20,21]
			TGFβ1	[20,21,65]

ECM1: extracellular matrix protein 1; EMILIN2: elastin microfibril interfacer 2; LTBP1: latent transforming growth factor beta binding protein 1; LTBP2: latent transforming growth factor beta binding protein 2; LTBP4: latent transforming growth factor beta binding protein 4; ITIH1: inter-alpha-trypsin inhibitor heavy chain H1; TINAGL1: tubulointerstitial nephritis antigen-like 1; HAPLN1: hyaluronan and proteoglycan link protein 1

tissue development and primary tumor growth, but it significantly suppresses breast cancer metastasis.[8,17] POSTN promotes cancer stem cell maintenance and lung metastasis by enhancing the WNT signaling pathway.[8,17] Fibronectin, a marker of epithelial-mesenchymal transition, enhances cancer metastasis through Src kinase and extracellular signal-regulated kinase/mitogen-activated protein kinase pathway.[55] Hyaluronan expression is upregulated in breast cancer, lung cancer, pancreatic cancer, melanoma cancer, and myeloma cancer.[22,23,27] Upregulation of hyaluronan is also associated with tumor progression and poor prognosis.[15,56,57] Hyaluronan receptor CD44 promotes survival of disseminated cancer cells during metastasis.[58] TNC is an oligomeric glycoprotein composed of individual polypeptides with molecular weights ranging from 180 kDa to 300 kDa. Expression of TNC in breast tumor is associated with lung metastasis.[8,16,18] Recent studies reveal that TNC is a critical component of metastatic niche and supports survival of disseminated cancer cells at secondary organs.[8,16,18]

Collagen is the major structural ECM protein in tumor tissue. It has been shown that women with dense breasts have a four- to six-fold increased risk of developing breast cancer, and the dense breast correlates with increased collagen deposition and crosslink. In addition, the crosslinked and orientated collagen in cancer tissue is a reliable marker associated with poor survival, regardless of tumor grade and size, tumor subtype, ER or PR status, and node status.[12,59] The abnormal deposition of collagen in tumor stroma promotes cancer progression. Increased collagen VI deposition stimulates cancer cell proliferation.[59-61] Col5A2 and Col11A1 are highly expressed in invasive ductal carcinoma compared to ductal carcinoma in situ. Both of them are involved in triggering cancer cells to disseminate.[62,63]

Collagen production and deposition is regulated by a variety of enzymes, including P4Hs, PLODs, and LOXs. Collagen deposition is regulated by hypoxia in tumor tissue.[47,48,61] Collagen modification enzymes, P4Hs, PLOD, and LOX, are activated by HIF-1α in cancer cells.[27,28,40,48] Expression of collagen P4H is significantly upregulated in breast cancer. Knockdown of P4HA inhibits mammary tumor growth and metastasis to lungs, and decreased P4HA activity depresses cancer cell alignment along collagen fibers.[31,32,50] PLOD2 expression is also associated with increased risk of mortality in breast cancer patient. PLOD2 is critical for breast cancer cell metastasis to lymph nodes and lungs because it increases fibril collagen formation and increases tumor stiffness.[30] In sarcoma cancer, inhibition of PLOD enzymatic activity suppresses metastases.[64] Secretion of LOX by metastatic breast cancer cells is upregulated in metastasis niche. Increased activity of LOX recruits bone marrow-derived cells (BMDCs) to metastasis niche. BMDCs are important in creating a microenvironment for metastatic cancer-cell invasion and growth.[43] Increased LOX expression results in increased ECM stiffening, which is essential for cancer cell expansion.[7] Inhibition

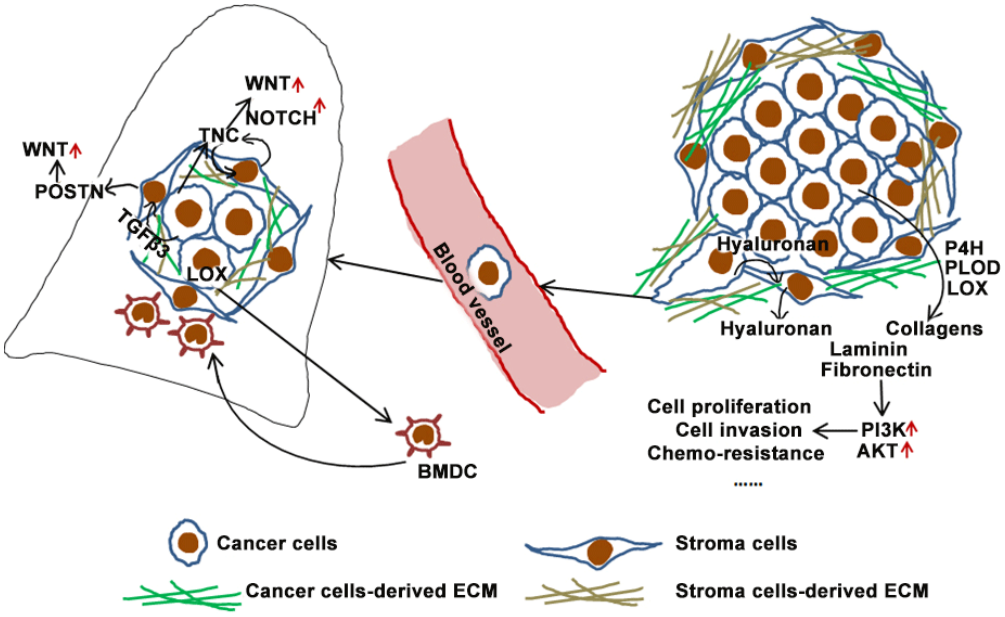

Figure 1: Stroma cell-derived extracellular matrix (ECM) and cancer cell-derived ECM collectively support cancer cell proliferation, invasion, and metastasis. ECM: extracellular matrix; BMDC: bone marrow-derived cells; PLOD: lysyl-hydroxylase; LOX: lysyl oxidase

LOX activation reduces collagen fibril formation and ECM stiffness, which depresses focal adhesions and PI3K activity, and consequently suppresses cancer cell invasion.[54] These results indicate that collagen modification enzymes P4Hs, PLODs, and LOXs play critical roles in cancer cell metastasis.

CANCER CELLS ARE CRITICAL SOURCES OF TUMOR ECM

The cellular components of tumor stroma include fibroblasts, endothelial cells, fat cells, and immune cells. It has been shown that cancer-associated fibroblasts produce and regulate the ECM remodeling in cancer tissue, and the roles of cancer cells in ECM deposition have not been appreciated until recently. Dr. Hynes's laboratory investigated matrisome (ECM and ECM-associated proteins) in colon tumor tissues, lung tumor tissues, and human breast cancer tissue.[20,21] They found that ECM components in tumor matrix are derived from cancer cells and stromal cells, and many of them are only expressed by cancer cells, including Col19A1, Col22A1, Col7A1, LAMA4, LAMB1, LTBP1, LTBP3, LTBP4, TINAGL1, and ECM regulators galectin 1 (LGALS1) and PLOD1.[20,21] Gene expression analysis of drug-resistant breast cancer cells has found that 25 ECM components' genes (including collagen, fibronectin, syndecan, and laminin) and integrin ligands are upregulated in drug-resistant breast cancer cells.[28] Gene expression analysis of drug-resistant ovarian cancer cells also discovered that molecules in ECM networks, including COL3A1, COL5A2, COL15A1, and LOX, among others, are very significantly upregulated.[65] Gene expression profile studies from other labs also reveal that expression of genes involved in synthesis and organization of ECM are upregulated in the epithelium of invasive cancer cells.[53,63,66-68]

LAMC2 (gamma 2 chain gene of laminin 5) is highly expressed in invasive cancer cells in mammary, colon, melanoma and sarcoma tumora.[24-26,69] Hyaluronan synthesis is increased in a variety types of cancer cells, including breast tumor, melanoma tumor, and myeloma tumor.[22,23,27] Thrombospondin-1 is expressed in the stroma and cancer cells.[70] TNC, a key metastatic niche molecule required for the metastasis initiation, is also expressed in breast tumor cells and stroma cells.[8,16,18] Collagens are mainly synthesized by cancer-associated fibroblasts in breast cancer, but cancer cells are also an important source of the collagen.[63] In addition, the expression of collagen synthesis regulating enzymes P4H and PLOD is induced by the HIF-1 pathway in cancer cells.[30,31,51,64] We have summarized ECM proteins and ECM-related enzymes derived from the stroma cells and cancer cells in Table 1. This evidence clearly shows that cancer cells are a major source of tumor ECM.

CANCER CELL-DERIVED ECM IN CANCER PROGRESSION AND METASTASIS

ECM deposited by cancer cells is crucial for cancer progression and metastasis. It has been shown that inhibition of LOX expression in cancer cell represses cell adhesion, migration, and invasion.[29,71] Hyaluronan

deposited by cancer cells promotes cell proliferation, migration, invasion, metastasis, multidrug resistance, and tumor-associated angiogenesis.[15,56,57] TNC that is derived from disseminated tumor cells promotes lung metastasis by enhancing NOTCH and WNT signaling pathways [Figure 1].[8,16,18] In addition, cancer cell-derived ECM proteins (fibronectin, collagen, and laminin) protect cancer cells from chemotherapy-induced apoptosis via activation of the PI3k/AKT pathway [Figure 1].[72,73]

Cancer cell-derived ECM proteins mediate the cancer cell-stromal cell crosstalk. Hyaluronan production by stroma fibroblasts is stimulated by factors secreted by cancer cells.[74,75] Metastatic niche molecule POSTN is secreted by stoma fibroblasts of breast tumor under stimulation from the tumor cells that are produced TGF-β3 [Figure 1].[8,16-18] Cancer cells also remotely recruit stromal cells to create a premetastatic niche before metastasis. Cancer cells-derived TNC initiates cancer cell metastasis, and then it stimulates stroma cell-derived TNC synthesis. Ablation of TNC expression in cancer cells at an early time in the metastatic process inhibits the outgrowth of lung metastases. Interestingly, inhibition TNC expression in cancer cells at a late stage of metastasis does not affect micrometastases expanding to macrometastases, because metastatic cancer cells have already induced TNC expression in stromal cells to promote tumor growth.[8,16,18] These results indicate that cancer cell-derived ECM molecules are critical regulators of the initiation of metastasis outgrowth through activating the stromal cells in the secondary organs [Figure 1].

CONCLUSION

In summary, tumor cells play critical roles in ECM deposition and remodeling during cancer development and progression. Accumulated evidence demonstrates that ECM molecules deposited by cancer cells promote cancer progression by enhancing cell survival and proliferation. However, it largely remains to be determined how cancer cell-derived ECM is regulated and how those ECM proteins function in tumor microenvironment remodeling. Answering those questions is critical for developing potential cancer treatment strategies by targeting the cancer cell-derived ECM and ECM-related enzymes.

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Conflicts of interest

There are no conflicts of interest.

Patient consent

No patient involved.

Ethics approval

This article does not contain any studies with human participants or animals.

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