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The matrix in cancer

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Abstract | The extracellular matrix is a fundamental, core component of all tissues and organs, and is essential for the existence of multicellular organisms. From the earliest stages of organism development until death, it regulates and fine-tunes every cellular process in the body. In cancer, the extracellular matrix is altered at the biochemical, biomechanical, architectural and topographical levels, and recent years have seen an exponential increase in the study and recognition of the importance of the matrix in solid tumours. Coupled with the advancement of new technologies to study various elements of the matrix and cell–matrix interactions, we are also beginning to see the deployment of matrix-centric, stromal targeting cancer therapies. This Review touches on many of the facets of matrix biology in solid cancers, including breast, pancreatic and lung cancer, with the aim of highlighting some of the emerging interactions of the matrix and influences that the matrix has on tumour onset, progression and metastatic dissemination, before summarizing the ongoing work in the field aimed at developing therapies to co-target the matrix in cancer and cancer metastasis.

Supramolecular
An entity consisting of a complex organization of more than one building block.

Dynamic reciprocity
The ongoing and bidirectional interaction between cells and their microenvironment, and in particular the extracellular matrix.

Desmoplasia
The dense fibrotic tissue that forms in response to insult to a tissue. It is typically observed in and around solid tumours characterized by the excessive or abnormal deposition of extracellular matrix.

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The extracellular matrix or 'matrix' is the ubiquitous, acellular component in all tissues and organs of the body. Matrix molecules are typically secreted and assembled into insoluble entities (although they can remain soluble until needed), and the matrix is crucial during organism development, tissue repair, and tissue and organ homeostasis throughout life. In addition to providing resident cells with architectural and mechanical support and protection, the matrix presents a diverse pool of cues that regulate and fine-tune every cellular process, including cell proliferation and survival, cell fate determination, cell migration and invasion, and tissue morphogenesis[1].

The matrix is composed of hundreds of different building blocks that group or bond together to form a vast three-dimensional supramolecular entity. The various geometries, shapes, structures and topologies formed by the interaction of the numerous matrix components span orders of magnitude from nanometres to millimetres (FIG. 1). Coupled with the post-transcriptional splicing of mRNA encoding matrix proteins and the extensive post-translational modification of matrix components, the body can produce an almost infinite array of matrices. The matrix is also 'dynamic', continually undergoing remodelling and renewal over time as well as in response to perturbations.

There is a delicate, reciprocal interaction between the matrix and the cells within it; cells deposit, break down and remodel the matrix, while the matrix simultaneously influences cell behaviour. This complex, mutually instructional interaction, which was termed 'dynamic reciprocity' almost 40 years ago[2], means that the matrix can be considered central to the entire physiology of tissues and organs.

In cancer, the matrix becomes highly dysregulated, playing both protumorigenic and antitumorigenic roles. Indeed, tumour desmoplasia is common in several solid tumours and resembles many of the facets of chronic tissue fibrosis[3] (BOX 1). The loss of correct matrix organization and homeostasis is often considered a hallmark of solid tumours. Furthermore, changes in the matrix that are associated with solid tumour onset and progression are often a driver and marker of transitional events. Importantly, both tumour cells and non-malignant stromal cells contribute to, and consequently are affected by, deposition and remodelling of the matrix[4].

In the past three to four decades, there has been an exponential increase in the study, and recognition of the importance, of the matrix in cancer. Furthermore, in the past 10–15 years the matrix has emerged as a reservoir of predictive, diagnostic and prognostic companion biomarkers, as well as of novel therapeutic targets, for cancer, with many laboratories working to develop and validate these new areas of research. This Review will briefly touch on many of the facets of the matrix in cancer, highlighting examples of its interactions and influences during tumour onset, progression and metastatic dissemination, before summarizing some of the ongoing work on therapies that target the matrix in solid cancers. Although many of the concepts discussed are relevant to a large number of solid tumours, examples are drawn from desmoplastic tumours (that is, those characterized by chronic inflammation, fibroblast expansion and activation, elevated angiogenesis, and in particular,

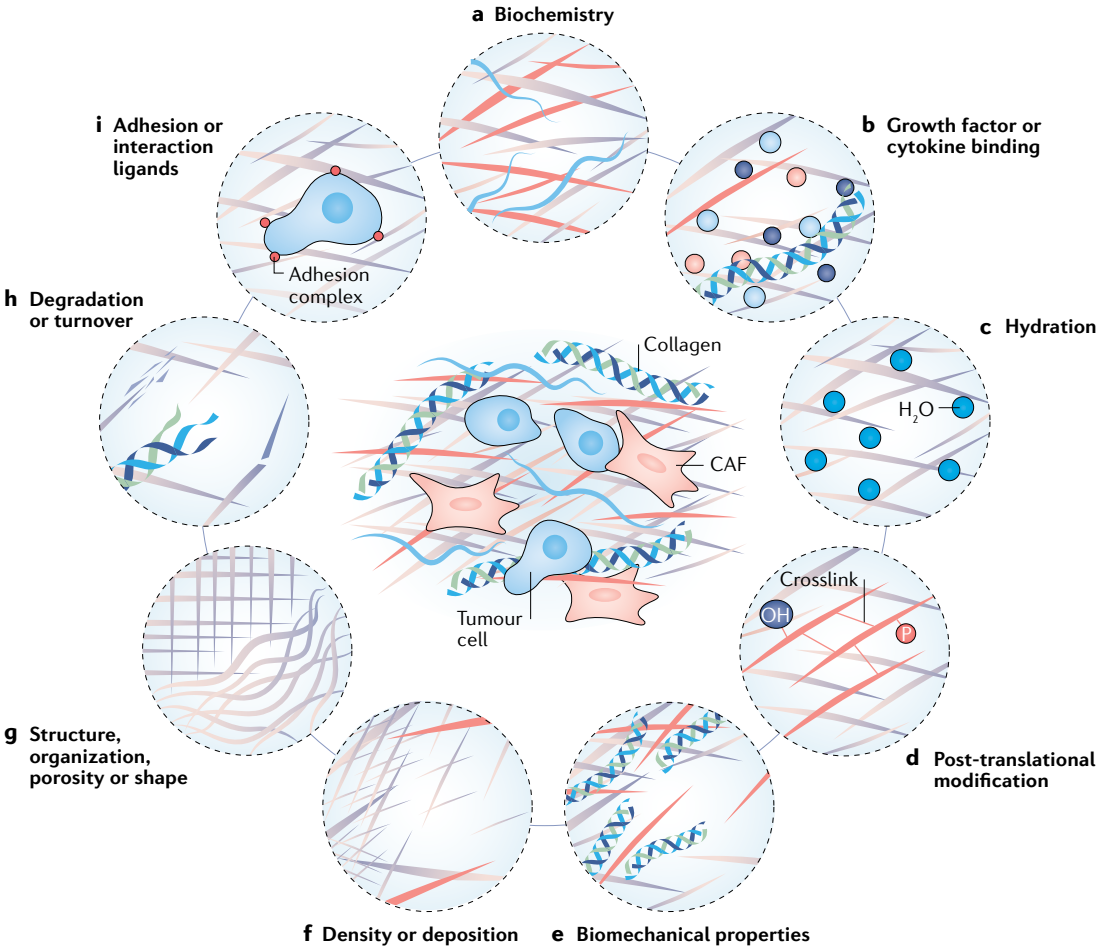

Fig. 1 | **Matrix changes in cancer.** The extracellular matrix is altered in many ways during tumour progression at both primary and secondary sites. In addition to biochemical changes, the biophysical properties of the matrix are also important and include nanotopography, microtopography and macrotopography, molecular density, stiffness, rigidity and tension. Localized changes in the biochemistry and composition of the matrix, which are predominantly driven by local and/or recruited non-malignant stromal cells, and to a lesser extent by tumour cells[4], create unique spatial compartments within the tumour (panel **a**) that will lead to, among other things, changes in the local sequestration of growth factors, cytokines and inorganic molecules (panel **b**). These changes also alter hydration (predominantly as a function of the levels of large polyanionic polysaccharides such as chondroitin sulfates and hyaluronan) (panel **c**), which affects the diffusion and perfusion of molecules throughout the tumour. Changes in the post-translational modification of the matrix, such as hydroxylation or enzymatic crosslinking (panel **d**), typically lead to changes in the biomechanical properties of the matrix (panel **e**), which, in turn, activate mechanosensing pathways within all cells that are present in the tumour microenvironment. Altered deposition of matrix components alters matrix density (panel **f**), triggering changes in matrix structure and organization as well as porosity (panel **g**), which, coupled with aberrant matrix degradation and turnover (panel **h**), markedly alters the number and spacing of adhesion and interaction ligands presented to cells (panel **i**). CAF, cancer-associated fibroblasts.

Matrisome
All of the extracellular matrix proteins that can potentially be expressed by the genome of a specific organism.

Basement membranes
Structures visible by light microscopy and, in addition to the basal lamina, that consist of layers that are typically secreted by cells from underlying connective tissue. Many basement membranes are rich in fibronectin.

Basal lamina
A molecularly defined part of the basement membrane comprising an electron-dense layer, ~20–100 nm thick, that consists of collagen IV and laminin, only visible by electron microscopy. It is made and maintained by the cells that sit on it, acting as the critical point of attachment.

increased levels of remodelled and often crosslinked matrix molecules), including breast, pancreatic and lung cancer, as these are usually associated with a dense fibrotic stroma and are some of the most well-studied tumour types.

Major components of the matrix

The term 'extracellular matrix' is used to describe the complex interconnected network of macromolecules that surround and support cells within organs and tissues. The entire set of matrix and matrix-associated proteins that can be potentially expressed by a genome has been termed the 'matrisome'[5]. There are ~1,100 matrix genes in the mammalian matrisome, which can be broadly split into core-matrisome molecules (~300 genes) and matrisome-associated molecules (~800 genes)[5]. The matrisomal proteins encoded by these genes are estimated to account for ~4% of the human proteome.

Extracellular matrices can be broadly divided into two major classes: interstitial matrix and highly specialized organ-specific or tissue-specific matrices, such as basement membranes (see also the glossary description for basal lamina). Both classes are highly compartmentalized and ordered assemblies of strength-conferring fibrillar meshworks, sheet-like networks and viscous compression-resistant molecules.

The macromolecules that make up different tissue and organ matrices are classified on the basis of their molecular composition (TABLE 1). Some of these classes, such as the collagens, constitute up to 30% of total body

Matreotype
The specific, acute state of matrix composition (and/or modification) at a given point, associated with, or causal for, a given physiological condition or phenotype.

Glycosaminoglycan
Also known as mucopolysaccharides, glycosaminoglycans are the most abundant heteropolysaccharide in the body. They are complex linear polysaccharides consisting of repeated alternating units of uronic acid and glycosamines.

protein and as much as 90% of some tissues, such as connective tissue[6]. Cells express different matrix components through time and space and in response to various cues, and the matrisomal proteins specific to a particular cell type, location or place in time and/or space have been termed the cellular 'matreotype'[7].

Each matrix or matrix compartment is uniquely characterized, and its properties are locally and globally shaped, by its biochemistry and biophysical parameters[8] (FIG. 1). The biophysical parameters enable the matrix to fulfil scaffolding and mechanical force-bearing functions, and its biochemistry (comprising its composition and post-translational modifications) enables the matrix to present ligands for cell attachment and interaction[9,10]. Collagens and glycoproteins are two major classes of matrix protein (TABLE 1).

Collagens. Collagens are the main structural element of the matrix and are generally the most abundant protein within it; 28 collagens have been identified to date[11]. They are typically the matrix component most commonly observed to have undergone a change in one of a number of parameters, including deposition, degradation, post-translational modification and organization in solid tumours compared with healthy tissues, likely due to their abundance and the range of tools available to study them. Collagens provide mechanical strength and bioactive sites for cell adhesion and regulate cell migration[12]. The deposition of fibrillar collagen in the extracellular space is typically coordinated by matrix glycoproteins (see later), such as fibrin and fibronectin, which are typically bound to cell-surface receptors. By providing organizational cues and binding sites, fibronectin concentrates collagen molecules to promote the interactions required for fibrillogenesis[13].

Box 1 | **The matrix in tissue fibrosis and the link to cancer**

The body's capacity to heal injured tissue is crucial for survival. However, chronic or repeated injury or irritation in any organ can result in a failure to heal and the onset of tissue fibrosis. Progressive fibrosis in organs shares common cellular and molecular pathways with the desmoplasia seen in many solid tumours, involving the excessive accumulation of a wide array of extracellular matrix components. Importantly, almost all cases of tissue fibrosis cause a progressive loss of tissue function. Organ fibrosis differs from tissue scarring in acute wound repair in both the composition and the volume of the matrix produced.

The close relationship between fibrosis and the progression of solid tumours is well documented[3,14,144], and involves many matrix molecules. In addition to playing a role in the progression of solid tumours, underlying conditions of aberrant tissue remodelling are important risk factors in the onset of cancer. For example, environmentally induced fibrotic disorders of the lung (such as chronic obstructive pulmonary disorder and emphysema) increase the incidence and progression of lung cancer[303]. Liver fibrosis, and in particular cirrhosis caused by alcohol abuse, nutritional deprivation, non-alcoholic fatty liver disease or hepatitis, also increases the risk of hepatocellular carcinoma[304]. In the breast, high mammographic density (characterized by dense breast tissue matrix) and fibrotic breast disease are associated with a predisposition to breast cancer[305,306].

Additionally, underlying fibrosis in secondary organs, driven by non-tumour events, can create tumour-supportive microenvironments in these tissues, enhancing the future colonization of circulating tumour cells[224]. The matrix remodelling in these fibrotic tissues is similar to that in the formation of premetastatic niches, an emerging concept in the field of metastasis research. Thus, matrix remodelling that occurs as part of non-tumour-associated tissue fibrosis may create microenvironments that facilitate primary and secondary overt colonization of the tissue; investigating these links is an area of intense research.

Fibrillar collagen type I is a major component of tumour desmoplasia and is causally linked to tumour cell survival and metastasis in many tumour types[3]. In healthy tissues, interstitial fibrillar collagens are usually isotropically oriented, whereas the collagen in tumours is often highly aligned and anisotropic[14–16]. However, as not all 28 collagens are protumorigenic, the absolute and ratiometric blend of the different collagens controls the compliancy, stiffness, porosity and viscoelastic and biochemical properties of the matrix in tissue homeostasis and in cancer.

Collagen- and laminin-rich basement membrane and basal lamina matrices define discrete boundaries within tissues, typically separating endothelial and epithelial layers from the interstitial matrix. Cancer cells must typically breach these specialized basement membranes to become invasive. Light microscopy studies have revealed that, compared with those surrounding healthy tissue, basement membranes surrounding premalignant lesions are typically thinner and contain less collagen, such as less collagen XV in human colon carcinoma samples[17] and less collagen XV and collagen XIX during progression from ductal carcinoma in situ to invasive carcinoma in human breast cancers[18]. These changes are in conjunction with the commonly seen loss of collagen IV (and the glycoprotein laminin) from basement membranes surrounding premalignant lesions, which is traditionally thought to facilitate tumour invasion and metastasis[19]. This early loss of collagens in cancer indicates that basement membranes, and in particular basal lamina collagens, may prevent cancer cell invasion.

Glycoproteins and proteoglycans. Glycoproteins and proteoglycans (which are encoded by ~30 genes) are composed of repeating carbohydrate (disaccharide) chains linked to a protein core. Proteoglycans are a specialist group of glycoproteins that differ from other glycoproteins in their structure, function and location. Indeed, glycoproteins consist of short, branched oligosaccharide chains covalently attached to a central polypeptide side chain. By contrast, proteoglycans are glycosylated proteins composed of a core protein and one of several covalently attached, long, unbranched, negatively charged, sulfated glycosaminoglycan chains. Glycosaminoglycan chains can be from a few thousand daltons to more than 1 MDa in size and their size plays a role in determining their widespread functions.

Glycoproteins, including proteoglycans, fill the interstitial space and buffer physical stress on the matrix owing to the high viscosity created by their side chains and their ability to resist compressive forces (FIG. 1). They also regulate cellular processes such as adhesion, motility, proliferation and differentiation[20]; help to create a cohesive network of matrix molecules by regulating the assembly and organization of other matrix molecules, and by binding and sequestering growth factors, cytokines and divalent cations due to their polyanionic charge (FIG. 1); and exhibit both tumour-promoting and tumour-suppressing roles in a number of solid tumours[21]. Furthermore, tissue hydration is mainly a function of large, polyanionic polysaccharides such as chondroitin sulfates and hyaluronan (also known as hyaluronic acid)[22].

Matricryptins
Also known as matrikines or cryptikines, these are biologically active fragments of matrix molecules that have undergone limited enzymatic cleavage and have a biological activity different from that of the parent protein.

Metzincin superfamily
The main endopeptidases responsible for matrix degradation, comprising matrix metalloproteinases (MMPs), a disintegrin and metalloproteinase proteins (ADAMs) and ADAMs with thrombospondin motifs (ADAMTSs).

Glycoproteins may also act as co-receptors to assist ligands in binding to cell-surface receptors, thereby modulating downstream intracellular signalling (FIG. 2). One of the most studied glycosaminoglycans in cancer is hyaluronan, which is typically overproduced by cancer and stromal cells and its molecular mass helps to determine whether it is antitumorigenic or protumorigenic[23]. Indeed, the hyaluronan receptor CD44 is a potent activator of intracellular signalling networks, and increasing the hyaluronan molecular mass up to 1 MDa has been associated with increased CD44 binding affinity and increased CD44 clustering[24]. CD44 activates a number of downstream signalling networks, including the PI3K–AKT and ERK pathways, as well those involving RhoA and RAC, RAS, NF-κB and SRC, and thus it can promote cell survival, cancer cell stemness, chemoresistance, cell motility and invasion, and epithelial–mesenchymal transition in several solid tumour types[25,26] (FIG. 2).

Other glycoproteins and proteoglycans typically dysregulated in cancer include agrin, laminin, fibronectin, fibrinogen, matrilins, vitronectin, tenascins, osteonectin (also known as secreted protein acidic and rich in cysteine (SPARC)), periostin, thrombospondins, nidogens (also known as enactins), aggrecan, decorin, lumican and biglycan (TABLE 1). The precise protumorigenic and antitumorigenic roles of glycoproteins and proteoglycans appear to be tissue-, context- and tumour-type dependent, and are likely influenced by the specific blend of matrix molecules at that precise time and location. Furthermore, cleavage of glycoproteins and proteoglycans, as well as other matrix molecules, can lead to the release of the bioactive fragments, known as matricryptins, such as versikine.

Matrix post-translational modifications

The biochemical and biomechanical properties of the matrix are regulated by the organ-specific composition, concentration and assembly of the different components (FIG. 1). These properties are then further modulated by the post-translational modification of matrix components by hydroxylation, glycosylation, transglutamination, sulfation and crosslinking, as well as by cleavage and degradation[27]. The post-translational modification of matrix molecules typically alters their interactions with other matrix molecules and cell-surface receptors, and may also change the charge of the molecule. Post-translational modification is performed by several families of intracellular and extracellular enzymes, including but not limited to, intracellular collagen prolyl hydroxylase 3 and prolyl hydroxylase 4 (REF.[28]), lysine hydroxylases[29], the extracellular lysyl oxidases (LOXs)[30], transglutaminases[31], sulfatases[32], heparanase[33], cathepsins[34] and the metzincin superfamily[35].

The continual post-translational modification of the matrix is crucial to its 'dynamic' nature and underlies the ongoing repair and renewal of the cellular microenvironment. As a result, dysregulation of any one or more of these enzyme families can lead to matrix changes that promote cancer progression. Importantly, matrix post-translational modification can be driven by both malignant and non-malignant cells, and can also affect cancer cells and non-malignant cells within the local vicinity, as well as cells arriving from other parts of the body (for example, immune cells) that can trigger further matrix deposition and remodelling[36]. In the context of cancer, excessive post-translational modification of the matrix contributes to a number of classical cancer hallmarks (see later and BOX 2).

Matrix crosslinking

Excessive matrix crosslinking is prevalent in most desmoplastic tumours[3]. Matrix components can be crosslinked in a number of ways, typically leading to the accumulation of a dense network of matrix molecules accompanied by progressive tissue stiffening[14]. This stiffening alters the activation of mechanosensing programmes (discussed later) in malignant and non-malignant cells. Crosslinking of matrix molecules can also reduce matrix turnover and increase the longevity of protumorigenic matrix molecules in the extracellular space[37]. Matrix crosslinking can be strong or weak; weak crosslinks coexist with stabler covalent 'strong' crosslinks and give rise to the viscoelastic properties and load-dependent dynamics[38] of the matrix, which facilitates stress relaxation in response to a deformation. Importantly, the combination of crosslink strengths likely explains why many cell responses are biphasic as opposed to linear[39].

Lysyl oxidases. LOX family matrix-crosslinking enzymes are essential for the deposition and stabilization of mature collagen fibrils and elastin in the extracellular space[40], and dysregulation of this family is implicated in a number of tumours, including breast cancer[41–47], colorectal cancer[48–51] and pancreatic ductal adenocarcinoma (PDAC)[52]. LOX family-mediated matrix crosslinking can also slow matrix degradation, alter cell migration and invasion, angiogenesis and therapy resistance, and increase intratumoural fluid pressure, reducing drug penetration[53]. The importance of LOXs in cancer has been covered previously[30,40].

Table 1 | **Non-exhaustive overview of matrix molecules**

Class of extracellular matrix molecule	Class members
Fibrillar and fibrillar-like collagens	Collagens I, II, III, V, XI, XXIV and XXVII
Network-forming collagens (for example, those in the basement membrane)	Collagens IV, VIII, X, XV and XVIII
Filament-forming collagens	Collagens VI and XXVI
FACITs (fibril-associated collagens with interrupted triple helix)	Collagens IX, XII, XIV, XVI, XIX, XX, XXI and XXII
Transmembrane and membrane-bound collagens	Collagens XIII, XVII, XXII XXIII and XXV
Elastic and microfibrillar proteins	Elastin, emilins, fibrillins, fibulins
Glycoproteins	Agrin, fibronectin, fibrinogen, laminin, matrilins, nidogens (also known as entactins), osteopontin, osteonectin (also known as SPARC), periostin, tenascins, thrombospondins, vitronectin
Proteoglycans	Aggrecan, brevican, biglycan, decorin, lumican, neurocan, perlecan, versican
Other matrix components	Hyaluronan, galectins, mucins, hyaluronan and proteoglycan link proteins (HAPLNs)

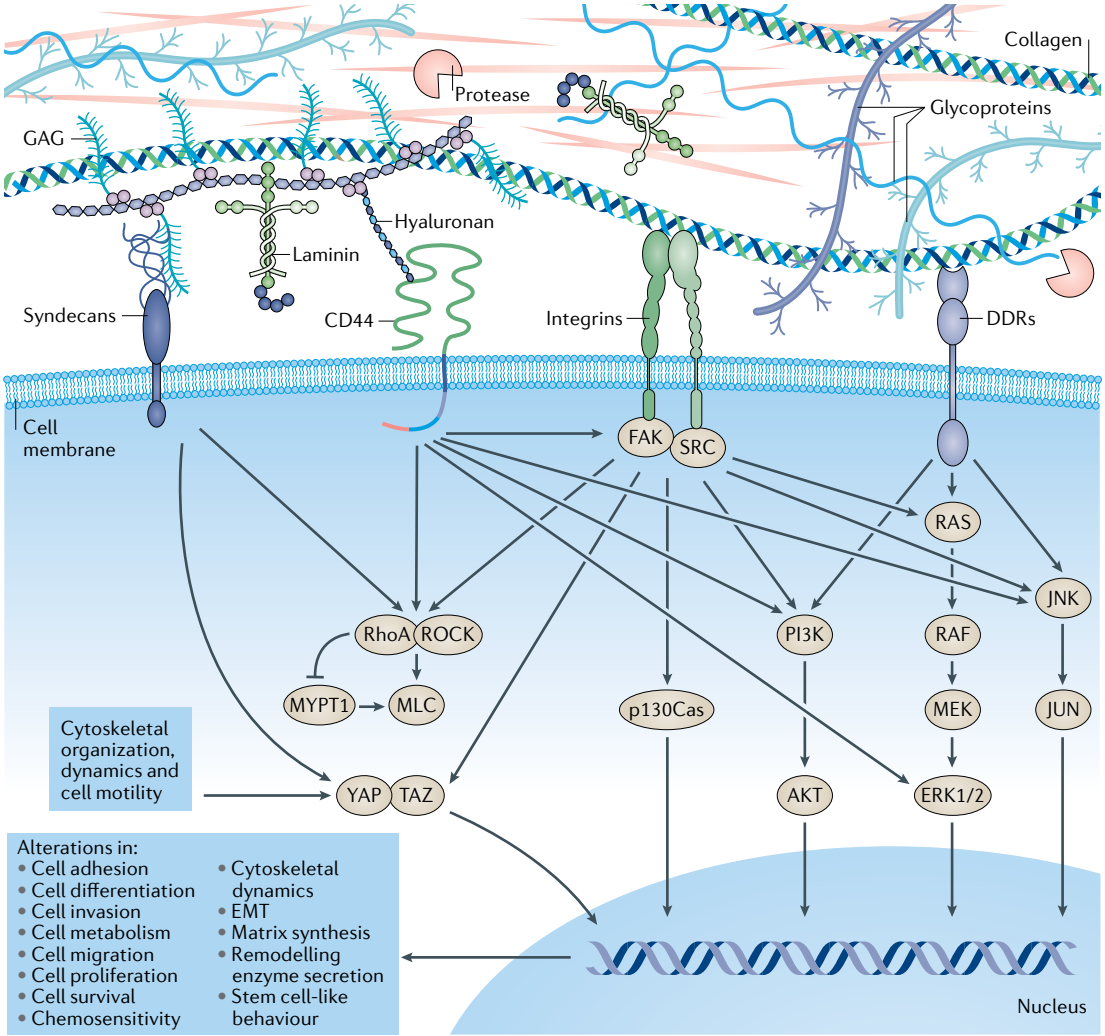

Fig. 2 | **Matrix changes modulate intracellular signalling in cancer.** Changes in the extracellular matrix modulate a number of intracellular signalling pathways. Cell-surface receptors are the major hubs of intracellular signalling initiation[299], triggering downstream changes in gene expression within the nucleus[300]. These changes ultimately regulate cellular adhesion, cytoskeletal dynamics, cell invasion and migration, cell proliferation and survival, differentiation, stem cell-like behaviour, epithelial–mesenchymal transition (EMT) programmes[301], cell metabolism, chemosensitivity, and the further secretion of matrix molecules and matrix-remodelling enzymes within the growing tumour. The major transducers of extracellular cues are the integrins, which integrate these biochemical and biomechanical cues with other inputs, such as growth factor signalling. Integrin heterodimers consist of one α-subunit and one β-subunit. To date, 18 integrin α-subunits and 8 β-subunits are known to heterodimerize into 24 different integrin pairs[302]. Other receptors that transduce matrix cues include epithelial discoidin domain-containing receptors (DDRs), syndecans and the hyaluronan receptor CD44. It is generally accepted that any perturbations in biochemical or biomechanical signalling as a result of a changes in the matrix (including of its dimensionality and molecular composition) exert important, interconnected roles that can be protumorigenic, antitumorigenic or both. FAK, focal adhesion kinase; GAG, glycosaminoglycan; JNK, JUN amino-terminal kinase; MLC, myosin light chain; MYPT1, protein phosphatase 1 regulatory subunit 12A; p130Cas, CAS scaffolding protein family member 1; ROCK, Rho-associated protein kinase.

Schiff base adduct
A subclass of imines with the general structure R2C=NR′.

Amadori rearrangement
Important in carbohydrate biology, this rearrangement is the isomerization event whereby the *N*-glycoside of an aldose sugar is converted to the corresponding ketone by acid or base catalysis.

Transglutaminases. Another important family of matrix-crosslinking enzymes is the transglutaminases[31]. Transglutaminases stabilize the matrix through transamidating and/or deamidating glutamine residues during the assembly of several molecules, including fibronectin, heparan sulfate proteoglycans[54], fibrinogen[55] and collagen VI (REF.[56]). Transglutaminases are important in matrix fibre alignment as well as in increasing matrix stiffness, and they can stabilize matrix molecules against proteolytic degradation. The actions of transglutaminases influence cell adhesion to and migration within the matrix[57]. Elevated expression of transglutaminase 2 in metastatic breast cancer models enhances fibronectin crosslinking and fibrillogenesis and may play an important role in pulmonary metastatic colonization[58]

Glycation. Glycation is a spontaneous, non-enzymatic form of matrix crosslinking driven by Schiff base adduct formation, Amadori rearrangement and subsequent advanced glycation end product (AGE) generation as a result of exposure to sugars. AGEs slowly accumulate with time and are an important biomarker of ageing

Box 2 | **Other post-translational modifications of matrix components**

Glycosylation
Numerous extracellular matrix molecules are glycosylated, often at multiple sites. This glycosylation involves the sequential removal or addition of individual carbohydrates (glycans) from or to proteins, respectively. In glycoproteins the attached glycans are classified as either N-linked (Asn) or O-linked (Ser/Thr) on the basis of the amino acid residue to which they are attached. Glycosylation plays an important role in folding of, and facilitating the orderly secretion and assembly of, matrix molecules. Aberrant glycosylation is common in solid tumours, and specific glycans actively drive tumour development and progression[307]. Targeting tumour glycans and glycosylation events could offer a new strategy for anticancer drug discovery[266].

Citrullination
Hypercitrullination (the conversion of arginine to citrulline) leads to changes in the electrostatic charge and folding of matrix molecules such as collagen and fibronectin. Elevated tumour citrullination is typically driven by overexpression of peptidylarginine deiminase 4. Hypercitrullination alters cell–matrix adhesion and enhances metastasis[308], partially through shifting integrin binding dynamics and subsequently activating the FAK–SRC and ILK–parvin pathways[309].

Oxidation
Matrix molecules are highly susceptible to oxidation, which results in changes to protein structure, function and turnover, as well as loss (or occasional gain) of activity. Accumulation of oxidized proteins, due to increased generation or decreased removal, is associated with ageing and cancer. Matrix oxidation is reviewed in depth in REF.[310].

Acetylation
The acetylation of matrix components such as glycosaminoglycans alters the complexity and size of carbohydrate chains. These changes have important consequences since most glycosaminoglycan functions are determined by their molecular structure and mass.

Phosphorylation
The phosphorylation of matrix components such as collagens, fibronectin and osteopontin has been reported[311]. Phosphorylation plays a role in regulating the biological properties of, and cellular interactions with, the matrix, including cell attachment and spreading[312]. The identity and intracellular and extracellular roles of physiological matrix kinases are under investigation[313], including the role their dysregulation plays in cancer.

Hydroxylation
Matrix hydroxylation, especially of prolines and lysines in collagens, is crucial for their correct folding, assembly, secretion and stability. Hydroxyproline and hydroxylysine are major components of collagens (the former is also present at lower levels in elastin), and they contribute to its unusual toughness and resilience. Procollagen-lysine, 2-oxoglutarate 5-dioxygenases (PLODs) hydroxylate procollagen lysines, allowing formation of the stable tropocollagen trihelices needed to form mature collagen fibres[29]. PLOD expression is increased in many cancer types and is typically associated with poor outcomes, including in breast cancer[314,315], oesophageal squamous cell carcinoma[316], pancreatic cancer[317], hepatocellular carcinoma[318] and colorectal cancer[319]. Ascorbate (vitamin C) is required for proline hydroxylation. Although the various roles of vitamin C in cancer have been discussed previously[320], it remains to be seen whether altered collagen synthesis as a result of severe ascorbate deficiency (scurvy) plays an important role in tumour matrix dynamics.

Sulfation
Sulfation of keratan, heparan and chondroitin chains is important for regulating matrix growth factor binding and hydration. Dysregulation of sulfatases such as SULF1 and SULF2 is seen in many cancers, leading to marked changes in proteoglycan sulfation that have both protumorigenic and antitumorigenic effects[32,321].

matrix (BOX 3). Cells also possess various receptors of AGEs (RAGEs), which activate downstream intracellular signalling programmes regulating, among other processes, cell motility and gene transcription[59]. Additionally, through their crosslinking action, AGEs prevent the normal function of matrix proteins and stiffen the matrix, thereby activating mechanotransduction pathways. Since RAGEs are expressed on monocytes, endothelial cells, macrophages, microglia, podocytes, pericytes, astrocytes and some tumour cells, their link to cancer risk and progression is an important area of investigation[60,61].

Mechanotransduction
A form of sensory transduction in which cells convert mechanical stimuli into biological signals and vice versa.

Matrix degradation and turnover

Degradation and turnover of the matrix is an important part of homeostasis (FIG. 1). Too little or too much degradation can lead to tissue fibrosis (BOX 1) and tissue destruction, respectively, both of which have potent effects in cancer. Matrix turnover is orchestrated by several families of enzymes (see later). Elevated matrix turnover in tumours results in the rapid degradation of 'normal' matrix, facilitating its replacement with tumour matrix that can reinforce aggressive characteristics. As matrix degradation can also remove physical barriers (such as basement membranes), destruction of the normal matrix facilitates malignancy and metastatic dissemination (BOX 4).

The matrix also functions as an important ligand reservoir through binding growth factors (FIG. 1). The cleavage of matrix molecules can locally release bound growth factors and activate intracellular signalling responses[10]. For example, in pancreatic neuroendocrine tumours, increased secretion of matrix metalloproteinase 9 (MMP9) releases sequestered vascular endothelial growth factor (VEGF) from the matrix, which switches vascular quiescence to active angiogenesis[62]. In addition to growth factors, chemokines and cytokines, the matrix is a reservoir for inorganic molecules. Divalent cations such as calcium cations can be released during matrix remodelling, facilitating calcium transport across the membrane and potentially modulating the activity of calcium-dependent, zinc-containing endopeptidases of the metzincin superfamily[63], including MMPs, a disintegrin and metalloproteinase proteins (ADAMs) and ADAMs with thrombospondin motifs (ADAMTSs).

Matrix metalloproteinases. Within the metzincin superfamily, MMPs are the major class of matrix-degrading proteinases. There are 23 human MMPs[64], and their domain structure typically comprises a propeptide, a Zn^{2+}-binding catalytic domain, four haemopexin-like domains and, in the case of MMP14 (also known as MT1-MMP), MMP15, MMP16 and MMP24, a transmembrane and cytoplasmic domain. MMPs extensively degrade matrix proteins or selectively release cell surface-bound cytokines, growth factors or their receptors, thereby impacting matrix integrity, cell behaviour and phenotype, and tissue turnover[65]. Historically MMPs have been grouped by their substrate specificity and/or cellular localization into collagenases, gelatinases, stromelysins and membrane-type MMPs[35]. However, these divisions are insufficient as some MMPs do not fit into any of these traditional groups.

MMP expression is heavily regulated at both the transcriptional level and the post-translational level, ensuring that their spatio-temporal distribution and action are highly restricted. In many cancer types, however, this regulation is lost[35], implicating MMPs in the development and progression of cancer. Moreover, the proteolytic degradation of matrix components in tumours can be both protumorigenic and antitumorigenic[66]. For example, overexpression of MMP8 (also known as

Endopeptidases
Peptidases that cleave peptide bonds of non-terminal amino acids within polypeptide chains and proteins (exopeptidases cleave only the terminal peptide bond of polypeptide chains and proteins).

neutrophil collagenase), which degrades type I, II and III collagens, is associated with increased survival in patients with oral squamous cell carcinoma (SCC)[67] but with poor outcome in patients with ovarian or hepatocellular cancers[68,69]. These conflicting results highlight why MMP inhibitors have underperformed in clinical trials to date (see later).

Adamalysins. The human adamalysin family includes 21 ADAMs and 19 ADAMTSs[70]. Similar to human MMPs and related to snake venom metalloproteinases, adamalysins are typically involved in the degradation of blood vessel basement membrane. In particular, expression and activity of ADAM8, ADAM9, ADAM10, ADAM12 and ADAM15 are dysregulated in solid tumours and they are involved in the turnover of several matrix proteins, including fibronectin, collagen IV and periostin, both directly and indirectly through upregulating the secretion of other matrix-remodelling enzymes, thus contributing to tumour progression[71]. ADAMTSs are secreted ADAMs with thrombospondin type I-like repeats in their carboxy-terminal sequences. Many ADAMTSs are proteoglycanolytic, whereas others process procollagens and are thus important for the deposition of collagen fibrils[71]. Adamalysins also regulate EGF–ERBB signalling through their ability to shed EGFR ligands from the cell surface. Indeed, ADAM17 is the principal sheddase for most EGFR ligands[72], highlighting the overlap between matrix signalling and other signalling pathways.

Box 3 | **The ageing matrix and cancer**

During ageing, extracellular matrix components are altered and become damaged through decreased or aberrant deposition, increased fragmentation and degradation, altered crosslinking, and the accumulation of protein aggregates. Together these changes contribute to a number of age-related diseases[7] by underpinning changes in cellular behaviour and phenotype. Since many matrix molecules exhibit very long half-lives, typically measured in years for some tissues, it exposes them to the possibility of cumulative modification such as by the generation of advanced glycation end products. A comprehensive review of the ageing microenvironment in tumour progression was recently published[322], including discussion of antagonistic pleiotropy in an ageing context. In particular, there is an emerging concept that certain matrix molecules drive phenotypes that increase cellular fitness early in life but become detrimental in an aged organism.

Loss of matrix integrity is a major hallmark of ageing tissues. It is estimated that collagen mass alone declines at a rate of 1% per year in tissues such as skin[323]. Placing senescent cells into a 'young' matrix can rejuvenate them, highlighting how the cellular microenvironment feeds into cellular ageing. Ageing of tissue matrix is also accelerated by extrinsic factors. For example, photo-ageing of the skin[324] markedly alters the proteomic composition of the matrix[325] such that it more closely resembles that of intrinsically aged skin.

Recently, the ageing matrix has been shown to be important in cancer. Young dermal fibroblasts secrete higher levels of various matrix constituents, including proteoglycans, glycoproteins and cartilage-linking proteins, compared with aged fibroblasts. In particular, age-related decreases in the level of the matrix molecule hyaluronan and proteoglycan link protein 1 (HAPLN1) alters the matrix alignment and invasion of melanoma cells[148], and reduces the suppression of immune infiltration. A reduction in the level of HAPLN1 also plays a role in increasing the permeability of sentinel lymph nodes, leading to increased distant metastases[147]. This work helps to shed light on why melanoma is typically more aggressive in patients older than 50 years of age, but also why they are typically more likely to respond to checkpoint inhibitor therapies. It is also beginning to address key questions, such as whether tumour-initiating events that occur in cells within 'young' matrices would exhibit the same penetrance and outcome as the same event within an 'old' matrix.

Cathepsins. Cathepsins are a family of 11 proteases made up of cysteine, serine and aspartic peptidases. In general, cathepsins are small (~20–35 kDa), with the exception of cathepsin C, which has a molecular mass of 200 kDa (REF.[34]). Most cathepsins are expressed intracellularly, found in lysosomes and involved in protein turnover. Importantly, cathepsins degrade a number of matrix precursors intracellularly, including collagens[34], elastin[73], laminins[74], fibronectin[75], tenascin C[76] and nidogen 1 (REF.[77]), thereby modulating the secretion and deposition of these components into the matrix in both normal and cancer settings[78].

Bone morphogenetic protein 1 and Tolloid-like proteinases. Bone morphogenetic protein 1 and Tolloid-like proteinases, which were originally named 'procollagen C-proteinases', were identified for their role in cleaving the carboxy terminus of collagen precursors to release mature collagen molecules. They function directly in matrix assembly, with little or no role in matrix degradation. These proteases are strictly regulated[79] and synchronize matrix assembly with growth factor activation to promote morphogenesis and tissue remodelling. Their overexpression in solid tumours (usually by cancer-associated fibroblasts and other stromal cells) is implicated in colorectal cancer[80], as well as in the metastasis of non-small-cell lung cancer (NSCLC)[81].

Hyaluronidase. Hyaluronan is rapidly turned over, with up to one third of hyaluronan in the body estimated to be degraded daily (often within hours of synthesis) by hyaluronidases[82]. The correct turnover of hyaluronan is important for health, and dysregulation of this turnover is typically seen in cancer, often leading to accumulation of high levels[83]. In addition to canonical hyaluronidases, transmembrane protein 2 (TMEM2) and CEMIP, both of which are overexpressed in a number of solid tumours, exhibit hyaluronidase activity[84,85], leading to increased turnover of hyaluronan and the formation of hyaluronan fragments. These bioactive fragments promote angiogenesis, stimulate the production of inflammatory cytokines and activate intracellular signalling pathways that can drive cancer progression[82]. In patients with cancer, high molecular mass hyaluronan may also aid the development of aggressive pancreatic tumours by impairing vascular function[86].

Heparanase. Heparanase is an endoglycosidase that cleaves heparan sulfate glycosaminoglycans from proteoglycan core proteins and degrades them to small oligosaccharides[87]. Anionic heparan sulfate glycosaminoglycans bind to matrix and cell-surface proteins, thereby providing a framework for matrix organization and cell–matrix interactions. Heparanase expression is typically upregulated in all major cancer types, including carcinomas, sarcomas and a number of haematological malignancies[88], where it is associated with increased metastasis and poor prognosis[89]. Dysregulated heparanase expression is thought to drive tumour growth and metastases by weakening heparan sulfate-containing matrix structures in the basement membrane, thereby facilitating tumour cell invasion[33,90]. Furthermore, heparanase

Box 4 | **The matrix in cancer cell migration and invasion**

Cell invasion through the three-dimensional tumour microenvironment is highly challenging, as cells must squeeze through complex and typically dense (highly desmoplastic) or specialized (basement membrane) matrices. When one is considering these challenges, it is important to consider both the local and the overall properties of the extracellular matrix. For example, both the stiffness of a single matrix fibre and the bulk properties of all the interconnected supramolecular matrix elements will influence the cells' ability to move through the tumour matrix[326]. Matrix pore size (FIG. 1) and pre-existing passageways will also alter the invasive properties of cells as well as the alignment of matrix structures.

Matrix anisotropy can instruct cancer cell migration and enable the global coordination of the cellular force required for movement[327,328]. Furthermore, the highly contractile nature of cancer-associated fibroblasts, which are found in and around tumours, can induce deformation fields in fibrillar collagen matrix; these fields provide long-range, force-mediated physical cues to cells, including macrophages[329] and possibly cancer cells, to direct migration. In some cases, cancer cells are unable to invade into the local matrix without the assistance of so-called leader cancer-associated fibroblasts, which physically and proteolytically generate tracks or highways through the matrix[330] that are exploited by cancer cells[331]. The role of the matrix in cancer cell invasion is elegantly covered in REFS[1,8,332].

Anisotropy
The property of being directionally dependent, whereby a particular characteristic (such as physical or mechanical properties) varies depending on the direction of measurement.

Vascular co-option
The process by which tumours hijack the vasculature of existing tissues of organs to obtain a blood supply independently of angiogenesis.

plays important roles in tumour angiogenesis[91], and it participates in some non-enzymatic tumour-promoting activities independently of its involvement in matrix degradation and remodelling[90,92–94].

Inhibitors of matrix turnover

The activity of each family of matrix degrading enzymes is regulated by inhibitors that are secreted by cells in an autocrine or paracrine manner.

Tissue inhibitors of metalloproteinases. Tissue inhibitors of metalloproteinases (TIMPs) are a family of four paralogues (TIMP1–TIMP4) initially characterized as inhibitors of MMPs[35]. However, they also inhibit ADAMs and ADAMTSs, which makes them critical regulators of both matrix degradation and the shedding of cell-surface molecules[95]. Dysregulation of TIMP expression and/or secretion, and the resulting imbalances in TIMP activity, is important in driving the progression of several solid tumours[96], including breast[97], colorectal[98] and pancreatic[99] cancers.

Cystatins. Cystatins reversibly inhibit cysteinyl cathepsins and also show inhibitory action against other peptidases, including papain and legumains[100]. Cystatins regulate biological processes inside and outside the cell, including matrix turnover and remodelling. In cancer, cystatins regulate cysteine peptidase activity, which is important at all stages of cancer progression. They also have proteolysis-independent effects, including modulating antitumour immune responses and in particular activation of adaptive immunity[100]. For example, cancer cell-derived cystatin B enhances metastasis in models of pancreatic cancer by elevating invadopodium formation and in vivo extravasation[101].

Serpins. Serpins of the serpin superfamily (more than 1,000 factors) regulate numerous proteolytic pathways, by irreversibly inhibiting many serine and cysteine proteases[31]. They control the degradation of collagens, heparan sulfate proteoglycans, heparin and hyaluronan by regulating the activity of the enzymes involved in their turnover. Given the large number of serpins and serpin targets, the role of serpins in cancer progression and metastasis remains controversial. Serpins exert protumorigenic and antitumorigenic effects on cancer progression and metastasis[31] by promoting cancer cell survival and vascular co-option[102] and inhibiting the release of matrix-bound proangiogenic factors[103].

Matricryptins

Many matrix proteins contain cryptic domains, the structures of which are highly similar to those of chemokines and cytokines[104]. The cleavage of these matrix molecules releases bioactive fragments called 'matricryptins' (also known as matrikines or cryptikines)[105], which have a bioactivity that differs from that of the full-length protein and is typically exposed only by proteolysis. These fragments regulate a wide array of processes, including cell migration, adhesion and differentiation[71]. Some fragments also act as proteolytic enzyme inhibitors and therefore help regulate matrix turnover. Matricryptins are comprehensively reviewed in REF.[105].

In cancer, the most well-studied matricryptins are derived from collagens. Endostatin (a matricryptin from collagen XVIII) can impair the function of androgen receptor in prostate cancer[106]. Other collagen-derived matricryptins such as canstatin, tumstatin, arresten and tetrastatin decrease tumour growth in in vivo models of glioma, renal cell carcinoma, colon adenocarcinoma, melanoma, breast cancer and prostate cancer[107–110]. Many matricryptins have historically been seen as antitumorigenic, through exerting antiproliferative effects on tumour endothelial cells, inducing senescence or apoptosis, or inhibiting migratory phenotypes. In contrast, in the last 5 years, work has shown that cleavage fragments generated from laminin 111 in the lung, driven by the secretion of proteases (namely MMP9 and neutrophil elastase) from neutrophils, awaken dormant disseminated breast cancer cells and facilitate overt colonization[111]. However, it is unclear whether this phenomenon is specific to metastasizing breast cancer cells or also important in other tumour types that spread to the lung or, indeed, in primary lung cancers[112].

Architects of matrix remodelling in cancer

Many advanced solid tumours are desmoplastic[14]. The principal producers of matrix within solid tumours are activated cancer-associated fibroblasts (CAFs)[113]. The phenotype of activated CAFs is reminiscent of that of fibroblasts during normal wound healing[114–116]. An increasing number of CAF subtypes and phenotypes have been described in various tumours[117] using approaches such as single-cell RNA sequencing[118] and mass spectrometry[119] and, in 2020, a consensus framework for defining CAFs was proposed[120]. Although the precise origin of many CAF subtypes remains elusive[121], CAFs are generally co-opted resident[122] or recruited fibroblast-like cells that are reprogrammed by the developing tumour[123]. Transdifferentiation of other mesenchymal cells, such as adipocytes into matrix-secreting adipocyte-derived fibroblasts, has also been reported in breast cancer models[124].

Exosomes
Extracellular vesicles, typically 30–150 nm in diameter, that are secreted by all cells, including cancer cells, and contain biological molecules, including DNA, RNA and proteins.

Exosomes contribute to the tumour cell-mediated activation of CAF-driven matrix remodelling. Exosomes (which are also rich in matrix-degrading proteases that can facilitate cancer cell dissemination[125]) can directly reprogramme CAFs and stromal fibroblasts in primary and metastatic sites, respectively, by transporting potent fibrogenic signalling activators, including transforming growth factor-β (TGFβ)[126]. However, the role of exosomes in cancer progression, in resistance to therapy and as potential diagnostic biomarkers is still emerging and under investigation[127].

In many tumours, an increase in the number of CAFs is correlated with poor prognosis[128]. However, the identification and classification of specific CAF subpopulations, such as inflammatory CAFs, myofibroblast-like CAFs[129] and antigen-presenting CAFs[130], suggests that not all CAFs are indicators of poor outcome. Thus, it is likely that there are specific, spatially segregated matrix-remodelling CAFs within tumours. This intratumoural CAF heterogeneity represents a shift in our understanding of CAF contributions to cancer, wherein different CAF subtypes make distinct contributions to progression. The specific location of CAFs within tumours likely also shapes their matrix secretomes and remodelling activities, which subsequently underpins much of the intratumoural heterogeneity of matrix composition. Critically, CAF subpopulations appear transient and can be reprogrammed by various growth factors and cytokines, such as TGFβ[131,132], IL-1 and leukaemia inhibitory factor (LIF), via pathways such as JAK–STAT signalling[133]. Other transcriptional regulators of the CAF phenotype include activation of nuclear factor erythroid 2-related factor 2 (NRF2) in SCCs[134].

Although CAFs are the major depositors of matrix within tumours (more than 90% in tumours such as pancreatic cancer[4]), most of the matrix components they deposit are classic, fibrotic matrix molecules[4]. Conversely, cancer cells tend to produce a large number (albeit in less abundance) of exotic matrix components[4] that likely promote tumour aggressiveness[101]. Such work suggests that elevated deposition of matrix by CAFs is not always indicative of patient outcome; cancer cell-derived matrisomal signatures may be better in predicting survival.

Chronic stress induces signalling from the sympathetic nervous system, which triggers stromal cell remodelling of the lymphatics in breast cancer mouse models[135]. It also enhances the deposition of collagens in ovarian cancer[136], and of osteopontin and tenascin C in breast cancer to promote metastasis and chemoresistance[137]. Surgical intervention in patients with cancer, which activates the sympathetic nervous system and wound healing responses, also causes matrix deposition and remodelling that can adversely affects outcome[138]. In this context, blocking surgery-induced expression of LOX reduces fibrotic scarring and the subsequent risk of lung metastases in breast cancer mouse models[139].

Finally, some aggressive tumours might actually deactivate matrix deposition and remodelling programmes to facilitate their progression. In some pancreatic cancers, tumour cells deactivate pancreatic stellate cells by secreting macrophage colony-stimulating factor 1 (CSF1)[140], which leads to downregulated collagen deposition and facilitates tumour progression. This finding is similar to findings in work in the *Ptf1a*–Cre;LSL–*Kras*$^{G12D/+}$;*Tgfbr2*$^{flox/flox}$ (PKT) mouse model of pancreatic cancer, which showed that the selective depletion of proliferating, α-smooth muscle actin-positive myofibroblasts from pancreatic intraductal neoplastic lesions led to decreased tumour desmoplasia and poorer outcomes in comparison with control mice[141]. Likewise, blocking Hedgehog-mediated activation of fibroblasts (by deleting *Shh* in pancreatic epithelial cells or by inhibiting Shh-induced signalling with the Smoothened inhibitor IPI-926) in LSL–*Kras*$^{G12D/+}$;*Trp53*$^{flox/+}$;*Pdx1-cre* (KPC) mice) decreased tumour desmoplasia and resulted in a poorer outcome in comparison with control mice[142] Disappointing results have also been observed in the clinic using inhibitors of the Hedgehog pathway in patients with pancreatic cancer[143]. More work is needed to understand how matrix composition, including the amount of matrix, and the source of secretion (cancer cells versus stromal cells) contribute to the protumorigenic and antitumorigenic effects that alter cancer development and progression. Thus, 'renormalizing' or 'deactivating' CAFs might be more beneficial than attempting to deplete CAFs in patients with cancer. However, given that the balance between protumorigenic and antitumorigenic matrix cues likely tips in favour of tumour- and metastasis-promoting roles as solid tumours progress[144], the timing of such interventions would also be critical.

The matrix in intracellular signalling

Widespread biochemical and biomechanical changes in the tumour matrix typically support the proliferation and survival of cancer cells and enhance aggressive features such as their resistance to chemotherapy[145]. Although changes in the matrix are sensed by cells over short timescales, their effects can initiate long-term responses. Indeed, as the same cell-surface receptors are often differentially activated by several matrix interactions, which the cell assimilates with other inputs to activate a functional biological response, cells receive an array of simultaneous signals across spatial and temporal scales[146]. Furthermore, the longevity of matrix components, which can be days, weeks or even months, might mean that cellular responses are activated long after the initial deposition of matrix. Indeed, this phenomenon has been shown in a number of studies into the ageing matrix and cancer[147,148] (BOX 3). The dynamic nature of the matrix means that cells also actively remodel the tumour matrix, leading to continuous changes in the clustering and activation of cell-surface receptors[149] (FIG. 2).

Intersection of matrix-centric and growth factor signalling. Adding to this complexity is the fact that matrix-centric signalling intersects and cross-regulates other signalling networks, such as growth factor signalling, both increasing and decreasing activation thresholds. For example, integrins activated by increasing matrix stiffness can increase the signalling through tyrosine kinase receptors, including EGFR, ERBB2, VEGFR and HGFR, in both normal and breast cancer models[150,151]. Furthermore,

collective invasion of SCC is driven by matrix-dependent mechanosensitization of EGF signalling[152]. ERBB2 activation may also be regulated by matrix stiffness, which, as a result, can potentiate resistance in some breast cancers[153]. Other pathways activated by changes in the tumour matrix include MAPK and YAP–TAZ signalling (FIG. 2), both of which promote chemoresistance in tumours[154,155]. Signalling cascades converging on Rho-associated protein kinase (ROCK) in both cancer cells and CAFs also play important matrix-responsive roles in pancreatic tumours[156–158], SCC[159] and breast tumours[160]. Therefore, targeting ROCK is a promising approach to uncoupling the protumorigenic reinforcement of matrix remodelling and increasing chemotherapy efficacy and improving patient outcome[161].

Integrins as matrix-binding receptors. Integrin heterodimers are the major nexus of cell–matrix communication, activating a large number of downstream signalling networks[162]. Several integrins, such as integrins αvβ3, αvβ5, αvβ6, α6β4, α4β1, α11β1 and α5β1, are overexpressed in various cancer cell types and activate tumour cell invasion and metastasis on binding their matrix ligands[163] (FIG. 2). Furthermore, integrin activation, such as of α5β1 in CAFs, and localization to the cell surface constitutes a stromal trait that is indicative of better patient outcome in renal cell carcinoma and pancreatic cancer[164].

The precise subtype and heterodimer formation of integrins, along with their nanospacing, affects drug sensitivity and might underscore the enhanced survival of some tumour cells following chemotherapy. Thus, 'integrin switching'[165] as the biochemistry of the tumour matrix evolves may contribute to progression and chemoresistance in some solid tumours[166]. For example, cancer cell engagement of αvβ3 integrin — rather than α5β1 integrin — with fibronectin in tumours, coupled with nanoscale alterations in fibronectin organization, has profound effects on increasing survival in breast cancer models[167]. Overexpression of α5β1 integrin in pancreatic cancer increases CAF activation and increases tumour desmoplasia, thereby decreasing tumour perfusion and reducing the efficacy of gemcitabine in in vivo models[168]; ongoing work should establish whether similar mechanisms occur in other tumour types.

Thus, the role of integrins in cancer depends on cell type and matrix biochemistry. Integrin switching is also linked to 'cadherin switching'[169] during epithelial–mesenchymal transition in solid tumours, where E-cadherin-to-N-cadherin switches appear coupled to a switch from α6β4 integrin to β1 and β3 integrins[170]. This switch allows cancer cells to shift between cell–cell and cell–basement membrane connections and more readily adhere to collagen I fibres, which facilitates local invasion[169] (BOX 4). Therefore, multiple integrin- and cadherin-based cell adhesion complexes, together with the cytoskeleton to which they are coupled (FIG. 1), form an integrated network that allows cells to sense, process and respond to their microenvironment[171].

Non-integrin matrix-binding receptors. Other matrix-binding receptors, such as discoidin domain-containing receptor 1 (DDR1) and DDR2 (REF.[172]), osteoclast-associated immunoglobulin-like receptor (OSCAR), syndecans (that is, cell surface-bound heparan sulfate proteoglycans), urokinase-type plasminogen activator receptor-associated protein (UPARAP; also known as ENDO180) and leukocyte-associated immunoglobulin-like receptor 1 (LAIR; also known as CD305), also coordinate extracellular–intracellular information transfer and the activation of downstream signalling programmes. For example, DDR1 and DDR2 activation in cancer cells and CAFs increases breast tumour matrix deposition and stiffness, enhances metastatic dissemination and decreases response to immunotherapy[173–176]. Syndecan 4, which is often deregulated in solid cancers, also tunes intracellular signalling in response to localized tension via a coordinated mechanochemical signalling response that involves activation of integrin β1 and downstream YAP signalling[177].

Biomechanical influence of the matrix

The role of matrix biomechanics in cancer, which has been extensively reviewed[178–180], will not be comprehensively discussed here. In brief, the sensing of external forces, followed by the transduction of this information into the cell, assimilation and then the activation (or deactivation) of particular signalling responses is termed 'mechanotransduction'[146].

Matrix biomechanics and cancer. Mechanotransduction is important in several developmental processes, including cell polarity, the regulation of gene expression and stem cell differentiation[181], and thus has implications for the cancer stem cell field. For example, therapies designed to soften tumours could adversely confer stem-like characteristics[182]. Furthermore, in addition to the behaviour of cancer cells and CAFs, tumour biomechanics can influence immune cells, including macrophages[183], and this is opening up an exciting area of research on mechano-immunomodulation. The matrix is a complex biomechanical entity[184], and instead of being a linearly elastic material, it exhibits complex mechanical behaviours, including viscoelasticity, mechanical plasticity and non-linear elasticity[39]. As a result, many cellular responses to the matrix are biphasic, and studies of the timescales of bidirectional matrix–cell interactions are giving critical insight into this complex relationship[185]; however, more work is needed to better understand the impact of matrix viscoelasticity in cancer.

The precise downstream mediators of mechanotransduction are numerous, including, FAK–SRC, MEK–ERK, YAP–TAZ and ROCK signalling[186,187] (FIG. 2). Of note, the long non-coding RNA nuclear paraspeckle assembly transcript 1 (*NEAT1*), which underpins subnuclear 'paraspeckle' bodies[188], might also be an important mechanosensor in cancer[189], highlighting the range of ways in which matrix biomechanics influence cells.

Biomechanics and metabolism. The biomechanical properties of the matrix modulate many elements of cancer cell behaviour, including metabolism[190]. Biomechanical changes in tumours can affect ATP/ADP and ATP/AMP ratios by altering the creatine–phosphagen

Viscoelasticity
A time-dependent response to loading or deformation.

Field cancerization
The process by which areas of tissue exhibit intracellular or extracellular procarcinogenic changes that lead to areas of premalignant cells or protumorigenic matrix, respectively.

ATP-recycling system in pancreatic tumours, leading to invasive migration, chemotaxis and enhanced liver metastasis[191]. Furthermore, signals transduced by cell–matrix adhesions alter the synthesis of neutral lipids[192], enhance glycolysis through reorganization of the cytoskeleton[193], dysregulate glutamine metabolism in both cancer and stromal cells[194] and facilitate aspartate–glutamate exchange supporting tumour growth and metastasis[195]. Some of these effects are modulated through canonical mechanotransduction pathways, such as FAK signalling, including in breast and pancreatic cancers[196]. Finally, the increase in levels of matrix components may also upregulate fatty acid oxidation, as exemplified by the elevation of collagen XI in ovarian cancer, which contributes to chemoresistance[197]. However, more work into the reciprocal interactions between the matrix and metabolism in solid tumours is needed.

The matrix in genotype–phenotype crosstalk

The matrix modulates the non-linear link between tumour genotype and tumour phenotype, not only in mutation-carrying cancer cells themselves (BOX 5) but also between cells within a heterogeneous tumour. The concept of tumour field cancerization has been around since the 1950s[198] and it has generally been explained genetically, whereby clones acquire genetic alterations that give them a growth advantage over neighbouring clones and are necessary, but not sufficient, for malignancy. This hypothesis predicts that a cancerization field is defined by the space occupied by a particular hyperplastic clone. Complementary to this hypothesis is the notion that, during field cancerization, alterations in the matrix are necessary to complement the genetic changes in the premalignant cancer cells[199]. This notion fits with observations that malignant cells can be reprogrammed by embedding them in normal, 'healthy' matrix[200].

The interplay between the intrinsic mutational burden in tumour cells and the properties of the matrix is also important. For example, RAS oncogenes can reprogramme normal, freshly explanted primary mouse and human mammary cells into tumour precursors; yet this process requires increased force transmission between oncogene-expressing cells and their surrounding matrix[201]. Thus, RAS oncogenes empower a disproportionate cellular response to the biomechanical properties of the cellular microenvironment, an affect termed 'oncogenic mechanosignalling'. In pancreatic cancer, genetic alterations that decrease TGFβ signalling elicit increased activity of STAT3 kinase and drive tumour desmoplasia. This desmoplasia increases tumour stiffness, facilitating malignant transition[202]. In breast cancer, matrix crosslinking by LOX promotes the metastasis of TGFβ-deficient mouse mammary carcinomas[46], highlighting the similar role of matrix changes in different tumour types.

The specific mutational burden of the p53 gene (that is, whether p53 is lost or mutated to be constitutively active) in pancreatic cancer cells alters their secretome, leading to alternate activation of local CAFs and differential remodelling of the tumour matrix, to create invasion-permissive and chemoprotective microenvironments[203]. Given the genetic heterogeneity of most solid tumours, different tumour cell clones might compete to subvert nearby non-malignant stromal cells to differentially remodel the tumour matrix, contributing to matrix heterogeneity within a tumour. In lung cancer, epigenetic silencing of the p53 gene in CAFs changes their secretomes and, in particular, the matrix components secreted, which modulates the behaviour of adjacent cancer cells to support invasion[204].

In triple-negative breast cancer, Hedgehog signalling from tumour cells locally reprogrammes CAFs, generating a collagen-rich, supportive niche for the acquisition of a chemoresistant cancer stem cell phenotype[205]. Finally, changes in the tumour matrix may also augment aberrant hormonal signalling, which has been shown to facilitate the metastasis of oestrogen receptor-positive breast cancers[206]. Together, these examples highlight how intrinsic cellular properties and extrinsic matrix properties create a crucial nexus that facilitates progression in solid tumours. However, more work is needed to separate cause from effect, as well as to understand whether these programmes occur across tumour types and between patients with the same type of tumour.

The matrix in metastasis

The concept of the 'hallmarks of metastasis' was introduced in 2019 to complement the classical 'hallmarks of cancer'[207]. These hallmarks of metastasis encompass the acquisition of four distinguishing features: motility and invasion, the ability to modulate secondary sites and/or local microenvironments, plasticity, and the ability to colonize secondary tissues. The matrix is central to all of these features, and ongoing work is addressing whether similar or discrete matrix-remodelling programmes are involved in each hallmark.

Box 5 | Matrix mutations and alternative splicing in cancer

A large number of mutations in extracellular matrix genes are reported in The Cancer Genome Atlas (TCGA) and other datasets, yet there has been very little focus on the function of these mutations within the context of cancer. Since many matrix components are large, multidomain molecules, they are often over-represented yet overlooked in genomic analyses. To date, most mutations in genes encoding monogenic matrix molecules have been studied in non-malignant congenital diseases such as Marfan syndrome and Ehlers–Danlos syndrome and, so far, none of these mutations has been identified as 'matrix drivers' of cancer. For example, mutations in *COL5A2* and *COL2A1* have been detected in subclonal secondary lung tumours following genomic doubling events in patients with adenocarcinoma and squamous cell carcinoma[333]. However, whether these mutations directly affect matrix architecture to influence cancer cells or are simply passenger mutations (cause versus effect) remains to be elucidated.

Work in the past year has shown that copy number alterations and mutations are frequent in matrisome genes and are predicted to impact gene expression and protein function[334]. Furthermore, the mutational burden of specific matrisome genes appears to be an independent predictor of survival in certain cancers[334], although this is still an emerging area of investigation.

Ongoing work is also exploring how the splicing of genes encoding matrix proteins affects solid tumours[335]. For example, carboxy-terminal splicing of the gene encoding the glycoprotein osteopontin leads to a truncated form of the protein, which appears to exert a more proinvasive effect than the full-length variant on non-small-cell lung cancer cells in vitro[336]. Tumour-specific splice variants of matrix molecules such as fibronectin, and in particular of extra domain B-containing fibronectin[337], are important in promoting a number of human cancers[338] and are also highly specific and sensitive targets for tumour detection and matrix-mediated therapies[264].

Indeed, two-photon imaging revealed that patients with breast cancer in which linearized and thickened collagen fibres orient themselves radially from their primary tumour mass have a higher predisposition for metastasis than patients with other collagen alignments[208]; this is likely because these perpendicular fibres act as highways that facilitate cell migration away from the tumour. Work in mouse models has shown that collagen I remodelling at primary and secondary tumour sites is highly similar[209], suggesting overlaps in matrix-remodelling programmes. Breast cancer cells also activate proteolytic mechanisms (including the secretion of MMP14) used by normal mammary epithelial cells during branching morphogenesis to degrade the matrix and facilitate invasion at the tumour periphery[210], an event that might occur in other tumours with a ductal origin, such as pancreatic cancer.

The matrix can promote metastasis without the need for it to be extensively degraded and/or remodelled. For example, activation of non-canonical DDR1 signalling in metastasizing breast cancer cells allows them to exploit the interstitial matrix component collagen I, which is already ubiquitous at secondary tumour sites, including lung, bone, and brain, to promote their survival in murine models[211]. In similar breast cancer models, metastasizing cells can also upregulate prolyl 4-hydroxylase, which hydroxylates collagen and facilitates its deposition and assembly within metastatic sites to support their aggressive outgrowth. Impairing collagen hydroxylation prevents overt lung metastases in these mouse models[212].

Finally, once cells leave the primary tumour and begin their journey to secondary sites, they can use matrix molecules to insulate themselves from the insult of haemodynamic fluid shear stress in the circulation[213,214]. Through interactions within fibrin–fibronectin microclots and platelets[215], or by upregulating fibronectin expression, cancer cells can reinforce integrin-dependent, adhesion-mediated survival signalling during transit[216]; whether this protective mechanism is accessible for the therapeutic targeting of metastasizing tumour cells, however, is unclear.

The matrix and the premetastatic niche. The preappropriation of secondary metastatic sites by primary tumours and the generation of tumour-supportive microenvironments, coined 'premetastatic niches', is an emerging concept that has gained notable interest[217]. Importantly, the generation of premetastatic niches appears to contribute to metastatic organotropism in some solid tumours[218]. Matrix remodelling contributes to the establishment of a premetastatic niche by reorganizing or degrading pre-existing matrix architecture, or by stimulating local matrix secretion.

Matrix-remodelling programmes in primary breast tumours share some similarity with those activated at premetastatic, and subsequently metastatic, niches[219]. Changes in structural fibrous matrix proteins such as collagens, as well as in glycoproteins (including periostin[220], fibronectin[221] and tenascins[222,223]) are important in premetastatic niche formation, as are LOX-mediated changes in matrix crosslinking[44,45,224] and the secretion of MMPs[225,226]. These changes typically lead to growth-supportive microenvironments that are usually immunosuppressive and facilitate metastatic colonization. In many cases, the earliest events invoke the recruitment of immune cells to forming premetastatic sites, where they activate further matrix-remodelling programmes[217]. To date, there is limited evidence of premetastatic niches in patients with cancer owing to the technical and ethical limitations of identifying subtle, non-malignant matrix changes in secondary tissues in the absence of tumour cells. However, it is generally accepted that changes in the matrix that precede, or occur immediately after, tumour cell arrival markedly contribute to overt metastatic colonization.

The matrix in dormancy. Metastatic colonization of secondary tissues does not necessarily require cancer cells to divide on arrival at the site, or the immediate development of clinically overt lesions. A large majority of disseminated tumour cells in secondary organs are solitary, mitotically quiescent cells[227]. Metastatic dormancy is common in a large number of solid cancers[228–231], where the tissue microenvironment, and in particular the matrix, supports tumour cell survival while suppressing colonization. Therefore, these niches appear to inhibit cancer cell proliferation and overt colonization[232] but protect cancer cells from immune surveillance and clearance.

Crucially, dormant disseminated cancer cells can reawaken months, or even years, after seeding to develop into macroscopic lesions[233], and the matrix plays an important role in this reawakening. For example, in breast cancer metastasis, expression of thrombospondin 1 in metastatic niches of the perivascular lung, bone marrow and brain induces breast cancer cell quiescence[234]. However, quiescence is lost on the initiation of neovasculature sprouting and the matrix remodelling that ensues, including the upregulation of periostin[234]. Thus, disrupting the interactions of cancer cells with the matrix of the perivascular niche[234], through, for example, targeting integrins, sensitizes disseminated tumour cells to chemotherapy[235]. In breast cancer lung metastasis, the interaction of indolent breast cancer cells with lung alveolar type I cells leads to the secretion and formation of fibronectin fibrils that trigger integrin-dependent, pro-survival signals in the tumour cells[236]. Again, whether this phenomenon extends to other primary tumours that metastasize to the lung and to primary lung tumours has yet to be investigated.

Finally, the release of neutrophil extracellular traps (NETs) can trigger dormant cancer cells to become aggressive metastases via the NET-mediated remodelling of laminin 111 (REF.[111]), which activates integrin-linked kinase signalling in cancer cells[237].

The matrix in overt colonization. To transition from single cells to microscopic and macroscopic metastatic deposits, matrix remodelling is usually required. Initial changes in the matrix might have already occurred if a premetastatic niche has formed; however, additional remodelling typically occurs as single, disseminated tumour cells divide and begin to overtly colonize the

Premetastatic niches
Specific microenvironments that are systemically induced within a secondary organ and thought to be important for overt colonization by metastasizing primary tumour cells.

Neutrophil extracellular traps
(NETs). Complex networks of extracellular fibres that are primarily composed of chromosomal DNA and histones, and have important roles in thrombosis, inflammation and cancer.

tissue. These matrix-remodelling programmes might be discrete from those that facilitated the aggressive growth of, and dissemination from, the primary tumour. Whether this difference is because the secondary tissue is physiologically different from that of the original primary tumour, due to the presence of different non-malignant stromal populations, or whether it reflects the known differences in clonality of primary and secondary tumours is unclear.

In breast cancer metastases, the secretion of glycoproteins, including tenascin C, periostin and osteopontin, is important for overt colonization[220,222], since they activate important intracellular signalling pathways in cancer cells, including Notch and WNT signalling. Colonizing breast cancer cells also evoke phenotypic changes in lung fibroblasts, activating the formation of a supportive metastatic niche through the secretion of collagens, glycoproteins and matrix-modifying enzymes such as LOXs[238]. Furthermore, while matrices that are rich in organized, fibrillar fibronectin facilitate dormancy, the MMP2-mediated degradation of this fibronectin supports breast cancer cell outgrowth[239]. Finally, during the metastasis of colorectal cancer to the liver, the activation of metastasis-associated fibroblasts drives matrix deposition and stiffening, enhancing angiogenesis and facilitating colonization[240].

Therapeutic implications of the matrix

The matrix as a regulator of therapy efficacy. Although most frontline therapies efficiently induce cell death, they can also activate a desmoplastic response, in particular in tumours being treated in the neoadjuvant setting. The surgical resection of neoadjuvant-treated tumours often yields a highly fibrotic mass with small islands or clusters of tumour cells within it[144]. Chemotherapy and radiotherapy can trigger matrix remodelling through elevating levels of profibrotic growth factors, such as TGFβ[241], and by activating local fibroblast and CAF populations to increase the secretion of, among other molecules, LOXs, fibronectin, fibrillar collagens and glycoproteins. This remodelling changes the biochemistry and biomechanics of the matrix, increasing metastatic progression, treatment resistance and the recurrence of cancer[242].

The clinical response to chemotherapy often correlates with tumour stiffness, with softer breast tumours (as measured by elastography) typically being more responsive to chemotherapy than stiffer breast tumours[243]. Therapeutic resistance is linked to the matrix through several avenues. In oestrogen receptor-positive breast cancers, gene expression patterns indicative of a reactive stroma predict resistance to chemotherapy[244]. Furthermore, post-chemoradiation tumour fibrosis is paradoxically associated with overall and disease-free survival in some cases, such as in patients with pancreatic cancer[245], suggesting that increases in non-malignant tissue fibrosis following neoadjuvant therapy could be indicative of a favourable outcome in some tumours[246,247].

Resistance to the tyrosine kinase inhibitor sorafenib is linked to increased matrix stiffness, and the resulting activation of JUN amino-terminal kinase (JNK) signalling, in models of human breast cancer and hepatocellular carcinoma[248]. Elevated deposition of laminin 322 in breast tumours can activate α6β4 integrin and is linked to trastuzumab resistance through the transmembrane protein CD151 and elevated signalling through FAK[249]. Resistance to doxorubicin in breast cancer is associated with therapy-induced increases in fibulin expression[250]. Together, these examples highlight how therapy-induced matrix deposition and remodelling can blunt the efficacy of the cancer therapies. However, where the same therapies are used to treat multiple cancer types, it is not yet clear whether similar or different matrix interactions promote resistance. Finally, changes in the deposition and density of matrix elements, in particular of large polyanionic polysaccharides such as chondroitin sulfates and hyaluronan, markedly increase matrix hydration due to their anionic nature. This increase in water content increases the hydrostatic pressure of tumours, which can adversely affect vascular patency and might also decrease the perfusion of therapies into, and their diffusion within, the tumour.

Diagnostic, prognostic and predictive matrix signatures. Given the prevalence of matrix in tumours and clinical biopsy samples, researchers are seeking to generate matrix-centric signatures with prognostic, diagnostic and predictive value. Pan-cancer analysis of matrix signatures has identified a subset of CAF-derived matrix molecules that not only differentiate normal from malignant tissue, but also correlate with poor prognosis across multiple solid tumour types[251]. This matrix signature is also a predictor of immunosuppression in otherwise immunologically active tumours, and thus might predict the efficacy of anti-PD1 therapy. Other work has identified matrisome-related or activated stromal signatures that can serve as predictive and prognostic indicators for patients with NSCLC[252] and pancreatic cancer[253], respectively. In breast cancer models, downregulation of the proteoglycans lumican and decorin is typically associated with poor outcome[254]; similarly, high levels of lumican in non-metastatic PDAC are associated with a more quiescent cancer cell state and prolonged patient survival compared with low levels[255]. There are also attempts to consolidate matrisomal signatures across multiple tumours to create 'consensus signatures', as was recently shown for ovarian cancer, lung adenocarcinoma, hepatocellular carcinoma, triple-negative breast cancer and PDAC[256]. Exosomes also contain matrix molecules, and it has been demonstrated that the levels of versican, tenascin C, and thrombospondin 2 can be used to distinguish tumour tissue exosomes from normal tissue exosomes, and therefore could be reliable biomarkers for cancer diagnosis[257]. Matrix signatures will likely also be important in the deployment of novel antistromal therapies, as well as in guiding the administration of stratified therapy to increase the fidelity of future clinical trials.

Notably, the distribution and/or organization of matrix molecules is equally as important in the tumour as their absolute and ratiometric amounts. Indeed, aligned collagen in and around breast tumours (tumour-associated collagen signature) is prognostic for survival[258]. Furthermore, there is an increased association

Neoadjuvant
Used to describe interventions given before a main treatment, or in the case of solid tumours, before surgery.

Elastography
A non-invasive medical imaging modality that maps the elastic properties and stiffness of tissues, and is predominantly used to characterize the biomechanical properties of soft tissues.

Vascular patency
The degree to which blood vessels of the vasculature are open and not blocked or obstructed.

with this aligned collagen of thrombospondin 2 and tenascin C, both of which have cell signalling and structural functions in the matrix[259]. Whether these principles apply to tumours developing in less-matrix-dense primary tissues, such as the brain, is unclear. Either way, assessing the spatial distribution and organization of the matrix as well as amounts will likely be important when tumour matrix signatures are being integrated into clinical situations.

Matrix signatures also have value in premalignant settings. Mammographic density is one of the biggest risk factors for the development of breast cancer. Increased mammographic density was originally thought to be underpinned simply by increased deposition of collagen; however, studies in the last decade have demonstrated that the specific spatial organization and topology of collagen fibrils is also important[260]. The altered matrix in patients with high mammographic density can also regulate microRNAs, which may increase breast cancer risk[261]. Finally, the role of the matrix in field cancerization is of particular interest for diagnosis, since it might be possible to identify biochemical and biomechanical matrix features (in otherwise normal or precancerous neoplasms) that underlie and precede overt clinically macroscopic changes and/or malignant transformation. However, developing non-invasive modalities to detect and quantify these matrix features in apparently healthy individuals will be challenging.

The tumour matrix as a 'homing beacon' for therapies. The marked alterations in the matrix at primary tumour and secondary metastatic sites can be exploited therapeutically to specifically target tumour cells within these altered matrices. For example, Abraxane, the albumin-bound nanoparticle formulation of paclitaxel that is used in the treatment of metastatic breast cancer, pancreatic adenocarcinoma,and NSCLC, has high affinity for osteonectin[262,263], which is highly upregulated in these typically desmoplastic tumours. Studies have suggested that albumin may accumulate in tumour tissue overexpressing osteonectin, thus increasing the concentration of Abraxane within them. Other notable examples include, NJB2, a nanobody specific for EIIIB$^+$, a fibronectin domain that is produced by alternative splicing and expressed only in the matrix and neovasculature of tumours, including triple-negative breast cancer, melanoma and PDAC. Through specifically targeting EIIIB$^+$, the delivery of imaging or therapeutic agents can be enhanced[264]. Furthermore, single-domain antibody (VHH)-based chimeric antigen receptor T cells that target EIIIB$^+$ appear to be effective in reducing the size of tumours in syngeneic, immunocompetent animal models of melanoma[265]. It may also be possible to target cancer-specific glycoprofiles, such as increased sialylation or branching of *N*-glycans, which are common in many solid tumours[266]. Therefore, antibodies targeting specific glycoforms, or inhibitors of glycotransferases associated with the synthesis of such glycans, have potential as tumour matrix-specific treatments.

Another therapy of note is BT1718 (Bicycle Therapeutics), a fully synthetic, short, double-loop, peptide–drug conjugate that exploits the high expression of MMP14 in many solid tumours. Initially developed as a tumour imaging approach[267], it is currently in phase I/IIa clinical trials to strategically deliver mertansine (also known as DM1) to advanced solid tumours, including NSCLC, sarcoma and oesophageal cancer (NCT03486730).

TNF ligand superfamily member 14 (also known as LIGHT) has been targeted to tumour vasculature using a vascular targeting peptide (VTP) that specifically binds heparan sulfates on angiogenic tumour blood vessels[268]. LIGHT–VTP reversed pathological vascular phenotypes, inhibited lung colonization by circulating cancer cells and exerted a direct inhibitory activity on pre-established metastases in models of Lewis lung carcinoma and melanoma[268]. Furthermore, LIGHT–VTP also resensitized refractory lung metastases to anti-PD1 checkpoint inhibitors.

The immunomodulating cytokine TNF can be targeted to tumours using a nine amino acid peptide ligand (CSGRRSSKC), which specifically binds to laminin–nidogen complexes in mouse and human carcinomas[269]. The selective delivery of TNF triggers robust immune cell infiltration, matrix degradation, reductions in tumour stiffness, dilation of tumour blood vessels, improved perfusion and greater intratumoural uptake of contrast agents. Finally, immune checkpoint therapies conjugated to a heparin-binding domain peptide, which binds glycoproteins and collagens, have also shown promise in preclinical melanoma studies[270].

Other strategies are using specific matrix-binding approaches to anchor agents directly within tumours to increase their accumulation. For example, the collagen-binding properties of lumican have been exploited to target collagen-rich melanomas with interleukins to potentiate immunotherapy[271]. Collagen-binding domains fused to IL-12 lead to its accumulation in the tumour stroma and can switch immunologically 'cold tumours' to immunologically 'hot tumours' in mouse models of breast cancer and melanoma[272]. Finally a tenascin C homing peptide coupled to iron oxide nanoworms has shown antitumour efficacy in glioblastoma and prostate carcinoma xenograft models[273].

Directly targeting the tumour matrix

Stromal targeting therapies typically directly target or modulate the secretion of matrix molecules or their post-translational modifications (that is, their crosslinking and stabilization); increase or decrease matrix turnover in tumours; or directly target the interactions of cells with the altered tumour matrix to break protumorigenic feedback. A number of therapies have been repurposed from other areas of research, working on the basis that tissue fibrosis and tumour desmoplasia share a number of common overlapping mechanisms[224].

Targeting matrix deposition. The antifibrotic drug pirfenidone, originally developed to treat idiopathic pulmonary fibrosis, is showing promise as a tractable antidesmoplastic therapy to treat solid cancers. Pirfenidone can suppress TGFβ activity in both malignant and non-malignant cells, leading to decreased deposition of fibrillar collagen I and hyaluronan in breast

tumours, suppressed angiogenesis and decreased lung metastasis[274]. Pirfenidone also reduced the activation of CAFs, decreasing collagen and periostin deposition and leading to slower tumour growth, reduced metastasis and enhanced efficacy of gemcitabine chemotherapy in mouse models of pancreatic cancer[275]. Another promising approach to targeting CAFs is blocking connective tissue growth factor (CTGF), a factor that increases matrix deposition in tumours[276]. The monoclonal antibody to CTGF, pamrevlumab (FG-3019), decreases matrix deposition and enhances the response to chemotherapy in models of pancreatic cancer[277], and has entered phase III trials in combination with chemotherapy (gemcitabine and Abraxane) in patients with locally advanced pancreatic cancer (NCT03941093).

All-*trans* retinoic acid can also induce CAF quiescence in models of pancreatic cancer, leading to decreased desmoplasia and slowed tumour growth[278,279]. Losartan, an inhibitor of angiotensin receptor II, can inhibit the secretion of collagen I by CAFs, restricting the desmoplastic response in mouse models of breast, skin and pancreatic cancer, and enhancing drug delivery and the efficacy of therapy in these models[280]. Losartan has also shown promise in recent clinical trials in increasing margin-negative resection rates in patients with previously unresectable pancreatic cancer[281].

Hedgehog signalling is implicated in several aspects of cancer progression, including desmoplasia. Targeting the Hedgehog pathway with the small-molecule inhibitor vismodegib has shown mixed results. Some studies report that vismodegib has antifibrotic effects that overcome resistance to docetaxel in breast cancer[205], while others report that it affords no increase in progression-free or overall survival when given in combination with gemcitabine in pancreatic cancer[282]; these observations highlight the complexity of targeting the matrix across different tumours. Another Hedgehog inhibitor, IPI-926, showed promise in depleting desmoplasia and improving the response to chemotherapy in pancreatic adenocarcinoma models[283], yet a trial in which IPI-926 was given in combination with FOLFIRINOX (5-fluorouracil, leucovorin, irinotecan, and oxaliplatin) led to shorter median survival time and an increased rate of disease progression[284].

Finally, in terms of modulating the secretion of matrix proteins, nintedanib, a receptor tyrosine kinase inhibitor targeting the VEGF, PDGF, FGF, and TGFβ receptors, can reduce fibrosis in solid tumours, including in hepatocellular carcinoma, renal cell carcinoma, colorectal cancer, prostate cancer, and gynaecological malignancies[285].

Targeting matrix remodelling. Blocking the post-translational modification of matrix elements is also a powerful approach to antagonizing its protumorigenic effects. Blocking crosslinking by LOXs[286] and transglutaminases[31] can decrease tumour desmoplasia and improve outcome in models of pancreatic cancer[287], colorectal cancer[49–51] and breast cancer[44,45]. The tumour stroma is also rich in proteolytic enzymes and their targets, a feature which could also be exploited for drug delivery. MMPs underlie many of the aggressive features of cancer, and thus should be a potent target for anti-tumour therapies. However, marimastat, a powerful broad-spectrum MMP inhibitor, showed no effect on patient survival either alone[288] or in combination with gemcitabine in patients with unresectable pancreatic cancer[289], and had only limited effects in NSCLC in combination with carboplatin and paclitaxel[290]. Similarly, tanomastat (also known as BAY-12-9566), an inhibitor of MMP3, MMP9 and MMP13, used in combination with gemcitabine showed low efficacy in phase III trials in patients with pancreatic cancer[291]. Although the trials to date have had disappointing results, they highlight the complexity of this large superfamily of proteases[35], which have both protumorigenic and antitumorigenic roles. More focused approaches, in terms of the specific family members being targeted and the timing of the administration of interventions, may be necessary. That said, engineering of MMP2-activated peptide–hybrid liposomes loaded with pirfenidone can specifically release pirfenidone within pancreatic tumours to down-regulate matrix deposition and, subsequently, increase the efficacy of gemcitabine[292].

Another notable approach to targeting the tumour matrix is the use of intratumoural injections of PEGPH20 (also known as pegvorhyaluronidase alfa; from Halozyme Therapeutics) in combination with chemotherapy, which has undergone a number of clinical trials (NCT03634332, NCT02910882, NCT02563548, NCT03481920, NCT02346370 and NCT01170897). PEGPH20 degrades hyaluronan, which accumulates in ~40% of pancreatic tumours[293]. Therefore, diagnostic screening for high levels of hyaluronan in tumours is a prerequisite for PEGPH20 administration. However, results have been mixed, with PEGPH20 plus FOLFIRINOX leading to poorer outcomes in patients with metastatic pancreatic cancer[294], and PEGPH20 as a first-line treatment in combination with gemcitabine plus Abraxane for patients with pancreatic cancer failing to reach the primary end point of overall survival[293]. This failing might be due to a number of reasons, including the release of highly bioactive hyaluronan fragments into the tumour. Regardless, there are ongoing trials for the use of PEGPH20 in combination with immunotherapy, although it is too early in these trials for any findings to have been reported.

Unexpected antistromal effects can occur with a number of other therapies. For example, tamoxifen treatment of pancreatic tumour-bearing mice, through agonizing the G-protein-coupled oestrogen receptor, reduced the deposition of fibrillar collagen and fibronectin, leading to altered collagen alignment, reduced tissue stiffness and improved outcome[295].

Targeting cell–matrix interactions. Finally, targeting the cellular response to the changing tumour matrix is also under intense investigation. As the main extracellular–intracellular signalling nexus, integrins recruit and assemble intracellular signalling complexes, initiating many downstream responses (FIG. 2). However, therapeutically targeting them in cancer has yielded limited success (reviewed in REF.[296]). Instead, targeting the signalling cascades downstream of integrins is showing efficacy

Basket trials
Clinical trials in which many tumour types carrying the same molecular or genetic aberration are grouped together and given the same treatment.

in disrupting the protumorigenic cues of the tumour matrix. For example, targeting ROCK signalling has shown in vivo efficacy in pancreatic cancer[156,157], SCC[159] and breast cancer[160]. FAK, another important signalling pathway downstream of integrins (FIG. 2), is activated in a number of solid tumours and promotes tumour progression and metastasis[297]. Inhibition of FAK signalling with an array of emerging small-molecule inhibitors has shown promise, not only through targeting of FAK in tumour cells and CAFs but also by targeting FAK signalling in endothelial cells to induce chemosensitization to DNA-damaging therapies in tumour cells[298].

Despite the often paradoxical protumorigenic and antitumorigenic roles of the matrix, it remains an underexplored, yet highly promising, therapeutic target in cancer. Although encouraging results have emerged, matrix and stromal targeting still faces many obstacles and challenges due to the enormous heterogeneity and dynamic nature of the matrix, and the relative scarcity of tools to interrogate it. Nevertheless, as our understanding of tumour matrix biology expands, we should see more matrix-centric therapies being successfully translated into clinical trials.

Conclusions and perspective

In almost all solid tumour biopsy samples there is matrix. Indeed, in biopsy samples with insufficient or no tumour cells to clinically stage them, the biopsy sample is often entirely matrix. This fact is especially true for highly fibrotic tumours, and also for tumours that have undergone neoadjuvant treatment. The development and refinement of high-throughput technologies to catalogue the composition, and map the spatial complexity, of the three-dimensional tumour matrix have rapidly expanded in the last 10 years (BOX 6). Therefore, as we begin to understand more about the role of the matrix in cancer, and develop prognostic, diagnostic and predictive matrix signatures, we might be able to revisit the millions of archived tissues to conduct detailed retrospective studies on the precise role of the matrix in cancer.

In the last decade a number of anticancer drugs have been approved for use in the pan-tumour setting. The approach of stratifying patients on the basis of a particular genetic aberration, rather than simply on the primary tumour site, as a means to administer specific treatments is proving effective. With that in mind, the ability to stratify patients on the basis of the nature of their tumour matrix also offers a powerful approach for personalized, patient-centric medicine. Similarly, since a number of solid tumours appear to activate similar programmes of matrix remodelling, the targeting of specific matrix molecules or remodelling events could be applied in a pan-cancer setting, which, in combination with already approved tumour cell-specific therapies, could increase the potency, efficacy and longevity of therapy, and subsequently result in improved patient outcome. Transitioning matrix-specific stromal targeting approaches into clinical trials may be tricky. However, just as we have basket trials for rare cancers, it may be feasible in the future to group cancers on the basis of their discrete matrix signatures to assess the efficacy of emerging antistromal therapies. Either way, more work is needed to understand how generalized matrix changes are across different solid tumours, and also among patients harbouring the same tumour type, to understand the extent of heterogeneity and the common mechanisms that may be at play. Furthermore, determining whether matrix changes are true drivers of tumour progression, markers of transitional events or indeed both will be important in targeting the matrix translationally in cancer.

Importantly, the non-selective depletion of the matrix and/or of matrix-producing cells is likely to have adverse outcomes and, paradoxically, can accelerate tumour progression and metastatic dissemination. Instead, more nuanced approaches of matrix normalization might prove more successful. Furthermore, distinguishing between tumour desmoplasia and tissue fibrosis might shed light on the protumorigenic and antitumorigenic roles of the matrix. Indeed, whether excessive matrix deposition and remodelling simply encapsulates tumour cells, thereby serving as a bona fide physical barrier, or permeates deeply throughout a tumour might also confer differential juxtacrine signalling cues to tumour cells. In addition to trying to block tumour fibrosis, the stimulation of antitumorigenic host tissue fibrosis may also offer a potential therapeutic avenue.

A final, yet crucial element to consider is the longevity of many matrix components, which leave a historical

Box 6 | Mapping the matrix in cancer

The extracellular matrix is highly compartmentalized, and this spatial organization is tightly regulated. Access to cutting-edge technologies, such as optical imaging and electron and atomic force microscopies, along with diffraction and X-ray-based spectroscopic methods spanning wide ranges of spatial scales and timescales, has provided insight into the composition and organization of the matrix[37].

Mass spectrometry-based proteomics can be used to comprehensively catalogue the matrix in health and in diseases such as cancer. This has led to the establishment of dataset resources, such as the Matrisome Project[5], Extracellular Matrix Atlas[339], MatrisomeDB[340], MatrixDB[341] and the Avner Australian Pancreatic Cancer Matrix Atlas, many of which are being deployed in the cancer setting. However, at present, due to technical limitations, many of these approaches do not capture the complex post-translational modifications or supramolecular structures of three-dimensional matrix assemblies. Advances in top-down proteomics and specialized mass spectrometry approaches, such as glycomics and glycosaminoglycan-omics, are allowing detailed characterization of matrix molecules that are known to have an important role in solid tumours.

Spatial proteomics using matrix-assisted laser desorption/ionization (MALDI) imaging–mass spectrometry is increasingly being applied to cancer, and has been successfully used to map the matrix in several tumour types, including breast and ovarian cancers as well as in tissue microarrays from patients[342–345]. The use of MALDI–Fourier transform ion cyclotron resonance is also emerging; this uses higher resolving power than time-of-flight machines[346], promising even deeper unbiased cataloguing of the spatial compartmentalization of the matrix in cancer.

Wholemount tissue preparation and clearing approaches that preserve the delicate three-dimensional structure of the matrix are also facilitating high-resolution optical imaging, allowing us to visualize the matrix in exquisite detail in its native confirmation[209,347]. A number of experimental and clinical imaging modalities also exist to measure and quantify the biomechanical properties of tumour across different scales (reviewed in REF.[179]). Finally, the ability to visualize the real-time dynamics of the matrix is also rapidly expanding. For example, the generation of small collagen hybridizing peptides, which specifically hybridize to degraded, unfolded collagen chains, can be used to image degraded collagen and inform tissue remodelling activity in various tissues and in cancer[348,349].

record of the evolution of the tumour. With a greater understanding of the role of the matrix, this record will likely give us crucial insight into the factors that may have shaped the development, evolution and cellular heterogeneity of the tumour or, for example, its response to a particular therapy. This knowledge will not only help us to better understand the complexity of tumours but should also allow us to better treat patients with cancer through the personalized selection of agents, timings and dosing regimens in order to improve outcome.

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