Role of extracellular matrix in breast cancer development: a brief update [version 2; peer review: 2 approved, 1 not approved]

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Abstract
Evidence is increasing on the crucial role of the extracellular matrix (ECM) in breast cancer progression, invasion and metastasis with almost all mortality cases owing to metastasis. The epithelial-mesenchymal transition is the first signal of metastasis involving different transcription factors such as Snail, TWIST, and ZEB1. ECM remodeling is a major event promoting cancer invasion and metastasis; where matrix metalloproteinases (MMPs) such as MMP-2, -9, -11, and -14 play vital roles degrading the matrix proteins for cancer spread. The β-D mannuronic acid (MMP inhibitor) has anti-metastatic properties through inhibition of MMP-2, and -9 and could be a potential therapeutical agent. Besides the MMPs, the enzymes such as LOXL2, LOXL4, procollagen lysyl hydroxylase-2, and heparanase also regulate breast cancer progression. The important ECM proteins like integrins (b1-, b5-, and b6- integrins), ECM1 protein, and Hic-5 protein are also actively involved in breast cancer development. The stromal cells such as tumor-associated macrophages (TAMs), cancer-associated fibroblasts (CAFs), and adipocytes also contribute in tumor development through different processes. The TAMs become proangiogenic through secretion of VEGF-A and building vessel network for nourishment and invasion of the tumor mass. The latest developments of ECM involvement in breast cancer progression has been discussed in this review and this study will help researchers in designing future work on breast cancer pathogenesis and developing therapy targeted to the ECM components.

Keywords
Extracellular matrix, breast cancer, metastasis, matrix metalloproteinases
Introduction
Breast cancer (BC) accounts for 25% of all cancer cases in women, and 12% of overall cancer cases worldwide. The extracellular matrix (ECM) plays a crucial role in BC progression, invasion, and metastasis; thus, elucidating the role of ECM will help in designing therapies targeting different ECM components. Comprehensive studies are currently going on related to the involvement of ECM in BC progression, and this review focuses on the latest developments in this regard with possible molecular targets for therapies.

The ECM (includes basement membrane (BM) and stroma) interacts with the cells mediated by ECM receptors like integrins, discoidin domain receptor, syndecans, CD44, dystroglycans, and Rhamm. The BM is mainly composed of laminins (laminin-111 is involved in milk protein synthesis and secretion), type IV collagen, entactin, and proteoglycans. The stromal cells, adipocytes, and immune cells produce many ECM proteins like type I, II, and III fibrillar collagens, fibronectin, vitronectin, elastin etc. and the stroma is highly charged and hydrated, providing tensile strength to tissues. It is observed fibrillar collagen I guides epithelial cell branching during the mammary gland development and macrophages are involved in this process of long fibre organization, required for branching morphogenesis (Rac1 acts as a modulator of collagen I orientation). Deregulation of the ECM dynamics is a hallmark of cancer. The ECM remodeling enzymes are deregulated changing the basic properties of ECM. There is more deposition of collagens (COL I, II, III, V, IX), and overproduction of ECM components like heparin sulphate proteoglycans and CD44 which promote growth factor signaling in cancerous cells. The ECM of cancerous tissue differs from that of normal tissue in following manners: the stroma of cancerous tissue seems to be stiffer than that of normal one; the COL I fibrils in BC tissue are highly linearized, and properly oriented, whereas relaxed nonoriented fibrils are observed in normal breast tissue; many MMPs are overproduced in cancerous tissues. In the BC development the collagen I stiffens the ECM, thus promoting the tumor invasion and metastasis; whereas the BM prevents invasion by acting as a barrier.

Epithelial-mesenchymal transition (EMT)
The EMT (process of losing epithelial characteristics and gaining mesenchymal properties) plays a significant role in the progression of tumor and metastasis, involving different transcription factors (TFs) and signals. This process is characterized by loss of E-cadherin (cell-cell adhesion molecule) and cytokeratins, along with gain of N-cadherin, fibronectin, and vimentin (mesenchymal cell associated proteins) and this is termed cadherin switching (E → cadherin to N → cadherin). The EMT is regulated by different signaling pathways such as TGF-β, notch, and wnt pathway. All these pathways converge to activate the EMT – specific TFs such as Snail (SNAI 1), slug (SNAI 2), Zeb, and Twist which differentially express in cancerous cells to promote EMT. Snail is a transcriptional repressor of E-cadherin (cell-cell adhesion molecule), and E-cadherin loss is a hallmark of EMT. Snail and TWIST cooperate inducing another TF, ZEB1 (significant inducer of EMT, invasion, and metastasis), which is triggered by extracellular hyaluronic acid (HA). Furthermore, ZEB1 induces HAS2 synthesis, promoting HA production in a positive feedback loop and its expression is correlated with ZEB1 expression in poor prognosis tumors. HAS2 also has a role in TGF-β-induced EMT. Platelets and platelet-derived TGF-β promote epithelial-mesenchymal-like transition and promote metastasis in vivo. Both the canonical (Smad dependent pathway) and noncanonical (Smad independent pathway) TGF-β signaling activate the TFs (Snail, Zeb, Twist, and Six1) responsible for EMT. The TGF-β induced EMT might be facilitated through enhanced expression of PARP3 (Poly ADP-Ribose Polymerase 3) protein which promotes cell motility, and chemoresistance in breast epithelial cells. The PARP3 seems to promote stemness of cancerous cells by inducing stem cell markers SOX2 and OCT4, and increasing the population of CD44+/CD24- tumor initiating cells. The notch signaling induces the TFs (Snail, Slug, Twist, and Zeb1/Zeb2) by acting through NF-kB, promoting cytokine production and cell survival. The wnt pathway induces Snail, thus down regulates E-cadherin via β-catenin. Besides these signaling, the hypoxic microenvironment also changes the function of mitochondria leading to HIF1 stimulation and subsequently increased expression of Zeb1 required for EMT. The inflammatory factors such as TNF-α and IL-1β showed to induce plasticity in nontransformed breast epithelial cells (surrounding the transformed tumor cells) by initiating EMT through Snail and Zeb1. Apart from this, the steroid nuclear receptors such as estrogen receptors, progesterone receptors, glucocorticoid receptors, and mineralocorticoid receptors are also observed to regulate the expression of TFs inducing EMT. A calcium-dependent phospholipid binding protein such as annexin A2 likely promotes EMT through activation of EGF/EGFR pathway. It is also observed annexin A2 directly binds to STAT3, which is a key EMT inducer up regulating the expression of TFs for EMT. Aberrant cancer metabolism promotes EMT which further aggravates metabolism (especially glucose metabolism) through Snail and Twist. A calcium-dependent phospholipid binding protein such as annexin A2 likely promotes EMT through activation of EGF/EGFR pathway. One of the TF Runx1 (highly expressed in epithelium) is found to stabilize the mammary epithelial cell (MEC) phenotype thus prevents the EMT. It is observed EMT is activated by ECM stiffness, which induces the release of TWIST1 from its anchor G3BP2 and this TF enters the nucleus and transcriptionally boost the EMT process through integrin clustering and activation.

The cancer cells need to overcome anoikis (apoptosis due to loss of attachment to ECM) for metastasis event as this is a crucial barrier preventing tumor cell migration to secondary sites. Induction of anoikis occurs through lysosome – mediated down
regulation of epidermal growth factor receptors (EGFRs) resulting in the termination of prosurvival signaling. It is observed the depletion of one of the kinases pre-mRNA splicing factor 4 kinase (PRP4K) promotes increased resistance to anoikis through reduced EGFR degradation (after cell detachment from ECM) with increased level of TrkB, vimentin, and ZEB1. Anoikis is evaded in ErbB2 expressing cells by multacellular aggregation during ECM detachment. EGFRs are stabilized by this aggregation, which results in ERK/MAPK survival signaling. EGFRs could be the therapeutic targets to eliminate the ECM-detached cancer cells.

**Enzymes in ECM remodeling**

Various ECM-remodeling enzymes are induced in BC promoting stem/progenitor signaling pathways and metastasis. The different pathways that are regulated by ECM during remodeling in BC development, are wnt, PI3K/AKT, ERK, JNK, Src-FAK etc. Major ECM proteins induced are fibrillar collagens, fibronectin, specific laminins, proteoglycans, and matricellular proteins and these could be potential drug targets for therapy. Matrix metalloproteinases (MMPs) degrade ECM proteins promoting invasion and metastasis. The MMP-11 (stromelysin-3) seems facilitating tumor development through apoptosis inhibition. However, it suppresses metastasis in animal models, exhibiting different roles in tumor progression. β-D mannuronic acid (BDM) is a MMP inhibitor, inhibiting MMP-2 and MMP-9 involved in invasion, metastasis, and angiogenesis. BDM possesses anti-metastatic activity and inhibits tumor growth by suppressing inflammatory chemokine and tumor-promoting cytokines. MMP-14 located on the cell surface, is a potential target to stop metastasis and a novel antibody-mediated MMP-14 blockade seems to limit hypoxia and metastasis in triple negative breast cancer (TNBC) models. The progression from ductal carcinoma in situ (DCIS) to invasive ductal carcinoma (IDC) exhibited up regulation of MMPs such as MMP-2, 11, 13, and 14 associated with invasion and ECM remodeling. The MMPs along with cross-linking enzymes LOX (Lysyl oxidase) facilitate collagen maturation, regulate expression and function of soluble factor like TGF-β which ultimately reciprocates through regulation of expression of many ECM proteins and modifying enzymes including LOXs. LOX is a copper-dependent amine oxidase which initiates the intra- and intermolecular collagen crosslinking through oxidative deamination of specific lysine and hydroxylysine residues located in the telopeptide domains. This crosslinking stiffens the matrix and promotes focal adhesions (Focal adhesion kinase level is increased), integrin clustering, PI3K signaling which ultimately facilitates ErbB2 – dependent breast tumor invasion. The increased stiffness of matrix (measured by elastography) showed low response to neoadjuvant chemotherapy as compared to patients with soft breast carcinomas. It is observed women with high mammographic density (MD) are more likely to develop BC as compared to women with low MD. It seems down-regulation of LOXL4 promotes BC growth and lung metastasis in mice. The LOXL2 protein catalyzes cross-linking of ECM components collagen and elastin and is involved in cancer progression and metastasis. The intracellular LOXL2 shows EMT induction and Snail-1 stabilization, and LOXL-2/Snail-1-mediated E-cadherin down-regulation promotes lung metastasis of BC without affecting ECM stiffness. Collagen is the major scaffolding protein in stroma providing tensile strength to the tissue and its metabolism is dysregulated in cancer with increased expression and deposition. The type I collagen is thought to provide barrier against tumor invasion; however enhanced collagen expression is observed with more incidence of metastasis.

The enzyme collagen prolyl hydroxylase, required for collagen synthesis, is over expressed in BC tissues with poor prognosis. Besides, the enzyme procollagen lysyl hydroxylase-2 involved in collagen synthesis, increases breast tumor stiffness, promotes metastatic tumors in lymph nodes and lungs. Matrix stiffness promotes tumor progression and invasion of ER+ type BC. The hardened ECM drives invasion and metastasis through ERK1/2 signal up-regulation and JAK2/STAT5 signal down-regulation. The enzyme heparanase cleaves heparan sulfate, promoting tumor invasion and metastasis. ER stress during chemotherapy enhances the heparanase activity. The MMTV-heparanase mice promoted growth and metastasis of breast cancer tumors to lungs suggesting a role for heparanase in BC progression. Elemene (extract of C urcuma rhizome plant), is an anticarcinogenic phytochemical showing effects by down-regulating heparanase expression (potential target for heparanase). The heparin and nanoheparin derivatives show their anti-cancer activities by reducing BC cell proliferation and metastasis. Loss of ECM integrity by plasmin facilitates cancer cell spread and plasin-induced ECM degradation may be controlled by lipoprotein-A (competitive inhibitor of plasminogen). Vitamin C seems to be very important curbing tumor growth, and metastasis as ECM integrity requires vitamin C.

The ECM proteins such as COL I, III, IV, VI, fibronectin, laminin 332, peristin, and vitronectin promote tumor progression and metastasis, whereas the proteins such as DMBT1, and SPARC suppress BC development and metastasis as reviewed by Zhu et al. (2014).

**Stromal cells in BC development**

Different types of stromal cells that inhabit in the tumor microenvironment (TME), are immune cells, fibroblasts, adipocytes, endothelial cells and bonemarrow derived stem cells. Tumor cells recruit tumor-associated macrophages (TAMs), which become proangiogenic by secreting VEGF-A which nourishes tumor cells and build a vessel network for their invasion. Hypoxia also induces macrophages to produce more VEGF and suppress immune response, promoting invasion. Cancer-associated fibroblasts (CAFs) are involved in tumor development, progression, inflammation, metastasis, and build resistance to cancer therapy through secretion of hormones, cytokines, growth factors, etc. and cross-talk with other stromal cells, cancer cells, and ECM. CAFs facilitate the invasion through paracrine signaling with cancer cells. The cross-talk between CAFs and cancer cells enhances IGF1 secretion by CAFs and PAI-1 (Serpine1) activity in cancer cells. These two molecules activate RhoA/ROCK signaling in cancer cells which increases cell scattering and invasion. Another study showed CAFs initially assembling an unfolded fibronectin matrix, later remodeled into a dense predominating collagen I matrix driven by...
MMPs. This remodeling resulted in structural and mechanical changes in the stroma, promoting proangiogenic signaling and breast tumor invasion. CAFs can be potential therapeutic targets in BC. Cancer cell proliferation and migration is induced by activated fibroblasts derived from endothelial-to-mesenchymal transformation. The cancer associated adipocytes (CAAs) have a significant role in cancer progression, ECM remodeling, phenotype changes of CAFs, and resistance to cancer therapy. They show tumor-modified phenotype with ability to modify cancer cell phenotype favoring metastasis. Comparative gene expression profiling of myoepithelial cells of cancerous (DCIS) and normal breast tissue showed up regulation of several proteases (cathepsin F, K, and L, MMP2, and PRSS19), protease inhibitors (thrombospondin2, SERPING1, cystatin C and TIMP3), and collagens like COL1A1, COL3A1, COL6A1 in DCIS tissue.

Various ECM proteins in BC progression
Integrins, the primary receptors of MECs for ECM, act as sensors of epithelial microenvironment. They are the transmembrane glycoproteins present as heterodimers of α- and β- subunits. Total 8 β – subunits dimerize with 18 α – subunits to form around 24 distinct integrins which specifically bind to different ECM proteins. Their altered expression seems to disorganize ECM and promotes metastasis. Increased MEC proliferation occurs due to enhanced activity of integrin signaling (β1-, β5-, and β6-integrins) by co-activating the oncopgenes which augment growth factor signaling. The β1 and β3 integrins play crucial role in BC progression and metastasis, hence therapy needs targeting these two integrins at once or their downstream cytokines like FAKs (focal adhesion kinases) and SFKs (Src family kinases) for effective treatment. One of the studies revealed integrin mediated BC invasion through integrin – uPAR (urokinase/plasminogen activator urokinase receptors) signaling which leads to FRA-1 (Fos-related antigen 1) phosphorylation and invasion. The ECM protein vitronectin engagement via integrin and uPAR receptors, ends in activation of SRC and MAPK signaling which ultimately enhances FRA-1 phosphorylation. The FRA-1 (a member of AP-1 family of TFs) targets (which promote tumor cell proliferation, invasion and metastasis) include plasminogen activator, MMP-1, MMP-9, Clca2 (Chloride channel accessory2), adenosine receptor A2B, and mir221/222. Protein ECM1 is involved in angiogenesis, promoting TNBC migration and invasion. Protein Hic-5 (focal adhesion scaffold/adaptor protein) promotes mammary duct formation. Focal adhesions of cells are attached to ECM and transduce signals from ECM to cell. Hic-5 is up-regulated in CAFs of BC, involved in EMT and invadopodia (F-actin rich protrusions of cancer cells) formation facilitating invasion, migration and metastasis. The sustained directionality of tumor cells to a vessel is promoted by a chemotactic gradient of hepatocyte growth factor (HGF) produced from vessel endothelium. This directional streaming is possible by HGF/c-Met signaling pathway between endothelial cells and tumor cells; and c-Met inhibitors could be a potential target to block tumor cell streaming and metastasis.

Tumor microenvironment (TME) as therapeutic target
Clinical trials are going on intensively at present to target the stromal cells of TME for BC therapy in combination with cancerous cell targets, reviewed recently by Bahrami et al. (2018). To name a few drugs: drugs that target CAFs are chloroquine, metformin (targets lipid metabolism), anti-Met (targets glucose metabolism), celecoxib (COX-2 inhibitor), PD0332991 (cell cycle arrest), XAV 939 (β-catenin pathway inhibitor), SB431542 (TGF-β1 receptor kinase inhibitor) etc. The drugs that target the immune cells are denosumab (Treg cell inhibitor), bisphosphonates (TAM inhibitor), indoximod (IDO pathway inhibitor) etc.

An anthracycline such as doxorubicin treatment of BC shows resistance to the drug mediated by ECM proteins as observed in the in vitro model. Hence, probably combinatorial treatment with integrin signaling inhibitors would be more effective in BC therapy. ABL kinase inhibitors like imatinib, nilotinib, and GNF-5 impede the invadopodia formation, decrease ECM degradation, and impair the matrix proteolysis-dependent invasion as observed in the mouse xenograft model.

The cytotoxic T-cells present in the TME kill the tumor cells. However, their infiltration is retarded by various factors. The chemokines such as CXCL-9, -10, -11 (production is induced by IFN-γ and chemokine gradient is established) recruit the T-cells in the TME. The tumor cells produce the extracellular galectin-3 (a lectin that binds to glycans of glycoproteins in ECM), which binds to the glycoprotein IFN-γ and prevents it from inducing secretion of above chemokines, thus impedes the cytotoxic T-cell recruitment in the TME. Immuno-therapy targeting the galectin-3 would be better strategy to control tumor growth and invasion.

The TAMs (M2 phenotype) are protumoral in nature suppressing the adaptive immunity (supresses CD8+ T cells), promoting angiogenesis, and matrix remodeling. The TAM can be a potential therapeutic target for BC therapy. Besides, the polarity switch from M2 to M1 phenotype (antitumoral function) could be a better strategy for treatment of cancer.

MMP-14 was found to be a valid target to control tumor progression and metastasis in triple negative breast cancer. MMP-14 blockade by IgG3369 revealed decreased tumor progression and metastasis in triple negative breast cancer. The TAMs (M2 phenotype) are protumoral in nature suppressing the adaptive immunity (supresses CD8+ T cells), promoting angiogenesis, and matrix remodeling. The TAM can be a potential therapeutic target for BC therapy. Besides, the polarity switch from M2 to M1 phenotype (antitumoral function) could be a better strategy for treatment of cancer.

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High amount of hyaluronic acid (HA) present in TME seems to act as a physical barrier restricting the antibody and immune cell access to tumor cells. It was observed pericellular matrix of HA tumor cells restricted NK cell access and antibody- dependent cell mediated cytotoxicity (ADCC). The hyaluronidase (PEGPH20) treatment showed enhanced trastuzumab-dependent ADCC and NK cell mediated tumor growth inhibition in the in vivo system, proving an effective adjunctive therapy for HA tumors.

Benias et al. (2018) observed presence of fluid-filled interstitial space in the submucosa of many organs (which are subjected to intermittent compression) supported by thick collagen bundles. Similar structures may also present in breast tissues.
facilitating the metastasis of cancer cells as opposed to dense connective tissues acting as a barrier to migrating cancer cells.

**Conclusion**

The ECM constitutes a complex of structural proteins and its reorganization is essential during cancer progression. ECM proteins provide biochemical signals to induce EMT, promote metastasis progression of cancer to advanced stage. ECM remodeling enzymes like MMPs play an essential role in these processes. The TME, platelet-derived mitogens and chemokines, granulocytes and stromal cells help cancer cells achieve intravascular transit and metastasis to target site. In addition, various ECM proteins such as integrins, collagen and fibronectin engage in cell adhesion, invasion and metastasis. All these elements of the ECM are critical for cancer progression and hence targeting ECM is a prospective approach for targeted drug discovery and cancer therapy.

**Data availability**

No data is associated with this article.

**Competing interests**

No competing interests were disclosed.

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Version 2

Reviewer Report 26 June 2018

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Andrew R. Craig
Cancer Biology & Genetics, Queen's Cancer Research Institute, Queen's University, Kingston, ON, Canada

The authors have improved the review sufficiently for indexing. While the review still suffers from a lack of depth in any particularly topic or target, it does provide a starting point for readers that are new to this field. The authors should be careful when discussing available inhibitors to relevant ECM or TME targets, as to whether they are research grade or clinical grade. Is their goal to inform future preclinical studies or clinical studies of these targets, or both?

In future, the authors are encouraged to find particular subtopics or protein family that warrants a deeper dive into the literature.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Cancer metastasis, tumor microenvironment, matrix metalloproteinases, immunotherapy

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 13 June 2018

https://doi.org/10.5256/f1000research.16643.r34930

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Yunus A. Luqmani
Faculty of Pharmacy, Kuwait University, Safat, Kuwait

As I said previously this is a purely descriptive review which does not add much novelty to the existing literature. However, the authors have added additional details, as per referee comments, to improve the depth of the paper and it affords a useful succinct summary of the interaction between the cancer cell mass and its extracellular milieu which influences invasion and metastasis. On this basis I can recommend its indexing.

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Molecular breast oncology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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**Version 1**

Reviewer Report 09 May 2018

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Ren Xu

Markey Cancer Center, Department of Pharmacology and Nutritional Sciences, University of Kentucky, Lexington, KY, USA

ECM is the major component of tumor microenvironment. The review briefly summarized function of ECM in breast cancer progression. Authors discussed function of MMPs and LOXs in ECM remodelling and cancer progression. The review also touched the roles of EMT and CAF in ECM remodelling. However, none of these aspects has been covered thoroughly and discussed in detail. The review provides some information about ECM and cancer progression, but lacks big picture of ECM function and detail information about ECM deposition and remodelling.

Major comments:

1. ECM degradation is only part of ECM remodelling during cancer development. Increased ECM deposition is also associated with cancer progression. The authors need provide more and detail information about what and how ECM deposition contributes/represses cancer progression.

2. Function of ECM in cancer progression is very complicated. Some ECM proteins promote cancer progression, some may suppress cancer progression. It is crucial to classify it in the review.
3. EMT and CAF are both involved in ECM remodelling. Detail information about how EMT and CAF regulate ECM remodelling need to be provided. It is also important to discuss the function of other stromal cells in ECM remodelling in cancer tissue.

**Is the topic of the review discussed comprehensively in the context of the current literature?**
Partly

**Are all factual statements correct and adequately supported by citations?**
Partly

**Is the review written in accessible language?**
Yes

**Are the conclusions drawn appropriate in the context of the current research literature?**
Partly

*Competing Interests:* No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

**Author Response 07 Jun 2018**

Manoj Jena, Lovely Professional University, Phagwara, India

All the queries have been answered in this version.

*Competing Interests:* No competing interests were disclosed.

**Reviewer Report 09 April 2018**

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Yunus A. Luqmani
Faculty of Pharmacy, Kuwait University, Safat, Kuwait

This is a very brief overview of the various complex processes that are thought to influence the migration of breast cancer cells from their primary site into and through the extracellular matrix, a pre-requisite for metastatic dissemination through the vascular system. The importance of the ECM in this process and its potential as a therapeutic target has only relatively recently been
recognized, but the extent of interest has grown rapidly. Although this report does not add much novelty to the existing literature it is nevertheless a useful succinct summary of some of the major influencing factors/processes that are involved in breast cancer progression that inter-relates to the ECM. It is well written and easy to follow, particularly for those less familiar with this field.

The title refers specifically to the role of the ECM. But EMT is a process occurring within the cancer cells prior to interaction with the ECM so it’s description is rather out of place here. Perhaps instead the authors may speculate as to how alterations or signals originating from the ECM might initiate EMT in some cells of the tumour mass (platelet derived-TGFβ is one factor mentioned but this is also secreted from cancer cells). This would add something new as little is known about the triggers (as opposed to the plethora of mediators) of EMT. A short description of the differences in the tumour ECM from that around normal cells would be also be useful.

Minor points:
- Statements that 98% of any cancer mortality and 90% of BC is due to metastasis should be removed or compelling evidence cited to support these figures. Whilst it may be true for BC, primary tumours in vital organs such as brain, lung, liver etc are likely to be as responsible for mortality, if not more, than their metastases.
- A few grammatical corrections: lysyl and builds and MEC abbreviation to be explained.

Is the topic of the review discussed comprehensively in the context of the current literature?
Partly

Are all factual statements correct and adequately supported by citations?
Yes

Is the review written in accessible language?
Yes

Are the conclusions drawn appropriate in the context of the current research literature?
Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Molecular biology, endocrinology of breast cancer

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 07 Jun 2018

*Manoj Jena*, Lovely Professional University, Phagwara, India

All the queries have been answered in this version.
The review by Jena and Janjanam provides some updates on progress in our understanding of the complex interactions between breast cancer cells and surrounding extracellular matrix (ECM) during tumor progression and metastasis. The review also mentions the growing appreciation of the interplay of stromal cells such as tumor-associated macrophages and fibroblasts on this process. While I applaud the efforts to synthesize some recent findings in this review, each subtopic within the review would warrant a deeper dive into the literature and what this means for developing new therapies. The recent discovery of the interstitium as a fluid compartment, as opposed to a rigid ECM barrier, that facilitates metastasis within lymph nodes and blood vessels\(^1\) would warrant mention in a future review on this topic. There is also evidence emerging that ECM can form a barrier to cytotoxic immune cell recruitment. What might this mean for therapies targeting matrix metalloproteinases or cancer-associated fibroblasts that alter the ECM? How can the altered ECM improve responses to chemotherapy or immunotherapy in breast cancer?

Overall, the topic is very interesting and timely, but the review needs to be more focused to achieve significant depth, and to truly guide thinking about new therapeutic strategies for metastatic breast cancer.

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Is the topic of the review discussed comprehensively in the context of the current literature?
No

Are all factual statements correct and adequately supported by citations?
Partly

Is the review written in accessible language?
Yes

Are the conclusions drawn appropriate in the context of the current research literature?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Cancer metastasis, tumor microenvironment, matrix metalloproteinases, immunotherapy

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 07 Jun 2018
Manoj Jena, Lovely Professional University, Phagwara, India

All the queries have been answered in this version.

Competing Interests: No competing interests were disclosed.

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