INTERNATIONAL JOURNAL OF Experimental Pathology

Int. J. Exp. Path. (2018), 99, 58–76

REVIEW ARTICLE

Charting the unexplored extracellular matrix in cancer

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INTERNATIONAL JOURNAL OF EXPERIMENTAL PATHOLOGY

doi: 10.1111/iep.12269

Received for publication: 19 July 2017
Accepted for publication: 26 February 2018

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SUMMARY

The extracellular matrix (ECM) is present in all solid tissues and considered a master regulator of cell behaviour and phenotype. The importance of maintaining the correct biochemical and biophysical properties of the ECM, and the subsequent regulation of cell and tissue homeostasis, is illustrated by the simple fact that the ECM is highly dysregulated in many different types of disease, especially cancer. The loss of tissue ECM homeostasis and integrity is seen as one of the hallmarks of cancer and typically defines transitional events in progression and metastasis. The vast majority of cancer studies place an emphasis on exploring the behaviour and intrinsic signalling pathways of tumour cells. Their goal was to identify ways to target intracellular pathways regulating cancer. Cancer progression and metastasis are powerfully influenced by the ECM and thus present a vast, unexplored repository of anticancer targets that we are only just beginning to tap into. Deconstructing the complexity of the tumour ECM landscape and identifying the interactions between the many cell types, soluble factors and extracellular-matrix proteins have proved challenging. Here, we discuss some of the emerging tools and platforms being used to catalogue and chart the ECM in cancer.

Keywords
cancer, extracellular matrix, imaging, metastasis, proteomics, remodelling

The diverse extracellular-matrix (ECM) landscape

Composed of hundreds of different building blocks, the ECM makes up the complex, highly cross-linked, three-dimensional (3D) network of macromolecules (proteins, glycoproteins and its subgroup of proteoglycans, polysaccharides (glycosaminoglycans), elastins and carbohydrates) that surround cells (Figures 1 and 2). Extracellular matrix molecules assemble into supramolecular structures, each with their own distinct biochemical and biomechanical properties (Pickup *et al.* 2014). Broadly speaking, the ECM falls into two categories: a) interstitial matrix and b) highly specialized organ or tissue-specific ECMs such as basement membranes (BM). The ECM is a salient feature of tissues and as such considered one of the most important regulators of cellular and tissue function in the body. That is to say that cells residing in, or passing through discrete tissue and ECM microenvironments activate or deactivate distinct sets of intracellular signalling events, such as clustering of integrin-based adhesion proteins into focal adhesions (Nicolas *et al.* 2004), Rho-ROCK activation/deactivation and downstream cytoskeletal reorganization (Chrzanowska-Wodnicka & Burridge 1996; Reffay *et al.* 2014), and nuclear localization of key transcriptional regulators such as YAP/TAZ (Dupont *et al.* 2011) and SNAIL1 (Zhang *et al.* 2016; Figure 2). Importantly, these signalling events can elicit both short- and long-term effects (Nasrollahi *et al.* 2017). Furthermore, the activation of these signalling pathways, such as Rho-ROCK, typically leads to successive ECM reorganization such as collagen remodelling (Rath *et al.* 2016). The result is a dynamic reciprocity between cells and the ECM (Bissell & Aggeler 1987; Roskelley *et al.* 1995; Kass *et al.* 2007; Alexander & Cukierman 2016; Boyle & Samuel 2016).

In most tissues, the structure of the ECM is highly dynamic and undergoes constant remodelling controlled by the delicate balance between synthesis and degradation. In doing this, the ECM provides continuously evolving topological, mechanical and biochemical cues to cells (Hynes 2009). Through engagement with various cell surface molecules including integrins, syndecans and discoidin domain receptors (Figure 2), the ECM activates and suppresses

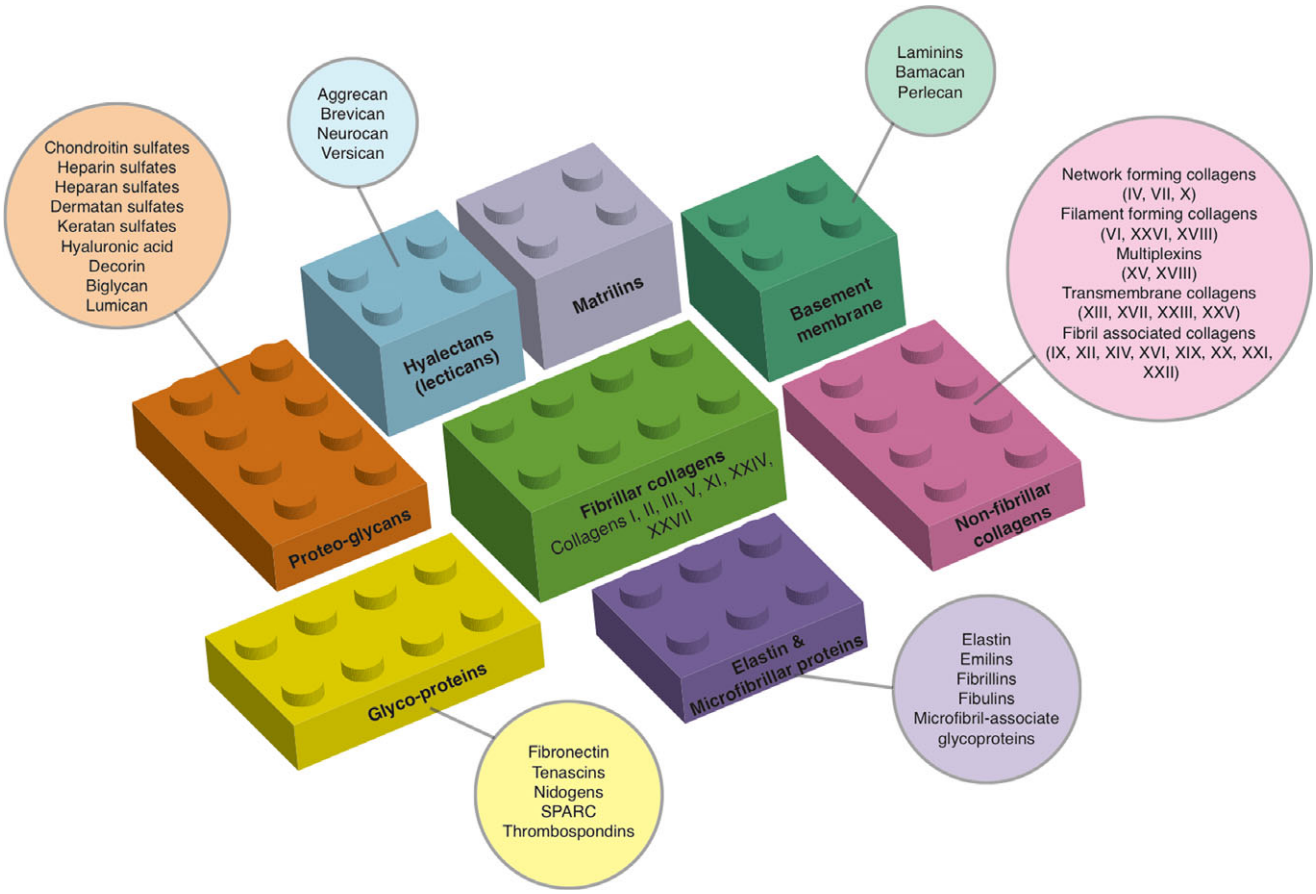

Figure 1 Overview of the basic building blocks of the extracellular matrix that come together to form the highly ordered supramolecular structures that contribute to the various interstitial matrix, microfibrils and the basal laminae within tissues. [Colour figure can be viewed at wileyonlinelibrary.com]

intracellular signalling pathways to regulate and fine-tune diverse cellular programmes including cell proliferation, survival, migration/invasion, differentiation, stemness and plasticity (Hynes 2002, 2009). For example, integrins and their associated receptor tyrosine kinase (RTK) containing focal adhesions (FAs) are critically involved in the cellular response to the ECM through the simultaneous integration of physical and biochemical cues to modulate downstream cellular decisions.

In addition to the canonical structural components, another group of highly disparate, non-structural matrix proteins exist within the ECM. Termed matricellular proteins (Bornstein & Sage 2002), these are capable of modulating a variety of biological processes within the ECM through altering cell–matrix interactions and cell regulatory functions. Acting via a number of unique mechanisms, matricellular proteins are typically highly context dependent, with many being organ specific. Examples include thrombospondin (TSP) 1 and 2, secreted protein acidic and rich in cysteine (SPARC), tenascin C, osteopontin and CCN family proteins (Figure 2). This group of proteins is dynamically expressed and is highly elevated during embryonic development but shows diminished presence in normal adult tissues. Thus, they are typically associated with tissue renewal and repair programmes and are thought to be important in normalizing the tissue (Chiodoni *et al.* 2010). For example, SPARC (initially called osteonectin) was originally identified in bone, where it binds collagen and Ca^{2+}, and is key to initiating nucleation during the process of bone mineralization (Termine *et al.* 1981). However, SPARC is also known to be secreted by non-epithelial cells in non-ossifying tissues (Sage *et al.* 1984) during both development and tissue repair. Here, it plays a role in ECM remodelling and turnover, as well as in mediating cell–ECM interactions, including preventing hypertrophy of adipocytes and hyperplasia of adipocyte progenitors (Engel *et al.* 1987; Sage *et al.* 1989; Funk & Sage 1991; Lane & Sage 1994; Murphy-Ullrich *et al.* 1995; Chlenski & Cohn 2010). SPARC has also been implicated in several solid cancers with SPARC overexpression being a marker of poor prognosis in cancers such as breast, prostate and melanoma (Podhajcer *et al.* 2008) and high expression correlating with tumour invasion through extracellular matrix in many aggressive cancers including breast invasive ductal carcinoma, glioblastoma, pancreatic ductal adenocarcinoma, clear-cell renal cell carcinoma, melanoma and prostate carcinoma (Morrissey *et al.* 2016). In

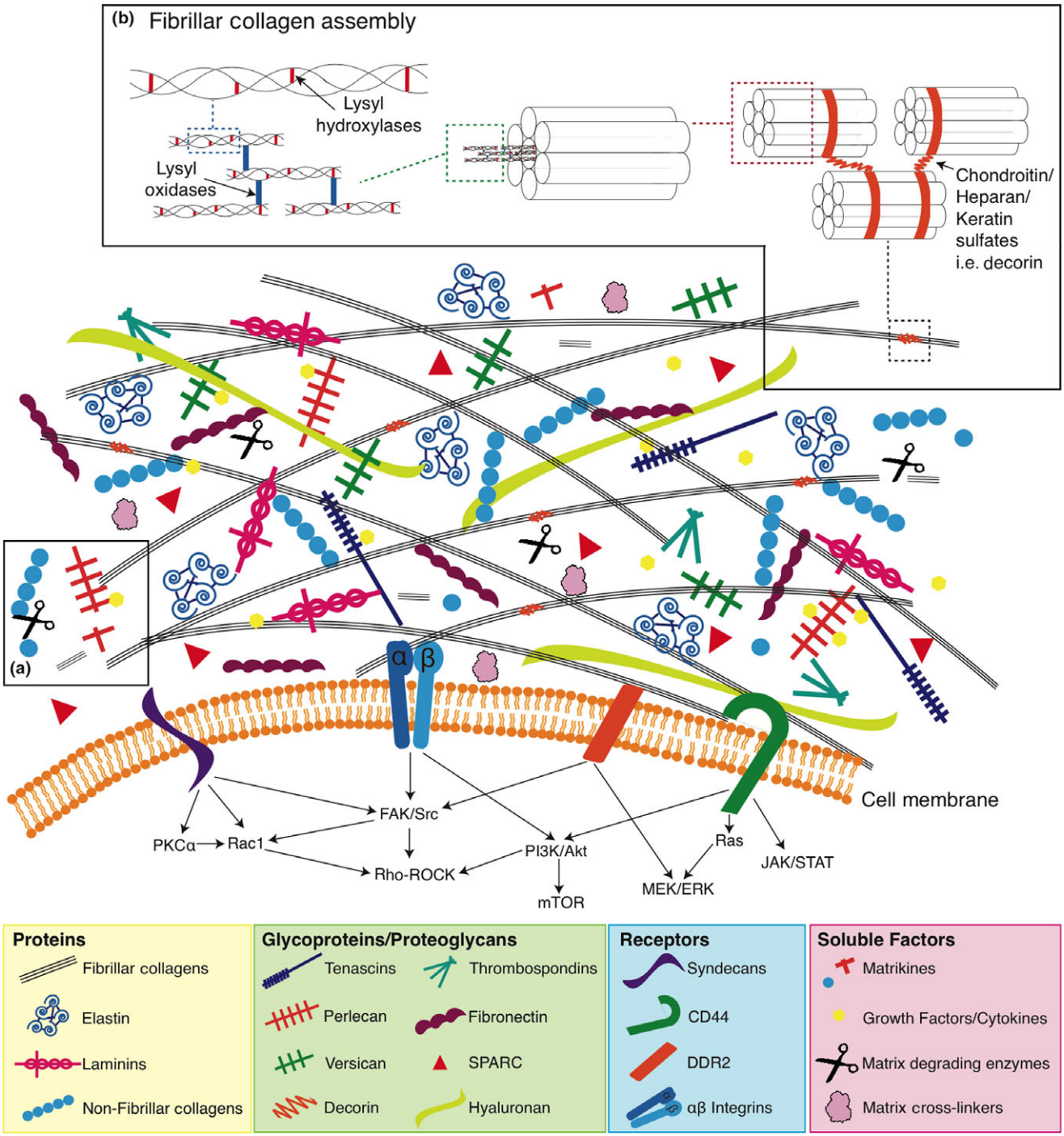

Figure 2 Simplified representation of the extracellular matrix. Made up of many molecules that come together to form the highly ordered supramolecular structures, and the extracellular matrix, through its various cell surface receptors, modulates a number of downstream intracellular signalling pathways to determine cell phenotype and behaviour. [Colour figure can be viewed at wileyonlinelibrary.com]

contrast, it has also been shown to be an inhibitor of cancer cell proliferation in some tumours including pancreatic cancer (Sato *et al.* 2003). Complicating the story further, SPARC is also known to be a chemoattractant for both breast and prostate cancer cells to home to the bone (Jacob *et al.* 1999). With this in mind, there is still much to do in order to uncover the precise role and function of matricellular proteins in the different stages of health and disease.

In recent years, there has also been increasing evidence that distinct fragments from specific ECM proteins and glycosaminoglycans (GAGs) are capable of regulating signalling through cell surface receptors and in turn regulating

biological functions. Known as matrikines (Figure 2, box a), these ECM fragments are released by partial or limited proteolysis of extracellular-matrix macromolecules. They are able to regulate cell activities such as proliferation, migration, protease production or apoptosis, leading to the modulation of physiopathological processes including angiogenesis, cancer, fibrosis, inflammation, neurodegenerative diseases and wound healing (Tran *et al.* 2005; Ricard-Blum & Salza 2014). Still subject to some debate, matrikines are defined as any ECM fragment which has biological functions distinct from those of the parental macromolecule (Arroyo & Iruela-Arispe 2010). Conversely, 'cryptic' matrikines or matricryptins are defined as enzymatic fragments of the ECM containing exposed cryptic sites (Davis *et al.* 2000). The term cryptic matrikine is restricted to biologically active fragments from the ECM-exposing functional cryptic sites that are not normally exposed in the full-length molecules. For more detailed reviews of matrikines and matricryptins, see Tran *et al.* (2005); Ricard-Blum and Salza (2014); Davis *et al.* (2000).

The biological activity of matrikines is typically mediated through the integrin, heparan sulphate proteoglycan and growth factor receptors. Some examples of matrikines include arresten, canstatin and tumstatin, which are cryptic fragments generated from the NC1 domain of collagen IV $\alpha_1/\alpha_2/\alpha_3$ chains respectively (Chong *et al.* 2012). Canstatin, tumstatin, endostatin and tetrastatin bind the $\alpha_v\beta_3$ integrin (Maeshima *et al.* 2000; Rehn *et al.* 2001; Magnon *et al.* 2005; Faye *et al.* 2009; Brassart-Pasco *et al.* 2012), whereas arrestin and anastellin are examples of matrikines binding β_1 integrins (Colorado *et al.* 2000; Mercurius & Morla 2001; Sudhakar *et al.* 2005). The physical size of matrikines has been shown to vary from as little as a few amino acids in the case of the classical RGD tripeptide, to over 700 amino acids in endorepellin, a matrikine derived from the C-terminal fragment of perlecan (Mongiat *et al.* 2003). Furthermore, the precise biological activity of specific matrikines may also be regulated by their size, an effect which was shown for different size fragments of hyaluronan oligosaccharides in wound healing (Ghazi *et al.* 2012) and angiogenesis (West *et al.* 1985; Cui *et al.* 2009). These findings reinforce the notion that the ECM is a highly dynamic entity that is highly regulated and compartmentalized at the post-translational level, as well as intracellularly at the transcriptional/translational level.

In addition to engagement with cell surface receptors, there is also emerging evidence that ECM fragments may act as a reservoir for critical nutrients under conditions of nutrient deficiency. Recently, Olivares *et al.* (2017) showed that in pancreatic cancer models, collagens are catabolized to release ECM fragments that are then taken up as a proline source when other nutrient fuels are limited. The collagen-derived proline is then metabolized by proline oxidase (PRODH1) to fuel the tricarboxylic acid (TCA) cycle and contributes to pancreatic ductal adenocarcinoma cell survival under restrictive nutrient conditions.

The changing ECM in disease

Remodelling of the ECM during tissue development and homeostasis, and in pathological diseases leads to the dramatic reorganization of biochemical and biomechanical cues presented to the cell. Alterations in the ECM have been linked to various pathologies such as fibrosis and skeletal diseases (Aszódi *et al.* 2006; Nelson & Bissell 2006; Bateman *et al.* 2009), and it has long been known that the tumour ECM is biochemically distinct from normal ECM (Cox & Erler 2011). In solid tumours, changes typically include both over- and underexpression of ECM molecules; increased and decreased degradation and/or post-translational modification; and complete loss of, and even *de novo* synthesis of new ECM molecules. Even small changes can lead to significant alterations in the properties of the tumour ECM. As such, the remodelling of the ECM that accompanies disease onset and progression leads to significant changes in the bioavailability and bioactivity of ECM cues. These cues include altered anchorage points for cells and/or other ECM molecules such as proteoglycans, altered ECM assembly and disassembly dynamics, altered sensitivity to degradation, and altered growth factor sequestration (Bissell *et al.* 2002).

Despite the rapidly expanding body of literature, there is still an enormous depth and complexity to the heterogeneous 3D ECM landscape that is perhaps currently underappreciated. Apart from the basement membrane, our current level of understanding of cancer ECM structure is very much akin to how the cytoplasm was considered an amorphous 'soup' 30 years ago. Just as the understanding of the intracellular space has increased, so the recent advances in matrix biology have begun to shed light on the premise that the tissue ECM is highly compartmentalized and composed of spatially distinct biochemical and biomechanical zones. In its basic sense, this compartmentalization charts distinct differences between basement membrane matrix, pericellular matrix and interstitial matrix, although these represent a gross oversimplification of the ECM organization. Recently, several new approaches have emerged to help us understand and map this spatial complexity and chart the global dynamics of the ECM in health and disease.

We have known for many years, and will discuss in more detail later in this review, how the changes in the tissue ECM of solid tumours is inherently linked to cancer progression from a small isolated tumour to large invasive and/or disseminated metastatic disease. For example, seminal work by Weaver and colleagues showed that post-translational cross-linking of the ECM in premalignant stages of breast cancer is required for malignant transformation to occur and will drive breast tumour progression (Paszek *et al.* 2005; Levental *et al.* 2009). At the same time, Erler and colleagues have shown that similar ECM remodelling is needed to drive breast and colorectal cancer metastatic dissemination and colonization of secondary organs (Erler *et al.* 2006, 2009; Baker *et al.* 2011, 2013a; Cox *et al.* 2013). However,

we still lack a detailed mechanistic understanding of the precise role that the tumour ECM plays at each point of this multistep process. With each transitional step in cancer progression, a number of ECM changes are involved, which modulate the intricate cross-talk between the cancer cells and their surroundings. Many of these changes are significant in terms of scale or magnitude, but it is now emerging that subtler changes in absolute amounts, relative ratios, and/or spatial organization and topology also lead to profound changes in biochemical and biomechanical properties of the cancer ECM that together promote disease progression. As we can typically only study these ECM changes at defined static endpoints, it makes understanding how the continual evolution of the ECM during disease progression incredibly difficult. However, deconstructing the complexity of the tumour ECM and identifying the interactions between cell populations, soluble factors and ECM proteins is an exciting yet challenging field of research.

Over the last 30 years, researchers have shown that tumour cells not only need to accumulate oncogenic genetic alterations, but also rely on permissive cues from a surrounding ECM for malignant progression (Hanahan & Weinberg 2000, 2011; Egeblad *et al.* 2010). For example, it has been shown that the ECM is capable of altering level of mRNA and the rates of synthesis and secretion of proteins consistent with the model of 'dynamic reciprocity' (Bissell *et al.* 1982; Bissell & Aggeler 1987). In this model, the ECM exerts an influence on gene expression via transmembrane proteins (including integrins) and cytoskeletal components. The cytoskeleton is closely linked to polyribosomes, which regulate mRNA stability and rates of protein synthesis, and also with the nuclear matrix, which regulates mRNA processing and rates of transcription. Disrupting these ECM cues alone can therefore alter oncogenic programmes to accelerate or delay tumour progression, as was recently shown in pancreatic cancer by Laklai *et al.* (2016) (Bissell *et al.* 1982, 2002; Bissell & Aggeler 1987; Nelson & Bissell 2006; Bissell & Hines 2011). Thus, the tumour ECM has rapidly become of great interest to cancer researchers who have begun interrogating the importance of aberrantly regulated ECM proteins produced by tumour-subverted stroma.

Extracellular matrix molecules such as tenascins (Mori *et al.* 1996; Hanamura *et al.* 1997; Yoshida *et al.* 1997; Pauli *et al.* 2002; Ilmonen *et al.* 2004; Brellier *et al.* 2009; Pedretti *et al.* 2009; Oskarsson *et al.* 2011; Saupe *et al.* 2013; Didem *et al.* 2014), periostin (Sasaki *et al.* 2001; Bao *et al.* 2004; Tilman *et al.* 2007; Fukushima *et al.* 2008; Puglisi *et al.* 2008; Takanami *et al.* 2008; Contié *et al.* 2011), hyaluronan (Sironen *et al.* 2011; Jacobetz *et al.* 2013) and versican (Ricciardelli *et al.* 2002, 2007, 2009; Pirinen *et al.* 2005; Ween *et al.* 2011; Keire *et al.* 2014) have all been shown to have important roles in the onset and progression of a multitude of different cancers. Furthermore, some of these individual ECM components have been implicated in controlling disseminated tumour cell dormancy (Barkan *et al.* 2010; Oskarsson & Massagué 2012).

Defining the ECM in health and disease

Many of the early ECM studies focused on extensively characterizing single ECM components in great detail. Whilst this led to enormous advances in our understanding of the role of these individual ECM components in health and disease, it adopts a reductionist approach to tackling the enormous complexity of the dynamic ECM landscape. However, given the inherently complex heterogeneous 3D nature of the ECM, including the extensive post-translation modification and cross-linking of ECM components, it has traditionally been very difficult to study the ECM and its function *in vitro* and *in vivo* at the global level. Much of our understanding of the 3D nature of the ECM has come from *in vivo* mouse models which leverage gene-targeting approaches, transgene expression and spontaneous mutations of ECM proteins (Aszódi *et al.* 2006). This has significantly contributed to, and accelerated our mechanistic understanding of the structure and function of the different ECM molecules in tissue homeostasis and disease. In particular, they have been critical in refining our understanding that not all ECM components contribute purely scaffolding roles to the matrix as basic structural elements, but many, if not all, act as organizers/modulators of the structural network and bioactive signalling cues.

The development of high-throughput 'omics'-based approaches has significantly accelerated the widespread global cataloguing of proteomes in almost every living organism. Thanks to the completion of the genomes of many species and to previous studies, it is now clear that vertebrate genomes contain hundreds of genes that encode, or potentially encode ECM proteins. Unbiased global approaches to mapping the proteome of tissue and organ ECMs are giving us new information into the make-up of tissues and how they are altered in the disease setting. In most tissues, it has been shown that the primary ECM scaffold is typically made up of organ- and tissue-specific blends of collagen fibrils or networks. Collagens are seen as the principal load-bearing elements in all tissues from skin to bone. The tissue-specific collagen networks typically stiffen under mechanical loads to prevent excessive and potentially damaging deformation of the tissues. To date, there are 16 different types of collagen, with collagen types I, II and III comprising approximately 75-80% of all of the collagen within our body (Figure 1). These collagens are packed together to form long thin, mainly structural fibrils. In contrast, collagen type IV forms a two-dimensional reticulum and is core to all basal laminae. Several of the other collagen types such as VI and XXVI then form filament networks associated with these fibrillar collagens and act to link them to one another or to other matrix components (Figures 1 and 2, box B). These different collagens and their resulting networks, along with the various other ECM components, undergo constant remodelling to provide required biochemical properties such as tensile strength and elasticity.

Importantly, the ratios of individual collagens and other ECM components to one another are critical in the

subsequent provision of assembly points or sequestration of various proteoglycans and glycoproteins in both a spatial and temporal manner. The result is the formation of highly ordered supramolecular structures that contribute to the various biochemical and biomechanical requirements of the tissue. For example, the specific make-up of glycosaminoglycans on proteoglycans will alter the hydration of the matrix (Figure 2), which in turn alters bulk tissue stiffness, interstitial pressure of tissues as well as rates of diffusion and perfusion within the tissues. At the same time, proteoglycans play a major role in signal transduction with regulatory functions in various cellular processes (Schaefer & Schaefer 2010). Therefore, the biomechanical properties of the ECM can synergize with and antagonize the biochemical cues to drive single-cell and collective cell behaviour resulting in correct tissue and organ function. Recently, there have also emerged exciting reports that tissue stiffness may even regulate cellular circadian clocks, with stiff extracellular matrix dampening the oscillations of the molecular clock within cells (Yang *et al.* 2017; Williams *et al.* 2018). As such, disruption of any single ECM component can, and often does, lead to an enormous array of downstream changes that typically force the establishment of a new dynamic equilibrium and underpin the gross changes in tissue and organ architecture and function typically associated with cancer progression (Mayorca-Guiliani *et al.* 2017).

For example, it was initially thought that increased mammographic density was caused purely by increased deposition of collagens within the mammary tissue. However, recent studies using approaches such as second harmonic generation (SHG) imaging, picrosirius red (PSR) and polarizing light microscopy have demonstrated that raised mammographic density is not associated with merely increased collagen deposition, but the specific spatial organization and topology of collagen fibrils (Huo *et al.* 2015; McConnell *et al.* 2016; Sherratt *et al.* 2016). This organization and topology are, in part, controlled by altered proteoglycan composition, and as a result, several laboratories are investigating how proteoglycans may underpin the physical properties of mammographic density and ultimately contribute to the associated increase in breast cancer risk. Despite numerous studies investigating proteoglycans in relation to mammographic density and breast cancer, the reported results are sometimes discordant. For a comprehensive review of the role of proteoglycans in mammographic density and breast cancer, see Shawky *et al.* (2015). At present, it is not known how localized ECM changes in composition and organization contribute to globally raised mammographic density and subsequently to increased breast cancer progression and metastasis at the mechanistic level. Likewise, there is still a knowledge gap in the specific changes in ECM composition and properties during tumour progression. Understanding how the changing ECM landscape is linked to alterations in mammographic density and subsequently to the onset and progression of breast cancer as well as survival is critically important. High mammographic density is not uncommon in the community, yet the biological basis of the ECM in high mammographic density, and how it raises breast cancer risk remains elusive.

There are many reports detailing ECM changes which have been shown to be involved in cancer progression, poor prognosis and malignant cell invasion at both primary and secondary sites. For example, elevated levels of fibronectin have been shown to promote ovarian cancer invasion and metastasis through $\alpha_5\beta_1$ integrin and activating downstream c-Met/FAK/Src-dependent signalling (Kenny *et al.* 2014). Increased levels of versican, fibronectin, collagen I and decreased levels of vitronectin and tenascin C have been shown in the premetastatic liver in models of pancreatic cancer (Costa-Silva *et al.* 2015). It is becoming increasingly clear that ECM remodelling in tumour progression does not only occur at the primary site of tumour cells, but can also be driven systemically through the release of tumour-secreted factors and exosomes to form so-called premetastatic niches. These premetastatic niches exhibit altered expression of ECM molecules as well as aberrant post-translational modification, which leads to the generation of a growth permissive milieu that acts to support tumour cell colonization of secondary organs. Premetastatic remodelling of the ECM has been shown to occur in several cancers including breast cancer (Erler *et al.* 2009; Hiratsuka *et al.* 2013; Cox *et al.* 2015), lung, pancreatic cancer and melanoma (Kaplan *et al.* 2005; Peinado *et al.* 2012) leading to increases in the resulting metastatic burden in these secondary organs.

Prolonged exposure to the extracellular proteoglycan, lumican, has been shown to restrain pancreatic adenocarcinoma growth (Li *et al.* 2017). In contrast, versican and tenascin have both been shown to be increased in the peritumoral stroma of breast cancer and predictive of poor relapse-free and overall survival respectively, in women with lymph node-negative breast cancer. In fact, versican is localized within the peri-tumoral stromal tissue of many solid cancers and is secreted into the ECM by both host tissue fibroblasts, or expressed by the cancer cells themselves. Several mechanisms have been proposed for regulating versican levels in the tumour ECM. For example, TGF-β-driven secretion of versican in the ovarian tumour microenvironment activates NF-κB signalling and upregulates expression of CD44, matrix metalloproteinase-9 and the hyaluronan-mediated motility receptor in the tumour cells to enhance aggressiveness (Yeung *et al.* 2013; Figure 2). Therefore, inhibiting versican synthesis may be a potential mechanism for reducing versican levels in cancer tissues. Treatment with the tyrosine kinase inhibitor, genistein, has been shown to inhibit versican synthesis induced by growth factors in malignant mesothelioma cell lines (Syrokou *et al.* 1999) and vascular SMCs (Schönherr *et al.* 1997). The biological role and regulation of versican in cancer are discussed in more detail in Ricciardelli *et al.* (2009).

Proteomic cataloguing of the ECM

Previous proteomic analyses of tumours have typically not focused on the ECM, although ECM proteins are usually

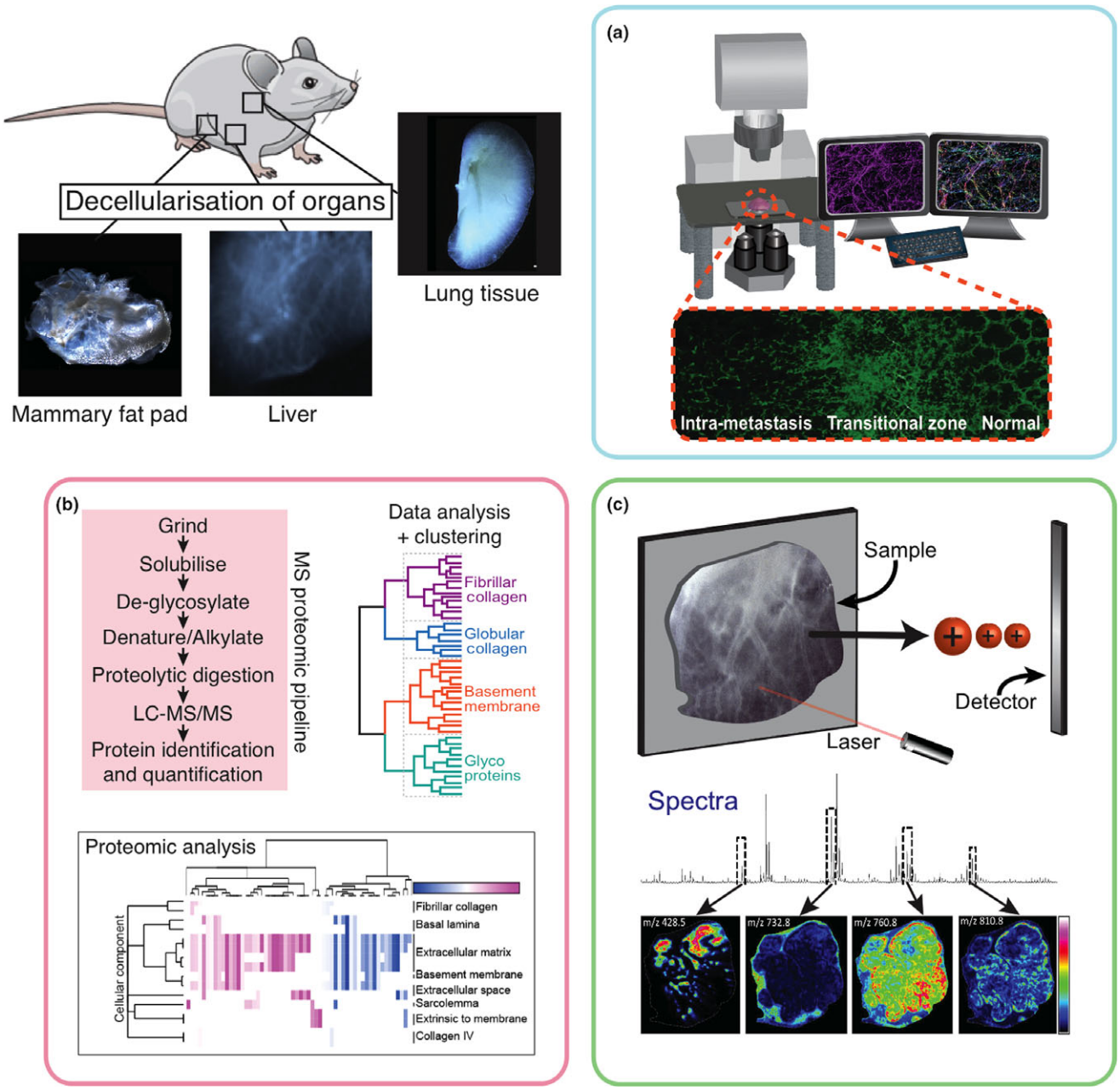

Figure 3 Multiplexing new platforms to study the extracellular matrix in health and disease. Approaches such as tissue decellularization significantly boost our ability to, and resolution at which we can study the extracellular matrix in downstream applications such as; (a) confocal and light sheet microscopy; (b) proteomic cataloguing using liquid chromatography-mass spectrometry and computational analysis; (c) MALDI-Mass Spec Imaging (MALDI-MSI) and spatial mapping of ECM components. [Colour figure can be viewed at wileyonlinelibrary.com]

detected among the tumour proteins analysed (Zanivan *et al.* 2008; Sutton *et al.* 2010; Shaheed *et al.* 2013). This is predominantly as a result of the physical and chemical properties of the ECM. ECM components are heavily cross-linked, highly insoluble and often strongly charged which poses significant technical challenges to mass spectrometry pipelines and have, until recently, prevented effective and routine analysis. However, new proteomic protocols and pipelines have been developed that selectively enrich for ECM proteins based on their relative insolubility compared to intracellular proteins. Quantitative proteomics is now seen as the gold standard method for comprehensively profiling the dynamic changes in the composition of the ECM that occur during disease progression. Selective enrichment for ECM components is primarily based on removal of soluble intracellular proteins from tissue samples in solution (Naba *et al.* 2012, 2015, 2017a) or more recently *in situ* using protocols such as ISDoT (Mayorca-Guiliani *et al.* 2017; Figure 3). In addition, the depletion of highly abundant ECM elements such as collagens, through treatment with collagenases, can enhance

the proteomic coverage of low-abundance proteins in decellularized tissues (Kuljanin *et al.* 2017).

In addition to proteomics-based cataloguing, Naba and colleagues have also pioneered efforts to combine proteomics-based methods with bioinformatic pipelines to reshape our definition of the so-called matrisome. The matrisome is classified as both the ECM and ECM-associated proteins which make up the protein composition of the tissue extracellular matrix (Naba *et al.* 2012). The establishment of the 'Matrisome Project' and the generation of reference matrisomes for different organisms (MatrisomeDB; http://matrisomeproject.mit.edu/) include proteins predicted (based on genomic sequence) to encode matrix proteins because they contain characteristic InterPro (https://www.ebi.ac.uk/interpro/) domain-based structures (Hohenester & Engel 2002), a classical hallmark of ECM proteins. Their inclusion criterion consists of 55 diagnostic InterPro domains commonly found in ECM proteins. The challenge now is to map expression patterns spatially to tissues and temporally to organ development and disease. For a comprehensive review of some of the new bioinformatic tools and experimental strategies for cataloguing the ECM in health and disease and how they can be exploited to provide novel insights in our understanding of ECM biology, see Naba *et al.* (2016).

Building on this, the recent establishment of the ECM Atlas (http://matrisomeproject.mit.edu/ecm-atlas/; Naba *et al.* 2016) also provides a rapidly expanding resource for matrix biologists. The ECM atlas is built through the compilation of proteomic data sets of ECM composition from multiple different tissues and disease types. The next release scheduled for later in 2018 will dramatically expand the ECM Atlas to incorporate new tissues and diseases and mark significant collaborative efforts in the matrix biology field to deepen our understanding of the ECM. Whilst it is becoming commonplace for journals to require that mass spectrometry data sets are made available upon publication, they are typically unwieldy and provide little information to non-experts. Web interfaces such as the ECM Atlas are now providing a common point of reference to easily access and extract useful information from this vast and rapidly expanding reservoir of data.

Moving forward, the next step is to determine the mechanisms leading to this presence of lower or higher abundance of specific ECM proteins in the microenvironment during normal development or disease. In particular, determining whether these changes are the result of transcriptional or translational control mechanisms, or altered secretion, assembly or stability of ECM proteins in the tissue microenvironment. We and others have previously identified novel ECM proteins and ECM modulators in tissue samples from tumour progression models that have previously not been associated with tumour progression (Mayorca-Guiliani *et al.* 2017; Naba *et al.* 2017a; Figure 3, box B). Refining our understanding of the build-up and breakdown of the ECM both in normal tissue and in disease will be critical to understanding how the dynamic ECM regulates tissue homeostasis and disease progression. Furthermore, the ability to define ECM signatures (Figure 3, box B) based on proteomic analysis will be incredibly useful in disease staging. Using label-based quantitative proteomics, Naba *et al.* (2017a) have achieved this and reported the characterization of the matrisome in pancreatic islets and identified a 35-ECM–protein signature that is characteristic of, and correlated with insulinoma progression, whilst in high-grade serous ovarian cancer (HGSOC), Pearce *et al.* (2017) reported a matrix index that could be used to predict poor prognosis. More recently, researchers have identified a nine-gene ECM signature (based on mining of gene transcriptional data sets), which strongly predicts outcome across multiple cancer types, although it still requires validation in prospective cancer cohorts to confirm its prognostic ability (Yuzhalin *et al.* 2018). Just as we now currently use genomic signatures to identify high-risk patients and predict outcome across a wide array of cancer types, it will not be long before we can use ECM-based proteomic signatures in a similar manner.

Seeing is believing: charting the matrix

Cataloguing the *in vivo* ECM composition of normal and diseased tissues using selective ECM enrichment has accelerated the pace of ECM cataloguing in disease (for a full review, see Byron *et al.* 2013). Whilst the information generated is invaluable in understanding changing ECM composition in disease, we are still lacking a matching repertoire of tools facilitating high-definition interrogation of global 3D topology of the ECM and the subsequent study of cell interactions with this native ECM. In addition to cataloguing the ECM, a better characterization of native ECM composition and its spatial and temporal distribution and organization is needed to provide a deeper understanding of its role in disease settings such as cancer.

Whilst several groups, including our own, have investigated ECM changes in 2D patient tissue sections, until very recently no 3D studies have thoroughly characterized or linked the spatial topology of the tumour ECM to disease progression. As a result, we have little insight into the global pathophysiology of the ECM in cancer, where ECM remodelling is a fundamental driver of cancer cell survival, proliferation and metastasis (Cox & Erler 2011). Currently, there are several approaches designed to facilitate high-resolution 3D microscopy of native tissues. These have almost exclusively focused on tissue fixation/clearing approaches and advances in microscopy to improve optical clarity of large volumes of tissue. These approaches include PACT, CLARITY, Sca*l*e/Sca*l*eA2, SeeDB, PARS, Clear^{T}/Clear^{T2}, 3DISCO, RIMS, FocusClear, CUBIC, LUMOS, DBE and BABB (Dodt *et al.* 2007; Tseng *et al.* 2009; Hama *et al.* 2011; Becker *et al.* 2012; Chung & Deisseroth 2013; Ertürk & Bradke 2013; Ke *et al.* 2013; Kuwajima *et al.* 2013; Moy *et al.* 2013; Susaki *et al.* 2014; Tainaka *et al.* 2014; Tomer *et al.* 2014). Some are specifically tailored to organs of interest such as the brain, whilst others such as 3DISCO can be applied more widely. Indeed, it was recently shown that RI-CUBIC (refractive index-optimized clear unobstructed

brain/body imaging cocktails and computational analysis) protocols enable whole-body examination of cancer models at the single-cell resolution (Kubota *et al.* 2017). The focus of these methodologies, however, is to increase imaging resolution whilst preserving cellular structure. These approaches are unfortunately not optimized for improving imaging resolution of the ECM architecture. Similarly, many of these approaches involve tissue fixation and swelling, which may affect tissue integrity and/or biomolecular structures, introduce artefacts, distort native ECM architecture and even irreversibly denature biomolecules.

In vivo the precise assembly of collagens into different fibrils and fibre organizations is also typically tissue dependent. For example, collagens can form thin fibrils in the region of 30 nm in diameter within in the cornea of the eye. However, they will also form much thicker fibres, in some cases several orders of magnitude larger, at several microns, in tissues such as tendons which experience very high tensile strains (Raspanti *et al.* 2018). Therefore, the preservation of the delicate 3D structure of the ECM is critical when attempting to dissect the processes underlying its spatial organization and construction, and the resulting effects on cell behaviour and phenotype. The overall organization of the ECM is largely determined by the positioning and orientation of the collagen fibrils to one another. The resulting ECM pore size, fibre alignment and visco-elasticity, as well as biomolecular composition in turn provide important cues controlling cell behaviour. The organization, alignment and spacing of collagen fibrils are determined, in addition to their abundance, by proteoglycans. This in turn is critical to controlling elements such as the mechanical properties of the tissue. The precise spacing between collagen fibrils is controlled by dermatan/chondroitin sulphate or keratan sulphate chains bound to the protein cores of small leucine-rich proteoglycans (Figure 2, box B). These proteoglycans bind to the specific binding domains of collagen fibrils (approximately every 65 nm), and their sulphated side chains bridge and control the interfibrillar space (Scott *et al.* 1998). Disrupting these proteoglycans significantly alters collagen fibre assembly and the organization of ECM architecture. For example, decorin, a small leucine-rich proteoglycan abundant in normal breast tissue, has been shown to coat the shaft of mature collagen fibrils preventing fibril fusion (Ishiba *et al.* 2014). Decorin knockout (KO) mouse models still show high levels of collagen fibrils within tissues such as the skin, similar to that shown in wild-type mice. However, in KO mice, these collagen fibrils exhibit an abnormal morphology which leads to altered tensile strength and dramatically increases skin fragility (Danielson *et al.* 1997).

In terms of disease, it has previously been shown that the precise organization and orientation of collagen fibres in the tumour microenvironment are also tightly correlated with breast tumour progression (Provenzano *et al.* 2006). Collagen fibre alignment perpendicular to and out from the tumour may be an enabling feature for breast cancer cells to migrate persistently along these fibres away from the primary site (Riching *et al.* 2014). It has recently been shown that increased confinement of MV3 melanoma and HT1080 fibrosarcoma cells, due to increasing collagen concentrations, can lead to 'cell jamming' and acts to drive a switch from single- to collective cell migration (Haeger *et al.* 2014). The importance of mechanoreciprocity, ECM organization and remodelling in determining cell migration and invasion and the concept of cell jamming are discussed in more detail in van Helvert *et al.* (2018), and a comprehensive review of cellular mechanotransduction at the cell–matrix interface is discussed in Jansen *et al.* (2017).

Decellularization of tissues to produce native ECMs is a powerful tool to study the matrix (Figure 3). Decellularized ECM (assuming the use of correct decellularization treatments) is identical to the composition of native ECM and is generally considered to possess native mechanical properties (e.g. stiffness) and microstructure. In the tissue regeneration and bioengineering field, decellularized tissues and organs are often used as scaffolds to facilitate the repair and reconstruction of tissues. Recently, researchers have also begun investigating the way that decellularization approaches affect the integrity of the ECM including the preservation of glycosaminoglycans (GAG), collagens and cytokines (Fischer *et al.* 2017). A clear benefit of tissue decellularization is that it improves the optical clarity of large volumes of tissue for studying the ECM using conventional approaches such as confocal and multiphoton microscopy as well as emerging techniques such as light-sheet microscopy. As such, tissue decellularization for matrix biology bridges the gap between *in vivo* whole-organ imaging and standard 2D histology.

We recently developed a fast and efficient approach to enhance the study of ECM composition and 3D structure of normal and diseased tissues in exquisite detail (Figure 3). Termed *In situ* decellularization of tissues (ISDoT), it allows tissues to be decellularized without collapse to leave the delicate native ECM architecture intact. The optically clear 3D ISDoT decellularized tissues can be studied using high-resolution confocal and state-of-the-art multiphoton microscopy without the need for extensive preparation, tissue fixation or sectioning. Furthermore, ISDoT acts to enrich ECM molecules making it perfectly suited to quantitative proteomic interrogation of the ECM to accurately catalogue global changes during cancer progression (workflow shown in Figure 3). Using ISDoT, we have shown that this enrichment facilitates an approximate order of magnitude increase in proteomic coverage compared to non-decellularized tissues (Mayorca-Guiliani *et al.* 2017). This therefore allows reliable identification and quantification of ECM molecules otherwise masked in the presence of cellular content. Specifically, it allows the accurate detection of subtle changes in ratiometric levels of ECM components. As discussed above, the changing ECM in cancer is not only characterized by increased ECM deposition. It is also important to distinguish between increased absolute amounts and increased concentrations of ECM components relative to one another during disease progression (Cox & Erler 2011). This can only be carried out with global 'omics' approaches and is not immediately possible when looking at single molecules in

isolation. Using matched ISDoT samples, we can both comprehensively catalogue the ECM and map the spatial distribution of components in 3D in high resolution in the normal *vs*. tumour setting. As such, this work was the first to provide detailed 3D characterization of the metastatic niche in breast cancer progression and identified several ECM components, which have not previously been implicated in cancer onset and progression (Mayorca-Guiliani *et al*. 2017). Recently, complementary decellularization approaches to study the ECM in development of whole murine embryos has also begun to visually map and interrogate proteoglycan-rich ECMs in multiple developing tissues, including the forelimb, eye and spinal cord (Acuna *et al*. 2018).

This work is part of the emerging trend of applying cutting-edge technologies to ECM research and is set to revolutionize the way in which we study the ECM in disease. For decades, the ECM was seen as 'the stuff outside cells', but we have shown that there is a highly ordered compartmentalization and localization of specific matrix molecules within the tumour microenvironment (Figure 3a, note the observed presence of the transition zone at the tumour interface). This compartmentalization is a fundamental feature of the ECM landscape and underpins the very essence of tissue and organ complexity. Each compartment constitutes a unique and specific context of biochemical and biomechanical cues based on its protein composition and spatial organization/topology. As such, the ECM is pivotal in contributing to both inter and intratumour heterogeneity. The ability to spatially map ECM heterogeneity will be critical to understanding how it modulates cancer cell phenotype, invasive/migratory behaviour, proliferative capacity and even stemness and plasticity (Bissell & Hines 2011). Similarly, by comparing and contrasting different ECM compositions and topologies across cancer types, we may be able to stratify and treat patient tumours using ECM-based signatures rather than the organ from which they originate.

Using 'omics to see the matrix – MALDI-MSI

Matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) has emerged as a powerful analytical technique that combines the specificity and sensitivity of mass spectrometry with spatial information to map the tissue-wide distribution of multiple analytes including proteins (Caprioli *et al*. 1997), peptides (DeKeyser *et al*. 2007), lipids (Woods & Jackson 2006; Jackson *et al*. 2007) and small molecules (Reyzer *et al*. 2003; Cornett *et al*. 2008; Dekker *et al*. 2009) directly from a single tissue section (Figure 3, box C). Current approaches can accurately identify and quantify hundreds of native (or modified) analytes simultaneously in a single experiment, with a typical spatial resolution of between 10 to 200 microns. MALDI-MSI allows direct label-free measurement of analytes from tissue sections, and spatial 'molecular histology' images can be reconstituted for each observed signal. Whilst the approach is conducted on 2D sections of tissue, it creates a digital MALDI image for each ECM component, which combined with serial sectioning and image registration and integration has the potential to generate 3D detailed reconstructions of the ECM topology and architecture (Figure 3, box C).

Cancer samples are highly heterogeneous with many different histological ECM structures on a small scale. As MALDI-MSI preserves the spatial distribution and the histology of the samples, it is perfectly suited for the analysis of these highly heterogeneous cancer samples. As such, MALDI-MSI is a highly flexible and powerful imaging technology and is not only proving to be a uniquely useful discovery tool for mapping tissue-wide distributions of analytes, but can also be used to look for specific molecules from pre-generated targeted lists. Moreover, subtle molecular ECM signals or patterns can be identified and correlated with tumour subtypes or other parameters such as outcome or response to treatment.

Recently, MALDI-MSI has been successfully combined with decellularization approaches to significantly improve identification rates and the resolution of spatial mapping of ECM components in tissues (Gessel *et al*. 2015; Figure 3). However, there are limitations to MALDI-MSI when it comes to high molecular weight (100's of kDa) components such as glycoproteins abundant in some tissues, because they are currently beyond the practical mass range for protein imaging experiments (Gessel *et al*. 2015). Whilst the spatial resolution of MALDI-MSI is typically less than that obtained by confocal, multiphoton and light-sheet microscopy, it does not rely on the availability of validated antibodies that may hamper other visualization approaches. It can in some cases also be used to map post-translational modifications such as protease cleavage, cross-linking, citrullination, nitrosylation, glycosylation and isomerization of ECM molecules.

Post-translational modification and regulation of the ECM

The natural ageing process of the ECM and various pathologies correlate closely with alterations in the post-translational modification of ECM molecules. These modifications affect the structural and mechanical integrity of the tissues, altering among other things their susceptibility to degradation. Classic examples of this are skin wrinkling, bone and cartilage deterioration, as well as cardiovascular and respiratory malfunctions. Typically, specific post-translational modifications are the result of local physiological or pathobiological processes. As such, the identification of specific post-translational modifications could be used as disease-specific markers for staging or ageing of the tumour. For a review on how post-translational ECM modifications are seen as key events in cancer progression, see Leeming *et al*. (2011).

Excessive post-translational modification of the ECM is associated with many solid tumours. In particular, the post-translational modification of fibrillar collagens has become an area of intense interest given its importance in tumour-associated desmoplasia. Normal collagen synthesis requires

several post-translational modifications, such as hydroxylation of proline and lysine residues, propeptide cleavage and the covalent cross-linking by lysyl oxidases (Figure 2, box B; Gerstenfeld *et al.* 1993; Myllyharju & Kivirikko 2004; Mäki 2009). The overexpression of the collagen cross-linking lysyl oxidases has been shown by us and others to be key players in the progression of several solid cancers including breast, pancreatic, colorectal, head and neck and more (Payne *et al.* 2005; Erler *et al.* 2006, 2009; Laczko *et al.* 2007; Bondareva *et al.* 2009; Levental *et al.* 2009; Sakai *et al.* 2009; Baker *et al.* 2011, 2013a,b; Taylor *et al.* 2011; Chu *et al.* 2012; El-Haibi *et al.* 2012; Cox *et al.* 2013, 2015; Pickup *et al.* 2013; Miller *et al.* 2015; Chang *et al.* 2017; Rachman-Tzemah *et al.* 2017; Reynaud *et al.* 2017). For a comprehensive full review of the role of the lysyl oxidase family in cancer see (Barker *et al.* 2012). Importantly, targeting the lysyl oxidase family has shown promising preclinical results and is seen to offer significant promise in targeting the remodelling of the ECM in cancer.

In addition to altered deposition, turnover or reorganization of the ECM, there are a large number of long-lived proteins that are prone to the accumulation of damage with both age and disease (Sherratt 2009). In fact, many structural ECM proteins exhibit a remarkable longevity *in vivo*, often measured in years as opposed to hours for intracellular proteins. This longevity also appears to be tissue specific and likely comes down to fibril organization and structure (discussed above). For example, types I and II collagen in human skin, articular cartilage and intervertebral disc exhibit 15-, 95- and 117-year half-lives respectively (Verzijl *et al.* 2000; Sivan *et al.* 2008). When we consider these timescales, events such as glycation-mediated collagen cross-linking, the normal and slow accumulation of cross-links that form advanced glycation end products (AGEs) become important and are thought to play a key role in many age-associated diseases, including degenerative eye disease, pulmonary fibrosis, arterial stiffening and cardiovascular disease, and neurodegeneration. Furthermore, these manifestations are increasingly accelerated in diabetes patients who exhibit chronically elevated blood glucose levels (Bunn *et al.* 1978; Frank 1991; Vitek *et al.* 1994; Sasaki *et al.* 1998; Glenn & Stitt 2009). The aberrant glycation of proteins leading to AGEs has been shown to be associated with increased tissue stiffness in tissues such as the prostate (Rodriguez-Teja *et al.* 2015), and such changes in tissue stiffness are also closely associated with cancer onset and progression (Bailey 2001; Paszek *et al.* 2005; Sharaf *et al.* 2015).

If we apply this to the cancer setting, it could be argued that the development of cancer in an aged individual may be more likely to be accompanied by accelerated progression and enhanced propensity for metastasis, than if the same cancer was to develop at a younger stage of life. A hypothesis of this nature would centre around a central theme that a slowing down of active ECM remodelling and the gradual replacement with passive cross-linked, and/or post-translationally modified ECM components would ultimately lead to a more aggressive tumour. In support of this, work by Maller *et al.* (2013) has attempted to address why clinically, there is a reduction in breast cancer risk in postpregnancy women. Following pregnancy, during involution, a vast developmental programme remodels the breast tissue following cessation of milk production. In their study, the authors reported an approximately 50% increase in abundance of collagen I in postparous rat mammary tissue and also in clinical samples. However, more importantly, they noted a significant decrease in the level of collagen I linearization as measured by second harmonic generation (SHG) imaging compared to nulliparous mice. Thus, it is clear that collagen I organization and not merely abundance plays an important role in parity-induced protection. These findings support the hypothesis that the active reorganization brought about by pregnancy-induced remodelling of the mammary gland may act to reset the accumulation of detrimental and/or protumorigenic post-translational modifications which slowly accumulate over time (Cox & Erler 2011).

The therapeutic repertoire of the matrix in disease

The vast majority of cancer studies place an emphasis on studying the behaviour and intrinsic signalling pathways of tumour cells. Their goal is to identify ways to target intracellular pathways regulating cancer. Indeed, most, if not all, of the chemotherapeutic regimens for cancer are directed at the tumour cells and not the ECM. We and others have shown that cancer progression and metastasis are strongly influenced by the microenvironment and in particular the ECM. As such, we argue that the ECM presents a vast, and at present, unexplored repository of anticancer targets and will briefly summarize some current and emerging approaches to targeting it in cancer.

One approach to therapeutically treating diseases such as solid tumours via their aberrant ECM is to exploit the presence of unique, or overexpressed ECM molecules as targeting beacons. For example, Ishihara and colleagues recently showed that they could conjugate immune checkpoint antibodies to the heparin binding domain (HBD) of placental growth factor-2 (PIGF-$2_{123\text{-}144}$) which shows exceptionally high affinity for multiple ECM proteins (Martino *et al.* 2014). Peri-tumoral injection of PIGF-$2_{123\text{-}144}$–αPD-L1 led to higher retention within tumour tissue. This in turn significantly reduced systemic toxicity and elicited a highly effective antitumour response in the B16F10 melanoma model (Ishihara *et al.* 2017). Similarly, collagen I targeting of cetuximab, an anti-EGFR monoclonal antibody, showed good therapeutic efficacy in the A431 epidermoid cancer model. By targeting cetuximab to the collagen-rich tumour ECM, it led to a longer retention time in tumours with no loss in potency (Liang *et al.* 2016). However, despite this encouraging data, it may prove more powerful to target the less ubiquitous molecules, such as those that are expressed exclusively in specific tumours.

A recent success of note in targeting ECM molecules directly in cancer to improve outcome is highlighted by the phase II HALO 202 clinical trial (NCT01839487). Metastatic pancreatic ductal adenocarcinoma is typically characterized by accumulation of excessive hyaluronan (HA), a large, linear polysaccharide within the tumour microenvironment. The increased HA leads to elevated interstitial pressure and as a consequence is thought to impair perfusion and chemotherapy delivery to tumours. In preclinical studies, pegvorhyaluronidase alfa (PEGPH20), a pegylated recombinant human hyaluronidase, was shown to successfully degrade intratumoral HA and remodel the tumour stroma to improve perfusion and drug delivery (Thompson *et al.* 2010; Provenzano *et al.* 2012; Jacobetz *et al.* 2013). Based on this, the recent phase II clinical trial in patients with previously untreated metastatic pancreatic ductal adenocarcinoma showed that in those with high HA tumours, treatment with PEGPH20 plus standard-of-care chemotherapy (gemcitabine + Nab-paclitaxel) significantly improved progression-free survival (Hingorani *et al.* 2017), highlighting how combinatorial approaches which target the ECM as well as cancer cells offer enormous promise.

The tightly controlled homeostasis of the ECM is sensitive to altered expression of matrix-degrading enzymes such as matrix metalloproteinases (MMPs; Figure 2), which are typically altered for prolonged periods of time in fibrotic diseases and cancer. Unfortunately, a range of matrix metalloproteinase (MMP) inhibitors have to date proven largely unsuccessful in the clinic. This is likely due (among other reasons) to their lack of specificity and ubiquitous nature of expression throughout multiple tissues in the body (Coussens *et al.* 2002). For a full and comprehensive review on MMP-targeting successes and failures, see Cathcart *et al.* (2015).

A recent example of the difficulties associated with targeting post-translational cross-linking in the matrix has been highlighted with the lysyl oxidase-like 2 (LOXL2) antibody, simtuzumab, which showed no significant improvement in overall survival in a phase II trial in late-stage metastatic pancreatic cancer (Benson *et al.* 2017), or in a phase II trial in metastatic KRAS mutant colorectal adenocarcinoma (Hecht *et al.* 2017). However, it should be pointed out here that in these trials, the late-stage terminal nature of disease in these patients was unlikely to yield significant results, and as such, earlier treatment will likely lead to more promising results in line with preclinical studies using lysyl oxidase inhibitors in breast (Erler *et al.* 2009; Cox *et al.* 2013, 2015; Chang *et al.* 2017), colorectal (Baker *et al.* 2011, 2013a,b) and pancreatic cancers (Miller *et al.* 2015).

In addition to targeting or exploiting ECM elements directly, other approaches have focussed on targeting the downstream cellular response to altered ECM in diseases such as cancer, multiple sclerosis and inflammatory bowel disease. The most prominent target is the major matrix engagers, the integrins and their downstream signalling machinery. However, success has been painfully limited on this front, and of the 24 known human integrins, three are currently targeted therapeutically by monoclonal antibodies, peptides or small molecules. To date, targeting the platelet $\alpha_{IIb}\beta_3$ integrin is used to prevent thrombotic complications after percutaneous coronary interventions, and compounds targeting the lymphocyte $\alpha_4\beta_1$ and $\alpha_4\beta_7$ integrins are showing promising indications in multiple sclerosis and inflammatory bowel disease. Building on this, new antibodies and small molecules targeting β_7 integrins ($\alpha_4\beta_7$ and $\alpha_E\beta_7$ integrins) as well as their ligands are currently in clinical development for the treatment of inflammatory bowel diseases. Generally, integrin-based therapeutics have shown clinically significant benefits in many patients, and as a result, this has led to the continued interest in further developing novel integrin inhibitors. Of note is that almost all integrin antagonists in use or in late-stage clinical trials target either the ligand-binding site or the ligand itself. As we begin to expand our repertoire of integrin-based therapies, it will be essential to co-develop companion biomarkers (discussed below) to ensure appropriate administration to maximize patient benefit. For a comprehensive review, including recent clinical trials, see Ley *et al.* (2016).

Downstream of integrin–ECM engagement is the molecular machinery, comprised of large complexes of multiple kinases that action change within the cells in response to exogenous ECM cues. As such, the use of small molecule kinase inhibitors that target these signalling pathways regulated by the ECM such as focal adhesion kinase (FAK; Lightfoot *et al.* 2004; Golubovskaya 2014; Jean *et al.* 2014; Lee *et al.* 2015) 89,90 and Rho-associated protein kinase (ROCK; Kular *et al.* 2015; Rath *et al.* 2016; Guerra *et al.* 2017; Vennin *et al.* 2017b; Whatcott *et al.* 2017) have proven to be effective in targeting the accompanying desmoplasia in cancer. Whilst these approaches show promise, without the addition of adjuvant cytotoxic therapy or radiotherapy, they do little to physically debulk and regress tumours. For a detailed discussion on the clinical potential of targeting ECM stiffness through downstream cellular signalling to combat diseases, see Lampi and Reinhart-King (2018). Finally, as mentioned above targeting intracellular signalling to prevent ECM secretion and deposition, such as treatment with the tyrosine kinase inhibitor, genistein, to inhibit versican synthesis, has also showed promise in preclinical models of malignant mesothelioma (Syrokou *et al.* 1999).

The call for better companion biomarkers to stratify patients for therapeutic intervention is clear. In our own studies in preclinical models of breast cancer, we have shown that during cancer progression and metastatic colonization of secondary sites, there are changes in expression of both unusual and unreported ECM proteins within the tumour and metastatic microenvironment (Mayorca-Guiliani *et al.* 2017), identifying them as potentially new diagnostic and/or prognostic markers for ECM-based therapeutics. The above-mentioned HALO 202 phase II clinical trial only reported benefit of administration of pegvorhyaluronidase alfa (PEGPH20) in HA-high pancreatic tumours showing little positive effect in HA-low pancreatic tumours (Hingorani

et al. 2017). In high-grade serous ovarian cancer (HGSOC), Pearce *et al.* (2017) recently reported a matrix index that could be used to predict poor prognosis, extending earlier work by Naba *et al.* (2017b) on the proteomic characterization of ECM changes in triple-negative breast cancer and adjacent mammary tissue, and omental metastasis from high-grade serous ovarian cancer and normal omentum. Importantly, these studies collectively illustrate that despite high levels of genetic heterogeneity within tumours, the changes in the ECM and the underlying mechanisms behind these changes, as well as the pathways they subsequently activate, may be more consistent and as a result represent more robust therapeutic targets.

Finally, the significant ECM remodelling that accompanies cancer progression has also been shown to be critical to facilitating metastatic colonization (Cox *et al.* 2013, 2015) and as such may represent markers for patients with already-disseminated disease. By leveraging this information, we can better stratify cancer patients, as well as identify and deploy new combination treatments that co-target the ECM and ECM remodelling alongside standard-of-care cancer therapies (Miller *et al.* 2015; Vennin *et al.* 2017a) to improve patient outcome.

Concluding remarks

The composition of the ECM and the distribution of its components define tissue architecture and modulate cellular responses to a plethora of exogenous cues. The complexity of the ECM makes it difficult to identify the precise functional repertoire of ECM proteins by biochemical or cell culture experiments alone. Hence, mouse models and human biopsies are critical to analysing the biological significance of ECM components in a wide variety of *in vivo* processes.

For the most part, we have developed sensitive methods to generate a list of building materials and can look at a final snapshot of the matrix composition and architecture. However, we must still conjecture the assembly dynamics. It would be the same as looking at a completed building and the materials list and piecing together how it was constructed. We are missing the process, and at present, we lack the tools required to watch in real time the assembly process of the ECM taking place *in vivo*. However, new approaches and platforms are continually being developed and it should hopefully not be long before we can watch ECM assembly and dynamics *in vivo* in 3D in real time.

From a therapeutic perspective, further studies are necessary to more clearly delineate deleterious and beneficial aspects of the ECM in the biology of cancer, but will likely offer a reservoir of new therapeutic avenues. Furthermore, rather than just identifying the presence of tumours in routine screening approaches such as biopsy, the ability to predict, based on a tumour's ECM landscape and genetic status, whether it may or may not have metastasized, and to where, and more importantly whether it may be refractive or responsive to a particular therapy will be of great significance in the clinical setting. This will significantly contribute to refining and personalizing the treatment regimens of patients and bring us closer to evidence-based, personalized medicine and improved patient care.

Acknowledgements

We apologize to authors whose work could not be discussed here because of space limitations. TRC is supported by a Susan G. Komen Career Catalyst award and the Cancer Institute of New South Wales (CINSW). JLC and ECF are supported by the National Health and Medical Research Council (NHMRC) of Australia. TRC was the recipient of the 2016 British Society for Matrix Biology (BSMB) John Scott Young Investigator Award.

Conflict of interest

The authors declare no conflict of interest.

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