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Review Article

The extracellular matrix in breast cancer predicts prognosis through composition, splicing, and crosslinking

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ABSTRACT

The extracellular matrix in the healthy breast has an important tumor suppressive role, whereas the abnormal ECM in tumors can promote aggressiveness, and has been linked to breast cancer relapse, survival and resistance to chemotherapy. This review article gives an overview of the elements of the ECM which have been linked to prognosis of breast cancers, including changes in ECM protein composition, splicing, and microstructure.

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Contents

1. Introduction

When metastatic breast cancer cells are mixed with murine mammary epithelial cells and injected into the mammary fat pad, one would expect to observe frank tumors [1]. However, instead of tumors, these cancerous cells incorporate into histologically normal ductal structures, respond appropriately to hormones, and even secrete milk proteins [2]. Furthermore, breast epithelial cells with surprisingly abnormal genomes can be found in histologically normal human breast ducts [3–5].

These studies, and many others, show that the correct context can induce non-malignant behavior, whereas, the abnormal environment in tumors can induce progressive genomic instability and tumorigenesis even in non-malignant cells, both in vitro and in animal models [6–8]. Recent work has linked the ECM in tumors to dormancy [9], resistance to chemotherapy or radiation [10–12], metastasis and metastasis tropism [13] again demonstrating the importance of understanding cell–ECM interactions. It has become apparent from both in vitro and clinical work that the ECM signals to cells through both biochemical and physical means with complex interactions between ECM composition, splicing, microstructure, and biomechanics.

This work gives a survey of the alterations to ECM observed in the progression from healthy breast to breast cancer with special attention to biomechanics. We will focus on data from the breast

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and breast cancer, as cell–matrix interactions have been studied extensively for this organ system and cell culture models of breast development and breast cancer show clear clinical relevance [14,15].

2. ECM in the healthy breast suppresses tumorigenesis

2.1. The basement membrane in the normal breast is a tumor suppressor

The epithelial structures in the breast originate at the nipple, form a branching set of ducts, and end in terminal ductal lobular units, where milk is synthesized. Breast ducts and lobules are bi-layered structures: the inner ring of luminal epithelial cells, which secrete milk during lactation, is surrounded by a ring of myoepithelial cells, which are contractile cells with the ability to secrete and organize ECM proteins. Subtending both these layers of cells is a highly specialized layer of extracellular matrix proteins termed the basement membrane (BM). Myoepithelial cells are lost with malignant progression [16–18] and are believed to play an important tumor suppressive role in the healthy breast due to their ability to secrete the specialized extracellular matrix proteins of the BM [16,19]. Myoepithelial cells surrounding tumors show a shift in ECM protein secretion, losing expression of tumor-suppressive laminins and increasing expression of collagens [16,20].

The basement membrane (BM), a complex, crosslinked layered structure of multiple laminins, collagen IV and other collagens, proteoglycans including perlecan/heparin sulfate proteoglycan nidogen/entactin, and others. Loss of an intact basement membrane is a key stage in malignant progression with high predictive value for prognosis [21], and animal models show that destruction of the BM results in genetic instability and tumorigenesis [7,8].The innermost layer of the basement membrane, at the epithelial cell surface, is a network of laminins [22,23]. In the presence of cell surface ECM receptors, such as dystroglycan, laminin-111 can polymerize into a soft, cohesive network [23,24], which then induces formation of a more structurally stable collagen IV network subtending the Ln-111 network [25], which epithelial cells do not typically contact. These independent networks are then linked by proteins such as fibronectin and nidogens [26], permitting formation of a cohesive mat of proteins.

Among BM proteins, laminin-111 is absolutely necessary for epithelial specific functions in 3D culture assays, including formation of polarity in human breast epithelial cells [16], and induction of milk protein expression (including beta-casein) in murine mammary gland epithelial cells [27]. Furthermore, tumor reversion, or induction of a quiescent phenotype in malignant cells requires laminin and induction of normal cell–ECM signaling [16,28]. Laminin-111/Ln-1 has three head domains which can crosslink into a soft cohesive 3D network, whereas other laminin isoforms with truncated head domains, such as laminin-332/Ln-5, laminin-511/Ln-10 or laminin-521/Ln-11, cannot form a network [22,29], and do not support normal epithelial cell function in vitro, despite the fact that all these isoforms present similar tail domains to cells [16]. Furthermore, some evidence suggests that laminin-332, or collagen IV may support tumor invasion or aggressiveness [30–32].

Both the biomechanics and composition of this laminin network regulate epithelial function: artificially stiffening the laminin network induces epithelial cells to enter an invasive phenotype due to disrupted clustering of β-4 integrin into hemidesmosomes [33] and increased β1 integrin signaling [34,35]. Increasing the density of laminin sites can overcome increased matrix stiffness [33], showing that cells integrate multiple aspects of ECM.

Given the small dimensions of the breast BM (30–50 nm in the human breast [36]), the biomechanical properties of the mammary gland basement membrane itself have never been experimentally determined (though breast stromal tissue has a modulus of 200–400 Pa [35,37]). The BM subtending the retina (which has a similar laminin-rich composition) has a modulus between 1 and 4 MPa, with a difference in matrix biomechanics between its two faces [38], suggesting that despite the thinness of this structure it specializes into sides.

2.2. Stroma

Surrounding the ducts and lobules of the glandular epithelium is the breast stroma, comprised of adipocytes, fibroblasts, and capillaries embedded in a different mix of ECM [39]. The stroma contains blood vessels, adipocytes and fibroblasts embedded in abundant collagen I, chondroitin sulfate and fibronectin [40] (note that the blood vessels have their own laminin-rich BM [41]). Despite their separation by the BM, stroma communicates with epithelia, and stromal changes are observed even in the early stages of malignancy [42]. Stromal ECM plays a major role in tumorigenesis: genetic work from both animal models [8,43] and the clinic [44,45] show that stromal gene expression can alter probablility of developing breast cancer. Importantly, gene expression patterns of normal stroma adjacent to breast cancers shows a different gene expression pattern from normal tissue from unaffected patients [46]. Changes in stromal ECM are observed even in ductal carcinoma in situ (DCIS), where carcinoma cells are confined within an intact basement membrane, including increased deposition of versican [47], loss of decorin [48], and altered expression of Col11A1 [46,49].

Direct contact between stroma and non-malignant epithelia is not observed except during involution [50]. Breast cancers arising during pregnancy and involution tend to be highly aggressive and metastatic [51], suggesting that the collagen-1 rich stromal ECM, along with inflammatory environment observed in lactation and involution, could be pro-tumorigenic [52]. Supporting this, mouse models of BM destruction or stromal collagen I overexpression, which would tend to increase exposure of epithelia to stromal ECM, show increased tumorigenesis [8,53]. Furthermore, non-malignant epithelial cells exposed to increased density of stromal-like collagen I and associated increases in ECM biomechanics undergo transition between formation of normal structures and loss of cell structure and increased growth [35]. The microarchitecture of the fibrilar collagen network (typically collagen I) in the stroma is believed to play a major role in specifying both risk of BC and the stiffness of the stromal ECM [37,50,54,55], suggesting that stiff stroma could encourage tumor initiation or progression. Depending on species, age and testing method, breast interstitial ECM has been measured to have a modulus of 167 ± 31 Pa [35], 0.4 kPa [37] and 1.13 ± 0.78 kPa [55], and the risk of developing BC has been linked to increases in total breast stiffness both clinically [54].

3. ECM in tumors

3.1. Altered ECM and altered cell response

In breast cancers, high levels of fibronectin and its splice variants, crosslinked collagen I, and tenascin-C are associated with poorer survival or time to progression for breast cancer patients, whereas high levels of laminins, high molecular weight hyaluronic acid, heparins, versican, lumican or decorin correlate with better outcomes (summarized in Table 1). While biological mechanisms for some of these links between ECM signatures and prognosis, many open questions remain.

Table 1
Clinical studies linking ECM to breast cancer progression survival.

Methods	Patient cohort	Results	Ref.
Transcriptomic			
RNA microarray for genes which predict metastasis	98 BC All under 55 yrs	231 genes were linked to prognosis including ColIVa2, MMP9	[118]
RNA microarray to distinguish between lobular and ductal cancers	106 IDC, 17 ILC, 6 normal	Among 11 genes differentially expressed between ductal and lobular cancers, Osteopontin/SPP1, expressed more in ductal tumors than lobular, Elastin, TSP1 observed in lobular but not ductal	[119]
mRNA-Seq to determine genes differentially expressed between DCIS and IDC and between normal and DCIS	4 normal, 7 DCIS, 12 IDC	Normal to DCIS did not show changes in ECM genes Between DCIS and IDC, 13% of differentially expressed genes classified as ECM Among 8 top genes differently expressed between DCIS and IDC, 7 were ECM related: COL1A1, SPARC, COL1A2, COL6A1, Lumican, COL3A1, FN1	[95]
mRNA seq of MEP isolated by CD10 MACS, LEP, stroma and immune cells	2 Normal, 2 DCIS 12 IDC,	Most consistent and dramatic gene changes from normal to DCIS to IDC were found in MEP Top 20 differentially expressed in DCIS MEP vs. Normal include: Col1A1, Col3A1, Col6A2, Thbs2, Timp3, CTSF, Decorin, biglycan	[20]
Microarray of unsorted tumor tissue for ECM related genes followed by unbiased clustering into 4 groups. Use of these gene signatures to predict outcome	114 early stage BC 59 NODE+ 74 Hormone Receptor+ 98 Tumor size > 2 cm 44 Grade III	ECM1: Hi in MARCO, ITGAs, PCAM, VECAM, MMP9 ECM2: Hi in PUNC, COL7A1 ECM3: Hi SPARC, FBLNs, COLs ECM4: Hi ADAMs, SERPINAs ECM1: Decreased survival or distant disease free survival, in all samples or luminal type cancers. PUNC and SPARC down and MARCO up: decreased survival ECM2: tumors were characterized by a more heterogeneous expression of ECM-related genes. Most likely to be luminal B, or Her2+ ECM3 tumors showed mainly up-regulation of genes encoding macromolecules involved in the maintenance of connective tissue; in particular, collagens, laminins, fibrillins, and the matrix-associated proteins. No change in overall survival, but predicted poorer outcome in luminal cancers ECM4: had a favorable outcome and was defined by the overexpression of a set of protease inhibitors belonging to the serpin family	[61]
RNA-seq of serum stimulated fibroblasts to determine a wound response gene siguature followed by RNAseq in BC samples	295 BC 10 Node neg 120 Node pos	Increased expression of genes induced by serum predict higher metastasis and lower survival	[120]
mRNA seq of matched laser capture microdissected tumor epithelia, tumor stroma, adjacent epithelia and adjacent normal	14 IDC 14 grade 1-3 IDC, 11 Er+, 5 Her2+, 10 node positive	Tumor stroma massively overexpressed ECM and ECM remodeling genes Enriched in stroma compared to tumor include: ~200 ECM components, 23 collagens, 26 MMPs Top 50 enriched in IDC compared to DCIS include: MMP11, MMP2, MMP14	[121]
mRNA microarray for genes which predict lung metastasis tropism	82 BC	54 gene signature predicted lung metastasis in all patients, ER- patients and poor prognosis patients, but did not predict bone metastasis Among 54 genes predicting lung tropism: COL6A1, MMP1 TNC, MMP2, SPARC	[13]
Previously published datasets were analysed for gene expression patterns in ER- BC	186 ER- and 527 ER+ BC	ECM enriched cluster had medium prognosis 7 gene signature in ER- cancers: High in and low in SPP1/Osteonectin predicted higher rate of death or distant metastasis (HR 2)	[59]
Analysis of genes predictive of tamoxifen resistance in ER+ tumors	112 ER+ primary BC	Among 81 gene signature predictive of tamoxifen resistance: TIMP3, FN1, LOX, Col1A!, SPARC, TNC	[122]
PCR for COL1A1, FN1, LOX, SPARC, TIMP3, and TNC	1286 primary BC 680 Node neg 73.3% ER+	All 6 ECM genes were correlated with each other FN, LOX, SPARC all were correlated with poorer survival Col1A1 hi: better histologic grade, smaller tumors, ErbB2 positive and EGFR hi FN1 hi: Small tumors, more lymph node involvement, ErbB2 positive and EGFR hi LOX hi: More	[86]
RNA seq in previously published dataset, along with array for differences between normal and BC	24 IBC, 38 ipsilateral normal, 3 contralateral normal, 28 normal	46 genes which distinguish normal from BC Cancers compared to normal include: Up: Col1A1, Col1A2, Col3A1, Col5A1, Col5A2, Col6A3, Col8A1, MMP 1, 10, 11, 12, 13 Down: CD44, MMP2 Luminal/Lobular include: CTGF, MMP2, AR, CFB, CD44, CDKN1B, ETAA1, FGFR2, TNFRSR10B Basal tumor specific include: MMP10, MMP12, SPP1/osteopontin	[81]

Table 1 (*continued*)

Methods	Patient cohort	Results	Ref.
nRNA seq of genes differentially expressed between DCIS and IBC	126 DCIS and IDC along with analysis of previously published work	Top 25 genes overexpressed in IDC compared to DCIS include: Col1A2, MMP11, Col11A1, Thbs2, Col6A3, POSTN, Col3A1, SPARC, Col5A2, Col8A1, Lumican, FN, Col5A1 Most differentially expressed gene classes: ECM and ECM organization	[82]
Microdissection of stroma and epithelial compartments for matched adjacent normal, DCIS and IDC, followed by whole genome sequencing	17 matched IDC and DCIS Of which, 13 ER+, 3 Her2+, 3 TNBC	Different between DCIS and IDC epithelia:. COL17A, COL5A2, COL22A1, COL8A1, COL12A1, COL10A1, COL11A1, MMP13, GPC6, KLK5, FREM1 Different between DCIS and IDC in both epithelia and stroma: COL11A1 Adjacent normal and normal tissue from unaffected patients do not cluster	[46]
RNA microarray to distinguish matched DCIS and IDC in concurrent cases	20 matched DCIS and IDC+(unmatched) 7 DCIS, 8 normal	Among genes 56 upregulated in IDC vs. DCIS: COL11A1, COL10A1, FN1, COL12A1, MMP13, THBS2, SPARC, LRRC15, ASPN, and MMP11, and MMP14	[123]
Multiple targets			
Laser capture microdissection of epithelial cells and LC/ mass spec for proteins differentially expressed between normal epithelia and ER+ BC	9 normal and 9 ER+PR+ IBC: 8 IDC, 1 ILC, grades 1-3, 1 Her2+	Among 1623 differentially expressed proteins Overexpressed in normal: LamA3, LamB2, LamC1, Col1A1, fibrinogen A	[124]
HC for TN, CD, Coll, LN,FN, CD44s and Ki-67 and correlation with survival	138 BC	Survival analysis showed an increased mortality risk associated with high levels of TN expression TN expression in the tumor stroma was positively correlated with tumor grade and size, CD44s expression, tumor and stromal CD expression as well as with FN, laminin and Coll expression in the same areas Among the ECM proteins, only TN expression was independently correlated with patients' survival	[87]
IHC for TN, versican, CS, HA and correlation with survival and relapse	86 node negative IDC, 54 ER+	Versican correlated with CS, TN correlated with HA in stroma. High TN tend to be high grade, large tumors, HA high tended to be low grade tumors Versican predicted relapse free survival	[125]
Laminins			
IHC and ISH for LN A5, B1 and B2 chains	3 normal, 18 IDC, grade 2 or 3, and 3 metastases	LN A5 observed in vascular tissue and BM of all epithelia LN A5 observed in cytoplasm of all tumors LNB1 and B2 observed in IDC only	[126]
PCR for LAMA3, LAMC2 and others in the tumor, invasive front and adjacent normal	4 DCIS and 7 IDC	LAMA3 and LAMC2, and ITGA6 and ITGB4 overexpressed in invasive zone compared to main tumor or adjacent normal, but not LAMA2, LAMB1, LAMC1 or SPARC	[30]
IHC for LAMB3 in cytoplasm of epithelia and comparison between TNBC and non-TNBC	243 BC, 80 TNBC	LAMB3 staining in cytoplasm observed in 70% of TNBC and only 15% of non-TNBC	[127]
IHC for human laminin	18 fibroadenomas 22 cases of fibrocystic disease, 96 IDC and 26 DCIS	Continuous staining for laminin observed in benign samples, 77% of DCIS showed continuous laminin staining and disrupted laminin staining observed in IDC. Small-sized tumors, those without lymphatic invasion and lymph node-negative tumors showed more complete patterns of laminin expression	[128]
IHC for LAMB3 LAMC2	25 BC patients 7 squamous, 4 sarcomatous, 8 chondroid, 1 fibromatosislike metaplastic carcinomas, and 5 cases with 2 metaplastic components	Both LAMB3 and LAMC2 observed in 96% of tumors. All ER- or TNBC expressed LAMB3 and LAMC2 where only 15% of high grade tumors expressed LAMB3 or LAMC2	[129]
IHC for laminin 5	55 patients	FGF-2 and laminin 5 expression were found throughout benign and atypical dedifferentiation in mammary tissue samples and were lost primarily with transformation to invasive cancer	[130]
Fibronectin			
ISH for total FN, ED-A and ED-B fibronectins	36 IDC and 13 benign lesions	FN and ED-A and ED-B expression observed in tumors, almost absent in benign lesions	[131]
IHC for β1 integrin, fibronectin and laminin-111	249 IBC	HR for increased grade: β1 integrin 1.43, FN staining: 1.03, Ln-111 staining 0.95	[132]
IHC for FN in epithelia (E-FN) and stroma (S-FN)	1596 invasive breast cancer samples	Epithelial FN associated with increased tumor grade and lymph node positivity, ER/PR- and Her2+ and worse overall survival and disease free progression E-FN predicted survival especially in ER/PR+ group S-FN did not predict survival	[64]

Table 1 (*continued*)

Methods	Patient cohort	Results	Ref.
Collagens			
MPM for aligned fibrillar collagens and correlation with prognosis	196 samples	Aligned collagen predicted poor survival, with a HR between 3.0 and 3.9, independently of tumor grade, nodal status or size, or ER, PR or HER2 status	[98]
Microarray for prolyl 4 hydroxylase 1 and 2	TCGA dataset	Higher prolyl4 hydroxylase 1 and 2 in cancer compared to adjacent, higher expression of either = > decreased patient survival	[133]
Microarray for Prolyl 4 hydroxylase 2, and Col1A1, Col3A1, and Col4A1	Previously published datasets	P4HA2 tends to be higher with increasing grade, higher in IDC than ILC, higher in ERBB2+ cancers, and P4H2 high predicts worse survival in all, ER+ and ER− cancers (HR 1.4)	[134]
Microarray for Prolyl 4 hydroxylase 2 and Col1A1, Col2A1 and Col4A1	Large microarray dataset (~2000 IBC and IDBC)	Higher expression of P4H2 correlated with higher collagen expression, higher clinical stage, Her2 positivity. Higher P4H2 expression predicted worse survival in all, ER+ and ER- populations	[134]
LS–MS for type XIV collagen	20 patients half with multiple LN+, half with LN−	type XIV collagen expression was predictive of lymph node metastasis along with type I collagen, MSTP161/ proline/arginine-rich end leucine-rich repeat protein (PRELP) or prolargin, angiomotin and hexokinase type I	[135]
IHC for Col11A1	201 core needle biopsies, 87 IDC, 14 ILC, 19 DCIS, 6 LCIS, 30 fibroadenoma,	Higher Col11A1 observed near tumor expansion areas. High Col11A1 predicted infiltration (sensitivity.93, specificity.97)	[49]
Tenascin			
IF for tenascin-C and correlation with lung metastatic potential	39 BC	High Tenascin predicted shorter lung metastasis free survival	[136]
IHC, ISH for all TN, truncated TN, TN extra exon 16 and TN extra exons 14–16	15 benign 5 fibroadenomas 13 DCIS, and 35 carcinomas (10 grade I, 9 grade II, and 9 grade III)	Although all tissues expressed the fully truncated TN, exon 16 (TN16) and exons 14 and 16 (TN14/16), associated with invasive phenotype	[76]
rtPCR for TNC and TN additional domain 1 and additional domain 2 isoforms	155 IDC, 62 IDC, 25 ILC, 14 benign, 33 normal 68 > 40 yrs, 62 idc < 40 YR, 25 ILC < 40 yr	Extra domain TNCs observed more often in younger women, and AD1 associated with ER− and high grade tumors	[137]
Hyaluronic Acid, heparin and versican			
IHC for syndecan-1	63 triple positive IDC and 61 matched adjacent normal	Triple positive IDC tended to express high syndecan-1. High syndecan-1 predicted low invasiveness and low grade	[138]
IHC for syndecan-1	72 patients, mostly DC, grade ii–iii	Syndecan-1 medium tumors tended to be ER+. Syndecan high tended to be ER-. High levels of syndecan-1 in epithelia predict poor survival	[139]
IS for Versican, CS, TN, HA	86 patients with node negative BC	High versican predicted shorter relapse free survival. Versican high tumors tended to be ER+ and PR+ Tenascin high samples tended to be larger and higher grade, HA in stroma predicted higher grade, HA in cancer cells predicted higher grade	[125]
IHC for versican	9 LCIS, mixed, and 28 DCIS	versican is strongly expressed in the stroma of some DCIS, and versican stromal staining predicts high grade category and comedo pattern	[47]
IHC for Versican	58 node negative BC	Decreased relapse free survival (HR 6.35)	[41]
IHC for HA	143 primary, invasive BC 91% ER+	HA positive cells correlated with lymph node positivity, low grade, more likely to be ER and PR negative, decreased survival	[140]
IHC for HA and CD44 (HA receptor)	156 IBC, 89 HER2−, 67 HER2+	High HA more likely to be seen in HER2 positive cases, High HA correlates with larger tumor size, lymph node positivity, ER−, PR− and poor differentiation	[141]
WB for lumican, decorin, ER and PR	140 node negative IBC	Low lumican associated with low ER and PR, and larger tumor size. Low Lumican and decorin both associated with with poorer survival Recurrence free survival HR: ER 6.1, PR 4.02, decorin 2.25, Overall survival HR: PR 12.28, ER 2.86, decorin 3.39	[142]
WB for lumican, decorin and PCR for lumican and decorin mRNA	15 BC	lumican was highly abundant relative to decorin, while biglycan and fibromodulin are only detected occasionally lumican mRNA was increased in tumors while decorin mRNA was decreased in neoplastic relative to adjacent normal stroma along with an increase in lumican, but not decorin	[143]
IHC for decorin	98 samples IBC and 22 were from patients with DCIS	Decorin was observed in stroma but not epithelia. Average decorin expression decreased from normal to DCIS to IDC	[48]
IHC for Syndecan 1,4, and Glypican 1	207 BC	Glypican-1 detected in a small sample of BC Higher level of Syndecan 1 and -4 predicted higher	[144]

Table 1 (*continued*)

Methods	Patient cohort	Results	Ref.
		size, grad, Ki67 and Er− status. Syndecan 1 hi also predicted lymph node positivity and worse survival	
Northern blot for glypican 1,2,3,4 and syndecan -1	20 BC with adjacent normal control, mostly stage 1	Increased Glypican-1, 3 and Syndecan-1 expression in cancers relative to adjacent normal	[145]
IHC for Heparanase-1	80 normal to metastatic BC samples	Heparanase-1 expression correlates with worse clinical grade	[146]
IHC for Heparanase and COX2	246 breast tumor samples	*overexpression of HPA and COX-2 was associated with increased likelihood of lymph node positivity in large, high-grade tumors*	[147]
SPARC/ OSTEONECTIN			
Microarray for 7 genes which correlate with SPARC	1729 systemically untreated patients from previously published datasets	SPARC tends to be highest in luminal-A type, followed by luminal-B followed by basal High SPARC predicts worse survival in HER2+, and basal cancers	[63]

3.2. Breast cancer subtypes

Some of the link between ECM and prognosis may relate to the phenotype or subtypes which associate with certain ECM configurations and the known differences in prognosis associated with these subtypes. Breast cancer is not a single disease [38]: breast cancer prognosis varies with expression of key hormone receptors: estrogen receptor (ER), progesterone receptor (PR) and her2/neu/ERbB2 (Her2) [56] histologic grade [57], and presence of metastasis. Recent evidence suggests that the microenvironment in these tumors is different [58,59].

For example, triple negative tumors which display mutant p53, typically have very poor survival [60]. These tumors tend to show complete loss of laminin and high levels of vascularization, which may explain some of the linkage between this form of ECM and poor outcome [61]. Stiff tumors, measured with ultrasound elastography, tend to be the most aggressive types, including triple negative or Her2+ tumors [62]. High levels of hyaluronic acid appears linked to HER2+ cancers, which tend to be more aggressive [61]. Patients with lumican and decorin polymorphisms appear to develop more ER+ cancers [45], which usually have better prognosis.

Similarly, some ECM signatures may only predict prognosis in certain subtypes: for example, SPARC mRNA level, though highest in luminal A tumors, predicts prognosis in basal and Her2+ tumors, but not in luminal types [63], whereas fibronectin, highest in TNBC/basal or Her2+ tumors, has the highest prognostic value in hormone positive cancers [64]. Alternatively, in vitro work has suggested that ECM can alter expression subtype markers, further confounding the link to prognosis [65,66] As a result, studies with different clinical samples can show very different results, and caution is needed in interpreting findings.

3.3. ECM protein splicing/structure changes

An estimated 75% of proteins have alternative splice forms [67], and changes to the spliceosome are observed with the progression from normalcy to malignancy [67,68]. Unsurprisingly, the ECM proteins with alternative splice forms are often observed to undergo isotype switching during development of cancer, though microenvironmental factors can normalize splicing in malignant cells [69]. The ED-A and ED-B fibronectin splice forms, i.e. the oncofetal splice variants or proangiogenic isoforms [70,71], display the integrin binding RGD domain differently, [72], and may show different assembly into fibrils [73]. Notably these variants are not found in soluble plasma fibrinogen, suggesting that these forms are more likely to polymerize [74]. Malignant cells express much higher levels of ED-A fibronectin and its receptor, $\alpha5\beta1$ integrin, both of which have been linked to radiation resistance [75]. Likewise, tenascin-c also has multiple alternative splice forms, which are not observed in normal adult breast tissue [76]. In both patient samples and cell culture models, these splice forms have been linked to invasiveness, potentially through MMP-based mechanisms [76,77].

Furthermore, the abberant ECM in tumors can alter fibronectin splicing even in non-malignant cells, whereas normalization of cell–matrix interactions in malignant cells normalizes fibronectin splicing[75]. Increased tissue stiffness appears to induce global changes in splicing, driving increased expression of ED-B fibronectin [78]. High levels of glucose in media likewise appear to alter expression of ED-B fibronectin splicing along with increasing total levels of fibronectin [79].

3.4. Microstructure, biomechanics and crosslinking

Among ECM components, fibrillar collagen I is believed to be the major determinant of breast and breast cancer stiffness [53], and has been proposed as a link between increased mammographic breast density (a well-known breast cancer risk factor) and increased risk of breast cancer [80]. Mouse models of increased collagen deposition confirm that increased collagen density likewise regulates breast cancer susceptibility [53]. Furthermore, increased expression of fibrillar collagens is observed in invasive breast cancers compared to normal or to DCIS [81,82] While collagen I is only one of many breast ECM components with cell regulatory effects, it represents the best characterized model for studying microstructure and its effects on cells.

The diverse microarchitectures of fibrillar collagen and the range of resulting biomechanics can result in very different microenvironments despite equivalent levels of collagen [83–85], which potentially explains the weak clinical link between collagen I and prognosis in older studies [86,87]. It remains difficult to decouple the effects of fiber diameter, pore size and biomechanics, but it appears that all three act on cells (reviewed in [88]). For example, crosslinking and crosslinking density each affect cell invasion, with a significant interaction, such that crosslinking loose ECM environments increased invasiveness, whereas crosslinking dense ECM decreased invasiveness [89].

Due to altered ECM microstructure and crosslinking, the increased stiffness of breast tumors is so different from the surrounding tissue that manual palpation remains an important diagnostic [90]. Tumor stiffness, assessed with ultrasound elastography or atomic force microscopy appears to increase with increasing grade [91] and predicts poorer prognosis [92].

Furthermore, successful chemotherapy decreases tumor stiffness whereas stiffening is observed in chemotherapy-resistant tumors stiffen after treatment [93].

In contrast, softer collagen I rich matrices appear to reduce breast cancer risk and progression. Parity appears to increase collagen I deposition, but in an unorganized, uncrosslinked form, resulting in softer ECM and reduced risk of breast cancers [83]. Collagen III disrupts formation of dense, organized collagen I networks, resulting in softer ECM [94]. Loss of collagen III in mouse models is associated with tumor aggressiveness [94], though Col III is often observed to be overexpressed with increasing tumor grade[20,81,82,95]. Furthermore, reductions in collagen I density via TGFβ blockade likewise suggest that altering collagen network structure is a potential therapeutic target [96].

A dense network of collagen fibers perpendicular to tumor border predicts invasiveness and poorer overall survival [97,98]. In mesenchymal cells, organization of fibriliar matrices is the major determinant of migration patterns, such that cell migration persistence tends to be highest in aligned matrices [97,99–101]. Both non-malignant and malignant epithelial cells respond to the biomechanics of their surrounding matrix [35], through multiple mechanisms [6,33,35,102]. Lysyl oxidases, by crosslinking collagens increase tumor stiffness [103], and predict prognosis [86].

3.5. ECM and chemotherapy

Resistance to chemotherapy has been linked to features of ECM through several avenues. In ER-breast cancers, gene expression patterns typical of reactive stroma predicted resistance to chemotherapy, though no link was found between this signature and outcome in untreated patients [10]. Similarly, tumor stiffness measured with ultrasound elastography predicted residual tumor burden after chemotherapy [104–106]. Interestingly, clinical response to chemotherapy involved a softening of the tumor site, whereas resistant tumors became stiffer after treatment [93]. Among elements of reactive stroma, increased ECM stiffness predicted resistance in vitro to the broad spectrum tyrosine kinase inhibitor sorafenib [107].

Integrins and many of the tyrosine kinase receptors (such as EGFR [108], ErbB2, VEGFRs, HFGRs, etc.) are known to cross regulate each other in stiff environments, potentiating both integrin signaling and often increasing receptor potency [107,109–111]. Her2 is acutely regulated by FAK, such that ECM stiffening is a powerful regulator of Her2 and of Her2 resistance [65,110,112]. Adhesion to laminin-332 through a6b4 has also been linked to trastuzumab resistance through the transmembrane protein CD151 [113]. We would argue that further work is urgently needed to understand the role of ECM in mediating chemotherapy resistance.

4. Conclusions how does ECM remodeling keep healthy tissues healthy and how did tumors get that way?

The natural history of breast cancer still remains poorly understood, and may differ by cell of origin [114,115], subtype or yet unknown factors. However, a preponderance of evidence now shows that the insoluble proteins comprising the extracellular matrix (ECM) can suppress tumor development and progression, whereas the abnormal ECM in tumors can promote progression of cancers and resistance to treatment. The diverse mechanisms by which cells sense and respond to their surrounding ECM represent an attractive target for new therapeutics for cancers [116]. However, the dramatic failure in clinical trials of one such class of treatment, namely MMP inhibitors [117] highlights the need for improved models and improved understanding of cell–ECM interactions. Many open questions remain about the complex interactions between ECM proteins, microstructure and biomechanics.

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