The cancer matrisome:
from comprehensive characterization to biomarker discovery.

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ABSTRACT

Tumor progression and dissemination critically depend on support from the tumor microenvironment, the ensemble of cellular and acellular components surrounding and interacting with tumor cells. The extracellular matrix (ECM), the complex scaffolding of hundreds of proteins surrounding and organizing cells in tissues, is a major component of the tumor microenvironment. It orchestrates cellular processes including proliferation, migration, and invasion, that are highly dysregulated during cancer progression. Alterations in ECM abundance, integrity, and mechanical properties have been correlated with poorer prognosis for cancer patients. Yet the ECM proteome, or "matrisome," of tumors remained until recently largely unexplored. This review will present the recent developments in computational and proteomic technologies that have allowed the comprehensive characterization of the ECM of different tumor types and microenvironmental niches. These approaches have resulted in the definition of protein signatures distinguishing tumors from normal tissues, tumors of different stages, primary from secondary tumors, and tumors from other diseased states such as fibrosis. Moreover, recent studies have demonstrated that the levels of expression of certain genes encoding ECM and ECM-associated proteins is prognostic of cancer patient survival and can thus serve as biomarkers. Last, proteomic studies have permitted the identification of novel ECM proteins playing functional roles in cancer progression. Such proteins have the potential to be exploited as therapeutic targets.

KEYWORDS

Extracellular Matrix; Tumor Microenvironment; Cancer; Metastasis; Proteomics; ECM Signatures

HIGHLIGHTS

- Precise ECM composition of tissues and tumors can be characterized by proteomics.
- ECM proteins or signatures are prognostic of cancer patient survival.
- Makeup of the primary tumor ECM primes tumor growth.
- Changes in ECM composition regulate distinct steps of the metastatic cascade.
- The ECM is a promising target for designing effective anti-cancer therapies.

1. INTRODUCTION

Worldwide cancer deaths are projected to increase from 8 million to 13 million by 2030, with 90% of these deaths resulting from metastasis – the spreading of tumor cells to secondary sites in the body [1]. Despite substantial increases in cancer awareness and the emergence of novel treatments, understanding and preventing tumor progression and metastasis remains a significant challenge. Over the past few decades, it has become clear that in order to progress, tumor cells not only need to accumulate mutations, but need to be surrounded by a permissive microenvironment [2–4]. The tumor microenvironment is composed of non-cancerous cells including normal resident cells, tumor-associated fibroblasts [5,6], immune and inflammatory cells including tumor-associated macrophages [7–10], and cells forming the tumor vasculature [11]. Additional parameters characterizing the tumor microenvironment include oxygenation levels [12] and pH [13]. Last, the tumor microenvironment comprises an acellular component: a unique tumor-associated extracellular matrix (ECM).

The ECM is a complex scaffolding made of hundreds of proteins that provide anchorage and support to the surrounding cells [14,15]. In addition to this architectural role, ECM proteins provide signals that cells interpret and transduce via cell-surface receptors such as the integrins, the discoidin-domain receptors, and the syndecans. Importantly, all these receptors have been shown to play a role in cancer [16–18]. ECM proteins are also capable of binding growth factors and modulate their signaling properties [19]. ECM signals activate pathways that govern cellular phenotypes including cell proliferation and survival, cell morphology, adhesion, spreading, and motility [20–22]. Alterations of the biochemical composition of the ECM, of its mechanical properties, or its integrity accompany or lead to diseases such as fibroses, cardiovascular, or musculoskeletal diseases [23–25]. Clinical observations have revealed that increased ECM content correlates with more aggressive tumors and poorer prognosis [26–29]. However, until recently we did not have a complete picture of the complexity of the tumor ECM, nor did we know the extent of its involvement in cancer progression.

Our appreciation for the ECM as a key player in tumor progression has grown rapidly. Indeed, the number of articles investigating the role of the ECM in cancer and metastasis has jumped over the past 30 years (Figure 1). This has been permitted by the emergence of novel animal models to study the complexity of the tumor microenvironment and its impact on the multi-step process of cancer progression which cannot be captured *in vitro* [30–32]. This has also been made possible by a better understanding of what the ECM is and better definition and prediction of which components constitute it [15,33]; and by the development of novel technologies allowing the biochemical characterization of the ECM composition of *in-vivo* samples (see section 2). It has become clear that the ECM impacts all the "hallmarks of cancer," all the cellular processes contributing to cancer initiation, progression, and

dissemination (Figure 2), [34,35]. It has become evident that the ECM represents an attractive new source of potential biomarkers and therapeutic targets that we are only now starting to explore and exploit. Recent reviews have discussed the importance of the biomechanical and physical properties of the ECM in cancer [36,37], the role of the ECM in the formation of metastatic niches [38,39], and the dynamic crosstalk that exists between the tumor ECM and other components of the tumor microenvironment [6,40]. The purpose of this review is to highlight how emerging bioinformatic and proteomic technologies have led to significant advances in the characterization of the ECM composition of tumor microenvironments and have allowed the identification of 1) novel or unsuspected ECM proteins playing causal roles in cancer progression and dissemination and 2) novel prognostic biomarkers.

2. -OMIC CHARACTERIZATION OF THE CANCER MATRISOME

We have previously defined computationally the "matrisome" as the ensemble of genes encoding core ECM proteins and ECM-associated proteins [41,33,15,42]. The core matrisome refers to the ensemble of close-to-300 genes encoding proteins that contribute mainly to the architectural organization of the ECM (including ECM glycoproteins such as the laminins, tenascins, thrombospondins, fibrillins, fibronectin, etc.; the collagens; and the proteoglycans), whereas matrisome-associated genes (~700 genes in the human genome) encode proteins involved in the regulation or modulation of ECM functions. Altogether the matrisome is encoded by nearly 1000 genes of 4% of the human or mouse genome. Since then, the usage of the term matrisome has been extended to refer to the actual protein composition of the ECM of biological samples, including tumors (see section 2.2). The purpose of establishing these lists of genes and proteins was to facilitate the annotation of ECM and ECM-associated genes and proteins in large genomic, transcriptomic, and proteomic data sets [42,43], and to enable the identification of novel ECM proteins that may play a functional role in tumor progression and thus be targeted or serve as biomarkers.

2.1. Identification of ECM signatures of cancers within gene expression data sets

The matrisome lists have proven to be particularly valuable to annotate cancer gene-expression data sets and identify ECM signatures of specific cancer types and subtypes, or characteristics of specific steps of cancer progression. For example, interrogation of gene expression profiles of breast cancers classified as luminal B2 and normal breast tissues defined the top 15 genes most up-regulated, of which 7 belonged to the matrisome (COL10A1, COL11A1, FN1, IBSP, INHA, MMP11, WISP1) and the top 15 genes the most down-regulated, of which 3 belonged to the matrisome (COL17A1, CX3CL1, SFRP1) [44]. In a recent study, Yuzhalin and collaborators interrogated the Oncomine platform and identified a 9-core-

matrisome-gene signature (COL1A1, COL10A1, COL11A1, AGRN, BGN, COMP, MFAP2, MXRA5, SPP1) consistently up-regulated in breast, esophageal, gastric, lung, ovarian, and colorectal adenocarcinomas [45]. Lim and collaborators conducted a computational analysis to identify genes differentially expressed between non-small-cell-lung cancer samples and normal tissues in a cohort of over 2000 samples [46]. Among the 103 differentially expressed genes, 29 were matrisome and matrisome-associated genes. They termed this 29-matrisome-gene signature the ECM-related prognostic and predictive indicator (EPPI) (see section 2.2 and Table 3).

The tumor angiogenic switch, the time point at which tumors start establishing their own blood supply, is a critical step during tumor progression [47,48]. Using the *in-silico* matrisome, Langlois and collaborators identified 110 matrisome genes whose expression was induced during the angiogenic switch and termed this gene signature the "AngioMatrix" [49]. They further showed that some of the AngioMatrix genes, including TNC, encoding tenascin-C, played a functional role in the angiogenic switch and tumor progression and were prognostic of cancer patient survival (see sections 3 and 4.3). The blood vessels established during the angiogenic switch are not only providing nutrients and oxygen supporting primary tumor growth, they also offer an escape route for tumor cells to metastasize. Up to now, we still do not have ways to predict whether a tumor will metastasize and if so, to which organ(s) it will disseminate to. In the early 2000s, the Massagué lab published the results of a series of studies aimed at identifying genes controlling metastatic potential and metastatic tropism, the preferential dissemination of tumor cells to certain organs [50–53]. Here, we examined these published gene sets and found that out of the 43 genes up-regulated in bone-tropic mammary tumor cells, 6 are matrisome genes (ADAMTS1, CTGF, FGF5, FST, IL11, MMP1); out of the 51 genes up-regulated in lung-tropic mammary tumor cells as compared to poorly metastatic cells, 14 are matrisome genes (COL1A1, COL6A1, LTBP1, MFAP2, SPARC, TNC, MMP1, MMP2, SERPINE2, ANGPTL4, CSF3, CXCL1, EREG, PDGFA); and out of the 17 genes up-regulated in brain-tropic cells as compared to poorly metastatic cells and that predicted patient survival, 9 were matrisome genes (COL13A1, LAMA4, LTBP1, MMP1, PLOD2, ANGPTL4, CSF3, HBEGF, TNFSF10). The partial overlap observed between these different gene sets highlight that some ECM components may be important for metastatic dissemination (for example LTBP1 and MMP1) whereas genes identified in a unique gene set may be contributing to the determination of metastatic tropism. These examples illustrate how consolidated and comprehensive lists of genes encoding components of the matrisome can be used to identify genes or gene signatures that may carry prognostic value and that may further be interrogated to discover novel proteins playing mechanistic roles in cancer progression (see sections 3 and 4).

2.2. Proteomic characterization of the ECM composition of tumor microenvironments

Proteomic pipelines to profile the protein composition of the ECM can be divided in three steps. The first step consists in tissue decellularization or the enrichment of ECM components and depletion of intracellular components from tissue samples. This can be achieved by using salts, detergents, or enzymes [41,54–59]. The second step consists of the solubilization and digestion of ECM-enriched protein samples into peptides and the analysis of the peptides by mass spectrometry [54–56,60]. The last step comprises the identification, annotation, and quantification of ECM proteins in a proteomic dataset which has been facilitated by the development of bioinformatic tools such as Matrisome Annotator and Matrisome Analyzer [42,43,55]. Over the past few years, these steps have been optimized by us and others to capture the complexity of ECM proteins (including high insolubility and ECM-specific post-translational modifications). As a result, mass-spectrometry-based bottom-up proteomics [61] has become the method of choice to profile the composition of the ECM of *in-vivo* samples [42,62,63].

Proteomic profiling of the matrisome of human tumor melanoma and mammary carcinoma xenografts grown in mice demonstrated that both the tumor cells and the stromal cells contribute to the production of the tumor ECM [41,64]. Furthermore, comparison of the matrisome of poorly and highly metastatic human xenografts of melanoma and mammary carcinoma cells revealed that the tumor matrisome changes with a tumor's metastatic potential and that both the tumor-cell and the stromal-cell contributions are altered [41,64]. Applied to mouse models or patient samples, these methods have permitted to identify ECM-protein signatures distinguishing normal tissues and primary tumors, including murine mammary tumors [57], non-small-cell lung adenocarcinoma [65], pancreatic ductal adenocarcinoma [66], insulinoma [67]; and human colorectal cancers [68], multiple myeloma [69], triple-negative breast cancers [55], and omental metastases originating from high-grade serous ovarian cancers [55,70] (Table 1). Such signatures can be further exploited to identify ECM protein playing functional roles in cancer progression.

Proteomics has also been used to profile the ECM composition of metastases and compare it to the ECM composition of the primary tumors they originate from. Using patient samples, we reported that the matrisome of colorectal-cancer liver metastases resembles more closely the matrisome of the primary colorectal tumors they derived from, rather than the liver matrisome (Figure 3A) [68]. A study from the Erler lab using a syngeneic mouse model of mammary carcinoma compared the ECM of normal mammary gland, lung, and lymph nodes with the ECM of primary mammary tumor and associated lung and lymph node metastases [57]. We reanalyzed their raw mass spectrometry data deposited in the ProteomeXchange repository [71] (dataset identifier PXD006579) and uncovered that the ECM of lung metastases is more similar to the ECM of the primary mammary tumor they originate from than to the

lung ECM (Figure 3B, left panel), whereas the ECM of lymph node metastases recapitulates the ECM of normal lymph nodes (Figure 3B, right panel).

Pearce and collaborators included matrisomics as part of a multi-omic approach to deconstruct the microenvironment of omental metastasis from high-grade serous ovarian cancer [70]. They analyzed samples from 36 patients and identified 145 ECM proteins, of which 6 core matrisome components (collagens I, III, and VI, fibrillin 1, lumican, perlecan) were found to be abundant in low-disease-scoring samples and several proteins (including fibrinogen, fibronectin, and proteoglycans) in high-disease-scoring samples. They further identified 22 matrisome proteins (Table 2) correlating with the disease score, tissue stiffness, and specific immune infiltrating cell populations. They termed this ECM signature the "Matrix Index" and further demonstrated its potential as a prognostic indicator (see section 3).

Recently, the implementation of label-based quantitative proteomics [72,73] has allowed assessment of the changes in ECM composition as tumors progress. The RIP1-Tag2 mouse in which the rat insulin promoter controls the expression of the oncogenic T-antigen of the SV40 virus is a model of insulinoma and is broadly used to study the tumor angiogenic switch [74,75]. Using label-based quantitative proteomics, we reported the identification of ECM proteins whose abundance varied from normal pancreatic islets to hyperplastic islets, to angiogenic and metastatic insulinomas [67]. Importantly, we identified several ECM signatures composed of proteins whose expression either increased (including the core matrisome proteins Efemp1, fibrillin1, and periostin) or decreased (including the core matrisome proteins decorin, Dmbt1, hemicentin, and Vwa5) as tumors progressed [67]. Label-based quantitative proteomics offers the ability to run multiplexed analyses and was recently used to profile in parallel the matrisome of non-small-cell lung primary carcinomas and derived lymph-node metastases and the matrisome of fibrotic lung samples in order to distinguish tumor-specific ECM changes from global desmoplasia seen in fibrosis [65]. This study has revealed that out of the 113 ECM proteins quantified, only 8 (fibronectin, tenascin-C, fibulin 5, perlecan, mimecan, Mfap4, cathepsin D, and S100A11) were detected in significantly different abundance in both fibrotic lung and non-small-cell lung adenocarcinoma samples as compared to normal lung, and an additional 40 proteins were found in significantly higher or lower abundance specifically in primary or secondary tumor samples.

The integration of ECM proteomic data in the ECM atlas revealed that certain ECM proteins have so far only been detected in tumor samples and not in normal adult tissues [42]. Among these are members of the insulin-like-growth-factor-binding protein family (IGFBP3, IGFBP4, and IGFBP5), matricellular proteins including members of the CCN family, thrombospondin-2, tenascin-N, and VWA9 [42]. The adhesive ECM glycoprotein fibronectin (and in particular the isoforms containing the domains encoded by the EIIIA and EIIIB exons) [76] and the adhesion-modulating matricellular protein tenascin-C [77]

are the two proteins consistently found in higher abundance in most tumor types [65,66,78–82]. Of note, the differential detection of ECM and ECM-associated proteins by mass spectrometry can result from changes in transcriptional, translational, or post-translational levels. Indeed, in addition to a change in protein abundance, changes affecting protein secretion or solubility, protein degradation, or the level of post-translational modifications can lead to differential detection.

For this review, we compared the 29-EPPI gene signature [46], the 9-core matrisome gene signature [45], and the Matrix Index [70] with the two proteomic-derived signatures of highly metastatic human breast tumors [64] and metastatic colorectal cancers [68] (Table 3). This comparison revealed that the four core ECM genes or proteins agrin, COMP (cartilage oligomeric matrix protein or thrombospondin 5), collagen XI, and SPP1 (Secreted Phosphoprotein 1 or osteopontin) are up-regulated or detected in higher abundance in more advanced diseased stages. This illustrates that we may be able to find within the ECM commonalities between vastly different tumor types, which may have significant impact on the development of anti-cancer strategies targeting the tumor ECM.

3. ECM PROTEINS AS PROGNOSTIC INDICATORS OF CANCER PATIENT SURVIVAL.

Because metastasis is the main cause of cancer-associated deaths, the ability to discover prognostic markers is imperative to better stratify and care for cancer patients. Matrisome analyses, whether at the gene or protein expression level, and whether conducted on mouse models of cancers or on patient samples, have the potential to identify novel candidates whose expression correlate with survival. For example, the AngioMatrix signature, identified in a mouse model of insulinoma, correlated with poorer prognosis, namely decreased relapse-free survival for colorectal cancer and glioblastoma patients [49]. The 9-core-matrisome-genes signature, identified by interrogating The Cancer Genome Atlas as consistently up-regulated in several types of adenocarcinomas, was prognostic of poorer overall and disease-free survival for ovarian, gastric, lung, and colorectal cancer patients [45]. The 29-ECM-related-gene prognostic and predictive indicator (EPPI) signature was used to stratify a cohort of over 2000 early-stage non-small-cell lung adenocarcinoma patients into low and high-risk groups and was strongly prognostic of overall survival [46].

The expression of the genes encoding 15 of the 43 ECM proteins characteristics of highly metastatic mammary tumors grown in mice (ADAM9, CST3, EGLN1 (also known as the gene PHD2), HTRA1, IGFBP4, IL16, ITIH4, LOXL2, LTBP3, P4HTM, PLXNB2, S100A10, SERPINA1B, SNED1, TIMP1) was prognostic of distant-metastasis-free survival of breast cancer patients, in particular those presenting with estrogen- and progesterone-receptor-negative breast cancers [64]. Similarly, high LTBP3 levels were prognostic of poorer overall survival in stages I-III head and neck squamous cell carcinoma [83].

S100A10 was also identified in a proteomic study characterizing the non-small-cell lung adenocarcinoma matrisome and a higher expression correlated with lower 5-year survival chance [65]. In the same study, the independent expression of TNC and S100A11 was associated with a lower 5-year survival. Importantly, the integration of the expression of these 3 genes in a signature predicted non-small-cell lung adenocarcinoma patient survival independent of age, sex, smoking history, and mutational load [65]. The Matrix Index, defined by a combination of transcriptomic and proteomic experiments, was used to stratify patients with high-grade serous ovarian carcinoma and a high expression of the genes of the Matrix Index significantly correlated with shorter overall survival. Importantly, a high Matrix Index was also a poor prognosis indicator for lung adenocarcinoma, kidney clear cell carcinoma, hepatocellular carcinoma, pancreatic ductal adenocarcinoma, and colon cancer patients. By multivariate hazard ratio analysis, the Matrix Index was further shown to have prognostic value irrespective of age, stage, grade, and primary treatment response in triple negative breast cancer, mesothelioma, ovarian cancer, liver hepatocellular carcinoma, lung adenocarcinoma, sarcoma, breast invasive carcinoma, colon and colorectal adenocarcinomas, head and neck squamous cell carcinoma, kidney renal clear cell carcinoma, lung squamous cell carcinoma, glioblastoma multiform, and skin cutaneous melanoma [70]. These studies highlight how bioinformatics and proteomics are powerful methods to identify novel genes and proteins of prognostic value for cancer patient survival. They also demonstrate that the ECM represents a large source of potential biomarkers that needs to be further explored.

4. CHANGES IN THE ECM COMPOSITION AFFECTS TUMOR PROGRESSION AND METASTASIS

In addition to the potential prognostic value of the ECM signatures, the distinct, quantitative changes identified by proteomics in the ECM composition of normal-versus-primary tumor as well as primary-versus-metastatic tumor provide insight into how the ECM influences cancer progression and dissemination. We will illustrate this here by providing examples of ECM proteins identified in proteomic screens (see section 2.2 and Table 1) and that have been incriminated in specific step(s) of cancer progression including tumor cell proliferation, tumor cell motility, invasiveness and intravasation, tumor angiogenesis, tumor cell extravasation, and metastatic outgrowth [84]. Although changes in the ECM do critically impact the functions of all the cells present in the tumor microenvironment including fibroblasts [6,40,85,86] and macrophages [87], which via cross-talk further influence tumor cell phenotype, our focus here is to describe the impact of compositional changes in the ECM on tumor cell phenotypes.

4.1. The ECM supports primary tumor growth.

In a proteomic screen comparing the ECM of poorly and highly metastatic melanoma, we reported that lysyl oxidase-like 3 (LOXL3) was found in higher abundance in highly metastatic tumors [41]. This finding was confirmed in a novel study and functional assessment of the role of LOXL3 in melanoma progression which demonstrated that it was required for melanoma cell survival and primary tumor growth [88]. Agrin, a basement membrane component, was shown to be present in higher abundance in highly metastatic mammary carcinoma xenografts [64] and in primary and secondary colorectal cancers [68]. Using SILAC-based quantitative mass spectrometry, Chakraborty and collaborators observed that agrin was produced in higher abundance by hepatocellular carcinoma cells as compared to hepatocytes and further demonstrated that agrin, by maintaining focal adhesions integrity, controlled the proliferation of hepatocellular carcinoma cells via the YAP signaling pathways [89,90]. Based on these observations, it would be interesting to test whether agrin plays a similar functional role in breast cancer and colorectal cancer.

Changes in ECM stiffness and composition supportive of tumor cell proliferation and primary tumor growth were also demonstrated in a model of colorectal cancers [91]. Using decellularized ECMs from normal colon or liver metastasis from colorectal tumor, Romero-López et al., demonstrated that colorectal tumor cells seeded on decellularized matrix from liver metastasis grew faster than when seeded on normal colon ECM. Proteomic comparison of the two types of ECM revealed the presence in higher abundance of fibronectin, fibulins 3 and 4, periostin, tenascin-C, thrombospondin 2, TGFBI, some laminins, versican, biglycan, collagens VI and XII in the ECM of liver metastases as compared to that of normal colon [91]. Interestingly, fibulin 4 (EFEMP2) and thrombospondin 2 were also identified in the proteomic screen comparing primary and secondary human colorectal cancers to normal colon and normal liver [68]. The ability of each individual proteins or of combinations of ECM proteins to promote cell proliferation has yet to be tested.

4. 2. Changes in the ECM makeup affect tumor cell invasiveness and intravasation.

In order to identify ECM proteins produced at the primary tumor site and promoting tumor cell dissemination, we compared the ECM of poorly and highly metastatic mammary carcinoma xenografts and reported the identification of 43 ECM proteins found specifically in highly metastatic tumor [64]. Of the 43 proteins, several had previously been shown to promote tumor progression (LOXL2, ANGPTL4, ADAMs) and we were further able to assess the functional role of four tumor-produced proteins: LTBP3, SNED1, EGLN1 (also known as PHD2), and S100A2. Knockdown of each of the four proteins inhibited the dissemination of cells implanted orthotopically. Interestingly, knock down of EGLN1 and S100A2 but not LTBP3 or SNED1 also prevented lung metastasis of cells injected in the

circulation, suggesting that LTBP3 and SNED1 played a functional role at early steps of the metastatic cascade, whereas EGLN1 and S100A2 impacted both early and late stages of the metastatic cascade. Histological analysis of the primary tumors also revealed that LTBP3 and SNED1 knockdown tumors remained encapsulated by a dense layer of collagen and failed to invade the surrounding normal tissues (mammary gland, adjacent skin and muscles) suggesting that these ECM proteins affect the invasive capacity of tumor cells. Although identified in the same proteomic screen and all being required for dissemination, we were able to pinpoint for each matrisome protein the specific step of the metastatic cascade to which it contributes [64]. In a recent study, Deryugina and collaborators further confirmed the role of LTBP3 in promoting the early steps of the metastatic cascade [83]. Using the chick chorioallantoic membrane assay and mouse models, they showed that LTBP3 was essential for head and neck carcinoma cell, sarcoma cell, and prostate cancer cell intravasation. The precise cellular mechanisms and signaling pathways activated downstream of these ECM proteins remain to be identified.

4. 3. The ECM modulates tumor angiogenesis.

The vasculature plays a critical role in cancer progression, and tumor angiogenesis is a rate limiting step in metastasis [48,74]. The importance of the ECM in regulating angiogenesis has long been recognized [92], however the emergence of novel technologies has permitted the identification of ensembles of genes and proteins working in concert to regulate this process. For example, one of the most up-regulated genes of the AngioMatrix, the set of ECM genes altered during the tumor angiogenic switch defined using the RIP1-Tag2 mouse model, is TNC, encoding the matricellular protein tenascin-C. Knocking out TNC in the RIP1-Tag2 mouse model resulted in decreased angiogenesis [49,79]. Further characterization of the pattern of deposition of tenascin-C in the tumor microenvironment revealed that it forms tracks enriched in niches where fibroblasts, endothelial cells and leukocytes accumulate [78]. Comparison of the AngioMatrix signature [49] with the proteomic profiling of the matrisome of pre- and post-angiogenic pancreatic islets [67] only showed a modest overlap which can be at least attributed to the reported lack of correlation between RNA and protein expression levels [93]. For example, the gene encoding the proteoglycan decorin was found to be up-regulated in the AngioMatrix but proteomic analysis found that the pool of ECM-associated, insoluble decorin present in lower abundance in angiogenic islets as compared to non-angiogenic islets [67]. Functional studies have identified both pro- and anti-angiogenic roles for decorin, although in the context of tumor angiogenesis, decorin acted mostly as an anti-angiogenic factor [94,95].

4.4. The ECM regulates extravasation and metastatic outgrowth in distant organs.

The establishment of a permissive environment supportive of tumor cell extravasation, seeding and metastatic growth in foreign organs is highly dependent on the ECM [38,39]. For example, Padua and collaborators showed that tumor cells produced the ECM-associated protein angiopoietin-like 4, which was critical to trigger vascular permeability in the lung and to allow tumor cells to extravasate and form metastasis in this organ [96]. Interestingly angiopoietin-like 4 was one of the proteins found in highly metastatic lung-tropic primary mammary tumors in the proteomic screen comparing poorly versus highly metastatic tumors [64]. This suggests that the makeup of the ECM of primary tumors affects distal events including extravasation in distant organs. Similarly, tenascin-C was among the genes up-regulated in highly metastatic mammary cells exhibiting lung tropism [50]. Oskarsson and collaborators further showed that mammary-cell-produced tenascin-C at a metastatic site contributed to the formation of a permissive niche supportive of metastatic outgrowth [82]. In a recent study, we showed that tenascin-C promoted metastasis of non-small-cell lung adenocarcinoma cells [65] and it would be interesting to test whether tenascin-C acts via similar mechanisms to promote breast and lung cancer metastasis.

These selected examples highlight how unbiased high-throughput proteomic screens aimed at characterizing the composition of the ECM of different tumor microenvironments resulted in the identification of proteins playing critically important roles in tumor progression and dissemination. It is now crucial to dissect the molecular mechanisms regulated by these proteins if we want to be able to devise novel therapeutic approaches aimed at targeting the ECM.

5. CONCLUSIONS

Once largely discounted from biomarkers and drug target discovery efforts [97], the ECM appears now to be a vast and appealing source of diagnostic and prognostic markers and drug targets [98,99]. The development of novel bioinformatics and proteomics approaches has shed light on this compartment, which is now being appreciated for its complex roles during cancer progression [100–102]. With a significant effort toward encouraging the deposition of proteomic data to public repositories, we will be able to expand the ECM Atlas [42] with the goal of building a "Tumor ECM Atlas" that will allow broad data dissemination and, importantly, help identify robust ECM signatures of solid tumors that may then be exploited for their prognostic, predictive, and therapeutic values. This will only become possible if we gain a better understanding of the fundamental mechanisms governed by the ECM.

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Table 1. **Summary of Proteomic Studies of the ECM in Cancer.**

Cancer Type	Experimental Design	References
Cell Injections		
Breast	Orthotopic or tail vein injections of human MDA-MB-231 or MDA-MB-231-LM2 triple negative breast cancer cells. *Model for poorly or highly metastatic breast cancer*.	[64]
	Orthotopic injection of murine 4T1 mammary tumor cells. *Model for metastatic breast cancer*.	[57]
Melanoma	Mouse B16-F10 melanoma cells implanted in mouse tongue. *Model for oral melanoma*.	[57]
	Subcutaneous injection of human A375 non-metastatic and MA2 metastatic melanoma cells.	[41]
Genetically-Engineered Mouse Models		
Non-small-cell lung adenocarcinoma	Primary tumors and lymph node metastases from $Kras^{LSL\text{-}G12D/+}$; $Trp53^{flox/flox}$ mouse model having received intratracheal administration of Cre recombinase.	[65]
Insulinoma	Hyperplastic islets, angiogenic islets and invasive insulinomas from RIP1-Tag2 mouse model.	[67]
Pancreatic ductal carcinoma	3 models: (Ptf1a-Cre; $Kras^{LSL\text{-}G12D/+}$); (Pdx1-Cre; $Kras^{LSL\text{-}G12D/+}$; $Tp53^{R172H}$); (Ptf1a-Cre; $Kras^{LSL\text{-}G12D/+}$; $Tgfbr2^{flox/wt}$ or $^{flox/flox}$).	[66]
Human Patient Samples		
Breast	Primary triple negative breast tumors.	[55]
Ovarian	Omental metastases from high-grade serous ovarian tumors.	[55]
	Omental metastases of different diseased stages from high-grade serous ovarian tumors.	[70]
Colon	Stage IV primary colorectal tumors and primary-colorectal-tumor metastases to the liver.	[68]
	Primary-colorectal-tumor metastases to the liver.	[91]
Multiple myeloma	Bone marrow aspirates from newly diagnosed and not yet treated patients and from relapsed patients.	[69]

Table 2. The "Matrix Index".

	Matrisome Category	Gene Symbol	Protein Name
Core Matrisome	Collagens	COL1A1	Collagen type I alpha 1
		COL6A6	Collagen type VI alpha 6
		COL11A1	Collagen type XI alpha 1 chain
		COL15A1	Collagen type XV alpha 1 chain
	ECM glycoproteins	COMP	Cartilage oligomeric matrix protein
		FN1	Fibronectin 1
		FBLN2	Fibulin 2
		LAMA4	Laminin subunit alpha 4
		LAMB1	Laminin subunit beta 1
		LAMC1	Laminin subunit gamma 1
		VWF	von Willebrand factor
		ABI3BP	ABI family member 3 binding protein
		TNXB	Tenascin XB
	Proteoglycans	HSPG2	Heparan sulfate proteoglycan 2
		VCAN	Versican
Matrisome-Associated	ECM regulators	CTSB	Cathepsin B
		CTSG	Cathepsin G
		AGT	Angiotensinogen
	ECM-affiliated proteins	ANXA1	Annexin A1
		ANXA5	Annexin A5
		ANXA6	Annexin A6
		LGALS3	Galectin 3

22-matrisome gene and protein signature identified in omental metastasis samples from high-grade serous ovarian cancer, predicting overall survival in several cancers adapted from Pearce *et al*. [70].

Table 3. Comparison of cancer matrisome signatures.

				Transcriptomics		*Proteomics*		
	Matrisome Category	**Gene Symbol**	**Protein Name**	**9-gene signature [45]**	**EPPI [46]**	**Matrix Index [70]**	**Mammary tumors [64]**	**Colorectal tumors [68]**
Core Matrisome	**Collagens**	COL1A1	Collagen type I alpha 1	+	-	+	-	-
		COL6A6	Collagen type VI alpha 6	-	+	+	-	-
		COL10A1	Collagen type X alpha 1	+	+	-	-	-
		COL11A1	**Collagen type XI alpha 1**	+	+	+	-	-
		COL22A1	Collagen type XXII alpha 1	-	-	-	+	+
	ECM glycoproteins	ABI3BP	ABI family, member 3 (NESH) binding protein	-	+	+	-	-
		AGRN	**Agrin**	+	-	-	+	+
		COMP	**Cartilage oligomeric matrix protein; Thrombospondin 5**	+	-	+	-	+
		EFEMP2	EGF-containing fibulin-like extracellular matrix protein 2	-	-	-	+	+
		MXRA5	Matrix remodeling associated 5	+	-	-	-	+
		SPP1	**Secreted phosphoprotein 1; Osteopontin**	+	+	-	-	+
		VWF	von Willebrand factor	-	-	+	+	-
Matrisome-associated	**ECM regulators**	CTSB	Cathepsin B	-	-	+	+	-
		HTRA1	HtrA serine peptidase 1	-	-	-	+	+
		LOXL2	Lysyl oxidase like 2	-	-	-	+	+
		SERPINE2	Serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 2	-	-	-	+	+
		TIMP1	TIMP metallopeptidase inhibitor 1	-	-	-	+	+
	ECM-affiliated proteins	ANXA5	Annexin A5	-	-	+	-	+
		C1QA	Complement component 1, q subcomponent, A chain	-	-	-	+	+
		SFTPD	Surfactant protein D	-	+	-	-	+
	Secreted factors	S100A2	S100 calcium binding protein A2	-	+	-	+	-

21 matrisome genes and proteins are found in at least two out of five of the matrisome signatures identified by transcriptomics or proteomics [45,46, 64, 68, 70]. COL11A1, AGRN, COMP, and SPP1 (bold) are up-regulated in three of the five studies compared.

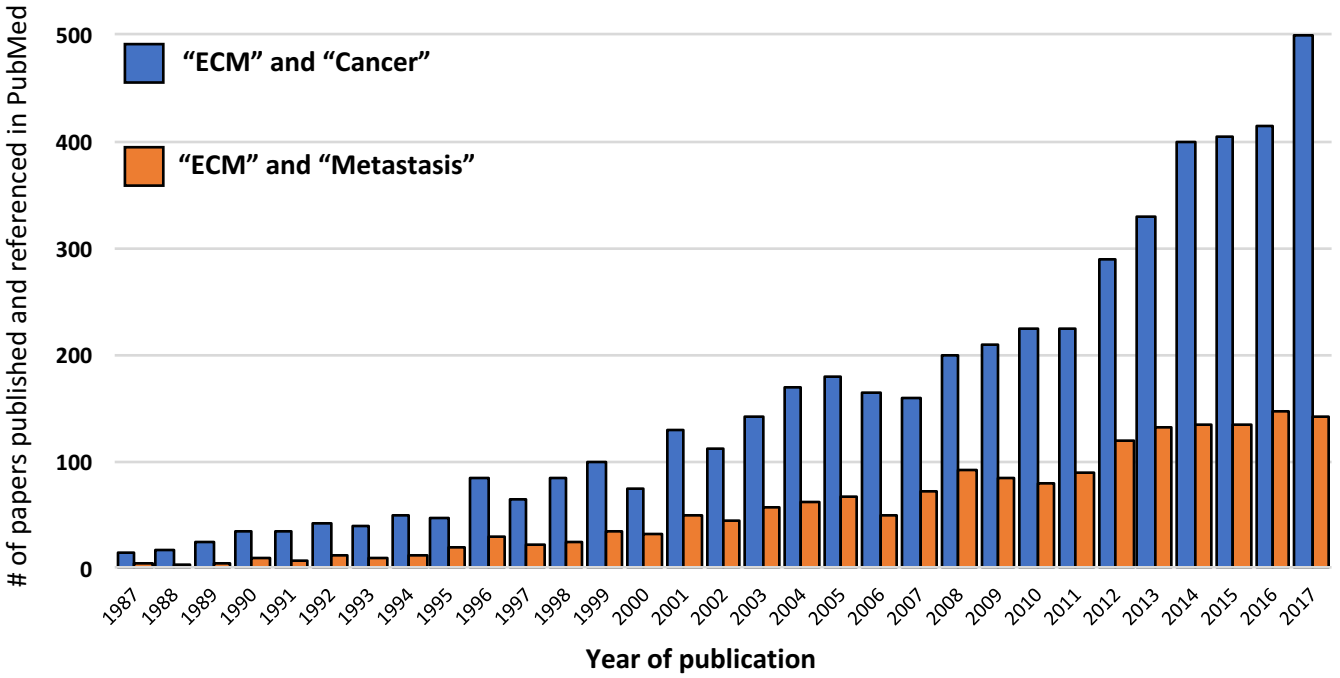
FIGURE LEGENDS

Figure 1. The contributions of the ECM in cancer progression are increasingly recognized.
PubMed searches using the following combination of keywords "ECM" and "cancer" or "ECM" and "metastasis" were conducted and data reporting the number of publications per year were downloaded in .csv format.

Figure 2. The ECM influences the hallmarks of cancer.
Adapted from Hanahan and Weinberg [34].

Figure 3. Comparison of the ECM composition of primary and secondary tumors
A. Venn diagram shows the comparison between the matrisomes of primary metastatic colon cancers, colorectal-cancer metastases to the liver, and normal liver (adapted from Naba *et al.*, [68]).
B. Venn diagrams show the comparisons between the matrisomes of primary mammary tumor, mammary tumor metastases to the lung, and normal lung (left panel), and between the matrisomes of primary mammary tumor, mammary tumor metastases to the lymph nodes, and normal lymph nodes (right panel). Venn diagrams were generated by processing the raw mass-spectrometry data from Mayorca-Guiliani *et al*. downloaded from the public ProteomeXchange repository (dataset identifier PXD006579) [57].

Figure 1.


# of papers published and referenced in PubMed
500
400
300
200
100
0
"ECM" and "Cancer"
"ECM" and "Metastasis"
1987
1988
1989
1990
1991
1992
1993
1994
1995
1996
1997
1998
1999
2000
2001
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2012
2013
2014
2015
2016
2017
Year of publication

Figure 2.

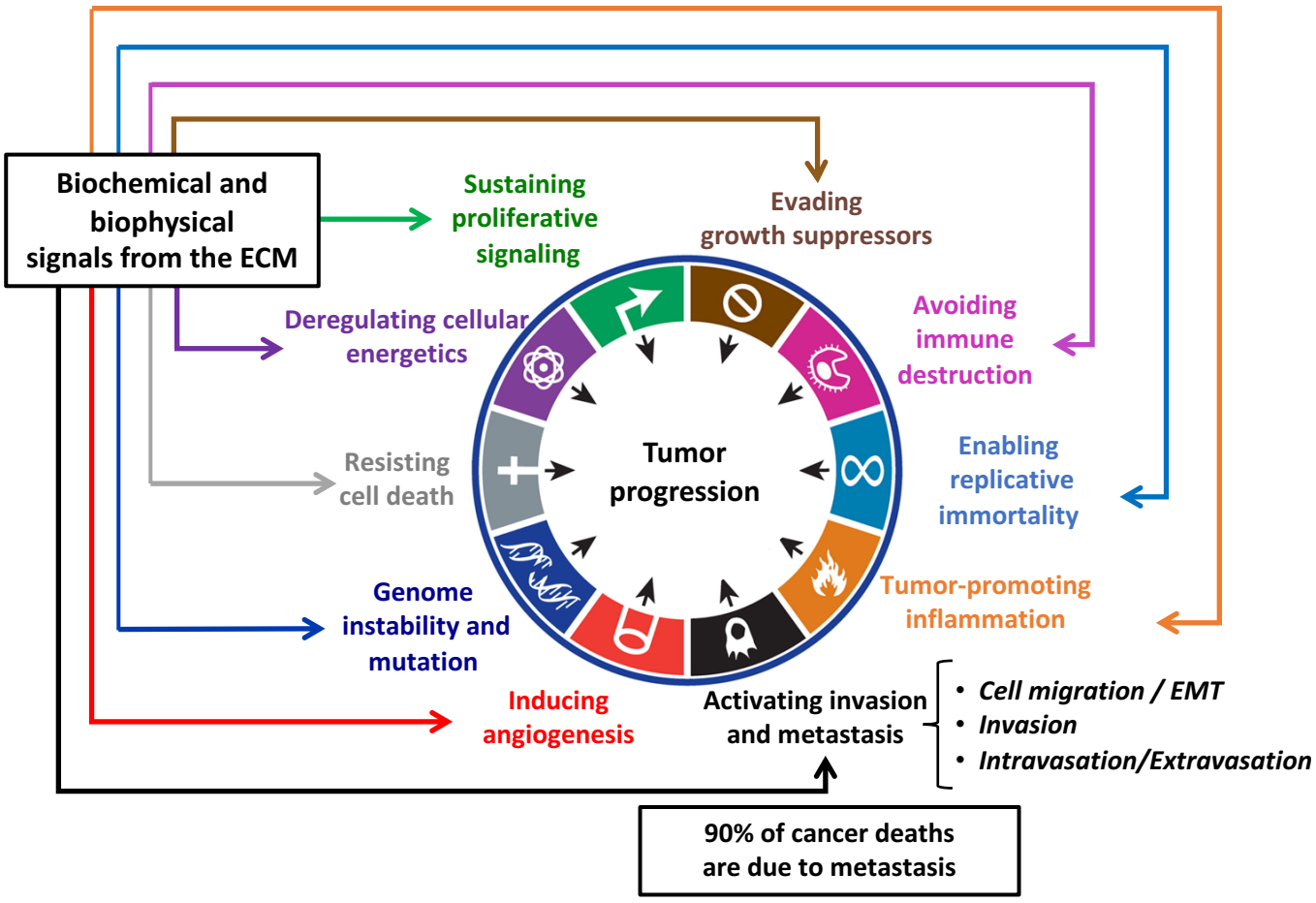
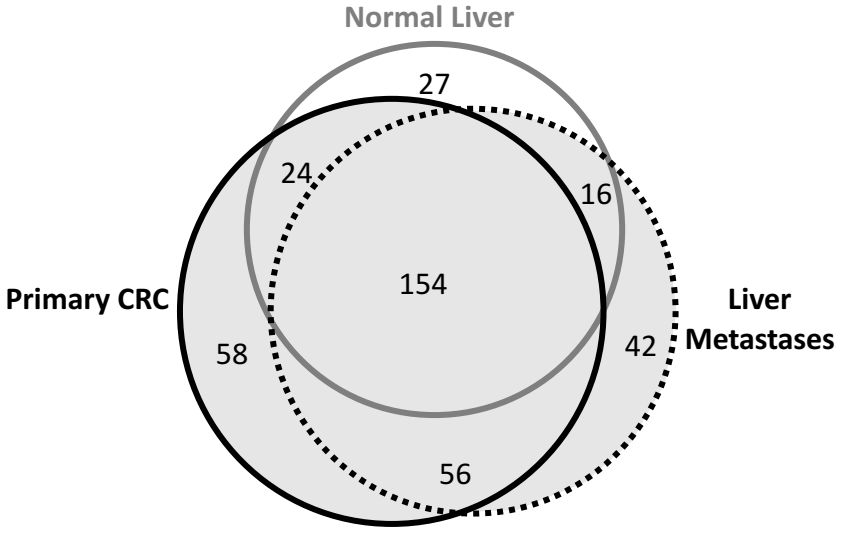
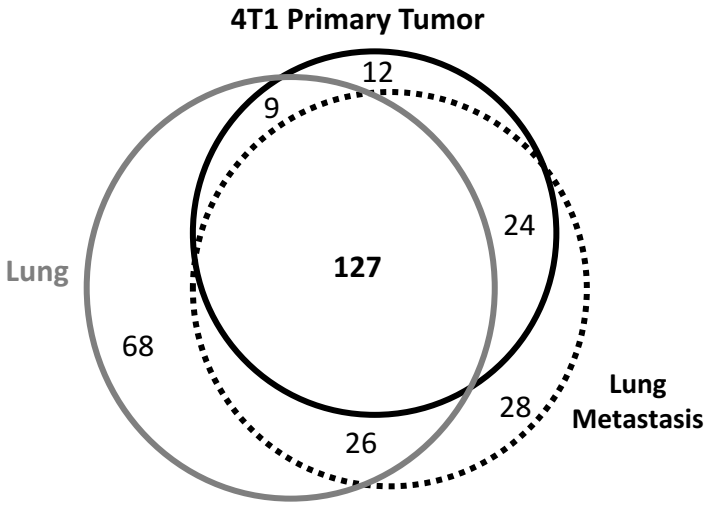

Biochemical and biophysical signals from the ECM
Sustaining proliferative signaling
Evading growth suppressors
Deregulating cellular energetics
Avoiding immune destruction
Resisting cell death
Tumor progression
Enabling replicative immortality
Genome instability and mutation
Tumor-promoting inflammation
Inducing angiogenesis
Activating invasion and metastasis
• Cell migration / EMT
• Invasion
• Intravasation/Extravasation
90% of cancer deaths are due to metastasis

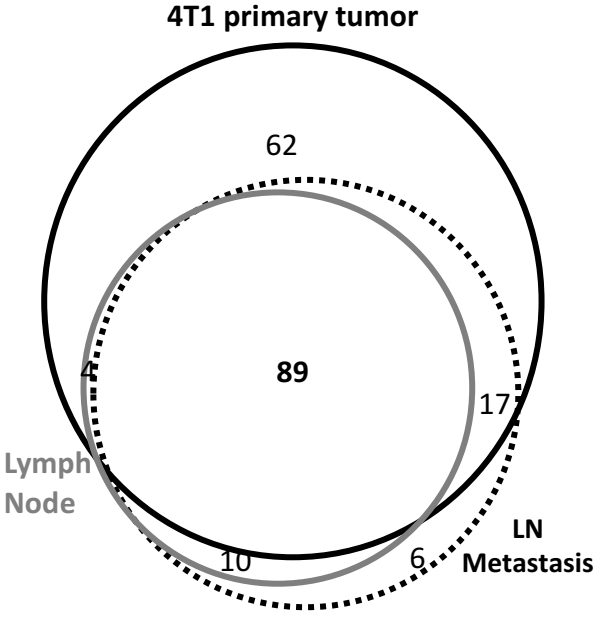
Figure 3.

A.


Normal Liver
27
24
16
154
Primary CRC
Liver
Metastases
58
42
56

B.


4T1 Primary Tumor
12
9
24
127
Lung
68
28
Lung
Metastasis
26


4T1 primary tumor
62
89
17
Lymph
Node
10
6
LN
Metastasis