The FEBS Journal

FEBS PRESS science publishing by scientists

VIEWPOINT

Extracellular matrix biomechanical roles and adaptation in health and disease

Marco Franchi[1], Zoi Piperigkou[2,3], Nicholas S. Mastronikolis[4] and Nikos Karamanos[2,3]

1 Department for Life Quality Studies, University of Bologna, Rimini, Italy
2 Department of Chemistry, Biochemistry, Biochemical Analysis and Matrix Pathobiology Res. Group, Laboratory of Biochemistry, University of Patras, Greece
3 Foundation for Research and Technology-Hellas (FORTH)/Institute of Chemical Engineering Sciences (ICE-HT), Patras, Greece
4 Department of Otorhinolaryngology-Head and Neck Surgery, School of Medicine, University of Patras, Greece

Keywords
adaptation; biomechanics; biosensing; cancer invasion; connective tissues; extracellular matrix

Correspondence
M. Franchi, Department for Life Quality Studies, University of Bologna, Rimini, Italy
Tel: +39 0541 434 600
E-mail: marco.franchi3@unibo.it
and
N. Karamanos, Department of Chemistry, Biochemistry, Biochemical Analysis and Matrix Pathobiology Res. Group, Laboratory of Biochemistry, University of Patras, Patras, Greece
Tel: +30 2610 997 915
E-mail: n.k.karamanos@upatras.gr

(Received 8 June 2023, revised 24 July 2023, accepted 22 August 2023)

doi:10.1111/febs.16938

Extracellular matrices (ECMs) are dynamic 3D macromolecular networks that exhibit structural characteristics and composition specific to different tissues, serving various biomechanical and regulatory functions. The interactions between ECM macromolecules such as collagen, elastin, glycosaminoglycans (GAGs), proteoglycans (PGs), fibronectin, and laminin, along with matrix effectors and water, contribute to the unique cellular and tissue functional properties during organ development, tissue homoeostasis, remodeling, disease development, and progression. Cells adapt to environmental changes by adjusting the composition and array of ECM components. ECMs, forming the 3D bioscaffolds of our body, provide mechanical support for tissues and organs and respond to the environmental variables influencing growth and final adult body shape in mammals. Different cell types display distinct adaptations to the respective ECM environments. ECMs regulate biological processes by controlling the diffusion of infections and inflammations, sensing and adapting to external stimuli and gravity from the surrounding habitat, and, in the context of cancer, interplaying with and regulating cancer cell invasion and drug resistance. Alterations in the ECM composition in pathological conditions drive adaptive responses of cells and could therefore result in abnormal cell behavior and tissue dysfunction. Understanding the biomechanical functionality, adaptation, and roles of distinct ECMs is essential for research on various pathologies, including cancer progression and multidrug resistance, which is of crucial importance for developing targeted therapies. In this Viewpoint article, we critically present and discuss specific biomechanical functions of ECMs and regulatory adaptation mechanisms in both health and disease, with a particular focus on cancer progression.

Abbreviations
3D, three dimensional; BM, basement membrane; CAFs, cancer-associated fibroblasts; CM, collagen membrane; ECM, extracellular matrix; EMT, epithelial-to-mesenchymal transition; FA, focal adhesion; GAG, glycosaminoglycan; GPCRs, G-protein-coupled receptors; HA, hyaluronan; MMP, matrix metalloproteinase; PG, proteoglycan; TACS, tumor-associated collagen signature classification; TME, tumor microenvironment.

Introduction

Cells adapt to changes in environmental stress to avoid harsh conditions and maintain key functions for their survival and tissue homeostasis. In biology, adaptation includes behavioral, metabolic, morphological, and physiological changes that keep or improve fitness under variable environmental conditions [1]. In cell biology, tissue adaptation consists of changes in cell behavior and phenotype, which, in physiological or pathological conditions, often respond by adjusting the composition and assembly of extracellular matrix (ECM) components. ECMs consist of molecular patterns of insoluble proteins, such as collagen and elastin, forming a fibrillar meshwork, the hydrophilic GAGs and PGs, as well as glycoproteins like fibronectin and laminin. Due to its dynamic nature, ECM in various connective tissues displays distinct architectures that regulate the unique tissue functional roles. In the skin, ECM acts as a scaffold for epithelial cells and interacts with the environment via epithelia by sensing physical external changes. In bones, ECM adapts to gravity changes, whereas in tendons, collagen fibers transmit forces and coordinate joint movement in ligaments. In joint cartilage, interarticular discs, and menisci ECM favors joint movement and partially dampens weight load. In arterial vessels, the elastic component of ECM smooths out blood pulsation pressure and favors blood flow. In the cornea, ECM contributes most of the eye's refractive powder, and in the pleural serous, it allows lung expansion for breathing.

The molecular and supramolecular array of ECM components forms a 3D dynamic network that interacting with water, regulates connective tissue hydration. ECMs also drive anatomical aspect, body shape and size among and within each mammal species. In solid tumors, ECM first opposes but then favors cancer cell invasion, metastasis, and multidrug resistance [2] (Table 1). Therefore, ECMs contribute to vital biomechanical functions, regulate mammalian health, and adapt to new functional requests by interplaying with their cellular components in different diseases or cancers. In this article, we discuss on the ECM as an elastic bioscaffold that continuously adapts to environmental stresses and plays biomechanical roles in regulating inflammatory infiltration, cancer cell invasion, and response to chemotherapy.

The ECM bioscaffold continuously adapts to external environmental signals

Extracellular matrices, as dynamic and adaptive 3D scaffolds, display regulatory roles in tissue morphogenesis and define the biochemical and biomechanical properties of all tissues and organs [3,4]. ECM provides physical cues to cells through its mechanical properties, including stiffness and elasticity. Cells respond to these mechanical stimuli, leading to changes in gene expression, cytoskeletal rearrangement, and cellular adaptation (Fig. 1). The basement membrane (BM) supports all epithelia and regulates their development, as well as bones, which form a mechanical scaffold that affects our body development and models our surface anatomy. Dynamic cell-ECM interactions play a pivotal role in various cellular processes, such as cell adhesion, migration, proliferation, differentiation, and tissue remodeling. ECM communicates with cells through a complex interplay of biochemical and mechanical signals, guiding essential processes like cell-matrix adhesion, force transmission, mechanosensing mechanisms, molecular signaling, matrix remodeling, and bidirectional cell-matrix communication. Mechanotransduction mechanisms, under the influence of matrix regulators, facilitate cells-matrix interactions. Additionally, cell membranes actively undergo conformational changes, achieved through pulling or pushing actions, often involving actin polymerization and microtubule formation (Fig. 1).

Table 1. The common and specific biomechanical functions of extracellular matrices in various connective tissues [2,36,72].

Connective tissue	Function
Skin and organs	Physical scaffold for epithelia and other tissues; interacting with the external environment by sensing physical changes; allowing accumulation of adipose tissue
Bone	Sensing the gravity force
Tendon	Transmitting forces for muscle contraction
Ligaments	Guiding and limiting joint movements
Hyaline cartilage	Favoring joint movement; shock absorber; opposing gravity force
Arterial vessels	Smoothing blood pulsation pressure and favoring blood flow
Cornea	Providing most of the eye's refractive powder
Tumor microenvironment	Opposing cancer cell invasion, metastasis, and drug resistance

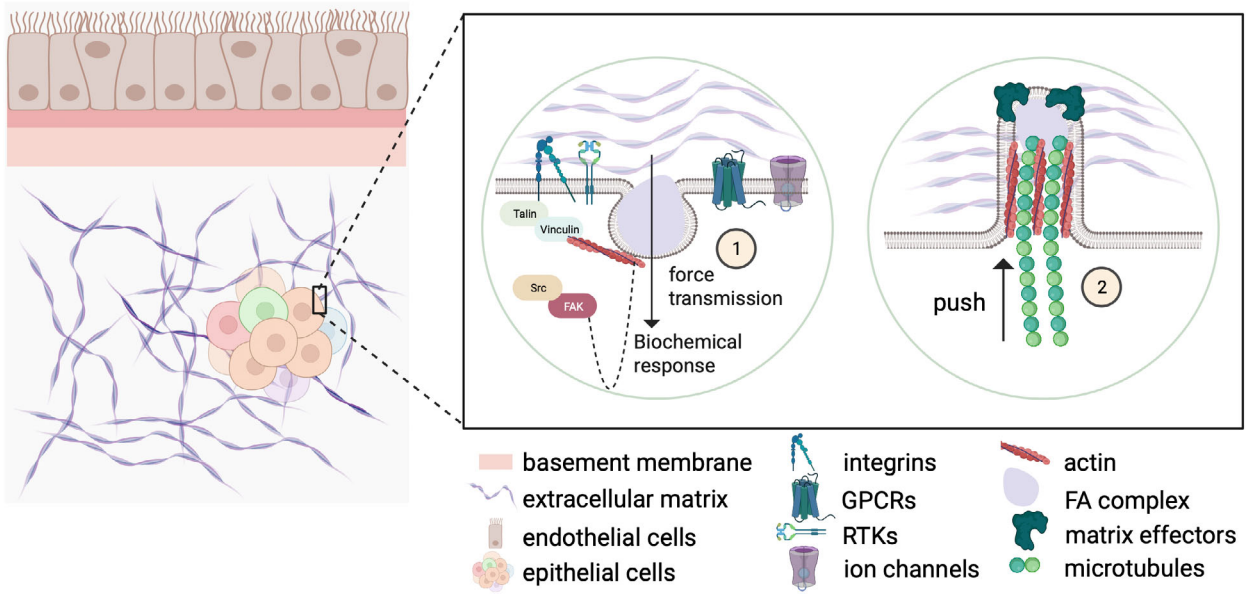

Fig. 1. Schematic representation of ECM biomechanical functionality and its role in cellular adaptation. The dynamic cell–matrix interactions are crucial for various cellular processes, including cell adhesion, migration, proliferation, differentiation, and tissue remodeling. The complex interplay of biochemical and mechanical signals that cells receive from the ECM guides crucial dynamic interactions, including cell-matrix adhesion, force transmission, mechanosensing mechanisms, molecular signaling, matrix remodeling, and bidirectional cell-matrix interplay. Cells interact with ECM through mechanotransduction mechanisms guided by matrix regulators, such as integrins, RTKs, GPCRs, stretch-activated ion channels, and the subsequent signaling molecules (i.e., FAK, Src). Cell membranes actively change conformation by pulling (1) or pushing (2), often via actin polymerization and microtubule formation. ECM, extracellular matrix; FA, focal adhesion; FAK, focal adhesion kinase; GPCRs, G-protein-coupled receptors; RTKs, receptor tyrosine kinases. Created with BioRender.com.

Aging is accompanied by changes in ECM composition. A decrease in collagen and elastin, as well as in GAGs, may affect body aspect over time. Skin dehydration, thinning of joint cartilages and intervertebral discs evoke pain and limited mobility, height reduction, and decreased elasticity in tendons, ligaments, and skin, which becomes stiffer [5]. Despite this, ECM can be improved through physical activity and medical procedures. Exercise balances the body proportions by modifying muscle mass and ECM, affects bone shape and size during growth in young people, and preserves or improves function of tendons and ligaments [6].

Medical procedures like dermal liposuction and hyaluronan (HA) injections in the dermis modify the connective tissues and improve skin appearance [7]. Collagen membranes are used for guided bone and soft tissue regeneration in periodontology and oral implantology, to repair joint capsules in orthopedic surgery, and in experimental 3D cultures [8]. Collagen grafts favor wound healing or restore skin in burning injuries, whereas implant insertion affects the gum ECM and peri-implant bone. A loss or change of synovial fluid in joints often occurring with aging or in autoimmune diseases causes pain and limit motion. Exercise and injections of HA into joint cavities reduce pain and improve recovering of joint movements [9].

ECM in physiological tissue biomechanics and adaptation

The ECM meshwork includes insoluble and hydrophobic collagen fibrils bound together with hydrophilic GAGs and PGs to form a hydrated dynamic bioscaffold, which mechanically allows all connective tissues to resist or transfer both pressure or tension and unidirectional or multidirectional forces. The presence of water contributes to the formation of elastic strings, which favor sliding among stretched fibrils or oppose pressure in cartilage [10]. Understanding the dynamic biosensing and adaptation properties of ECM has significant implications in biomedical research. The high molecular weight HA is the most distributed GAG in many ECMs and is particularly present in skin, whose hydration is related to HA content. A single HA molecule accommodates up to 500 water molecules, so that HA mechanically provides lubrication and pressure absorption in synovial joints [11]. ECMs sense changes in physical status in the external environment and

respond by activating chemical signaling cascades to activate a functional adaptation [12], and the first mechanical epithelial cell-ECM crosstalk occurs at the dermo-epidermal junction.

Moreover, connective fasciae and serous membranes adapt to environmental requests. In deep fascia, HA is concentrated in an intermediate loose connective tissue ensuring lubrication and gliding between two fibrous fascial layers. Movement promotes HA turnover, whereas immobilization favors accumulation [13]. Mesothelial cells secrete HA which play chemo-mechanical roles favoring lubrication and water homeostasis in pleural, pericardial, and peritoneal fluids, therefore contributing to vital physiological functions [14,15].

Extracellular matrices providing different anchorage sites for cell adhesion and migration, represent a physical barrier that regulates cell movement by separating or connecting different tissues through changes in hydration. ECMs physically support the anatomical development of nerves and blood or lymphatic vessels, providing gaseous and metabolic nutrition to other tissues. Plasma, the blood ECM, transports cells, playing a strategic role in wound healing and immune defenses. ECM macromolecular networks also oppose and regulate the diffusion of viruses or bacteria in inflammatory infections. This alters their uptake by living cells, while regulating hydration and the physical array of ECM. Modulations in water content significantly influence the elasticity of connective tissues [16]. These tissues encompass a composite structure consisting of collagen fibers and proteoglycans, with an interstitial fluid phase of incompressible water.

Collagen, the most abundant protein in mammals, accounts for up to 30% of total human protein mass. The collagen superfamily includes 28 members that contain at least one triple-helical domain and provide 3D structures for mechanical support of tissues and organs. Collagens can be divided into two subgroups: fibril-forming and nonfibril-forming collagens [17,18] The mechanical properties of single collagen fibrils allow walking, running, and body motions against gravity. In bones, they represent more than 90% of the organic component and form an active scaffold for bone mineralization. Collagen resistance to tensional loads is evident but it differs in tendons and ligaments. In tendons, they transmit muscle contraction forces, whereas in ligaments, they passively guide and limit joint movements. A repeated, differentiated, and hierarchical handedness from basic tropocollagen chains to fibers increases the mechanical resistance of fibrils to elongation [19,20].

The biomechanical roles of fibrils change with different collagen types, which have different distributions in various tissues. Type I and III collagens differently play the major role in mechanics of human tissues [21], as collagen I accounts for almost 70% of total collagen, whereas type III represents the 5–20% [22]. In mammalian evolution, this might represent an adaptation to improve motion; hence, type I collagen fibrils are thicker (150–300 nm) and stiffer vs. collagen III fibrils, which exhibit a smaller diameter (25–100 nm) and higher flexibility [21,23]. The large, straight type I collagen fibrils predominate in highly tensile structures like tendon, ligament, bone, and skin, and are unidirectionally stretched along their axis. The more flexible and pliable type III collagen fibrils subjected to multidirectional loads are a major structural component in hollow organs, connective fasciae, and sheaths [24,25] (Fig. 2A,B). Bending stiffness and flexibility of fibrils are related to the supramolecular array of microfibrils forming the single fibril: large and stiff type I collagen fibrils show an almost parallel microfibril arrangement (5° angle), whereas thinner and more pliable collagen III fibrils display a helical microfibril array (angle of 17°) [26]. This was confirmed by experimental fibrillogenesis; incorporation of collagen III caused the formation of thinner, more flexible fibrils with shorter D-banding [21]. Rigid collagen I fibrils can bend only in limited regions, "fibrillar crimps," forming fiber crimps that act like elastic biological hinges in tendons and ligaments (Fig. 2C,D) [20,27]. In few examples, collagen fibrils or fibers resist pressure if laterally compressed. Parallel fibrils inside bone osteon lamellae are laterally loaded under gravity in compact bone, and in intervertebral discs, fibrils of the annulus fibrosus ensure a mechano-biological barrier limiting extrusion of the nucleus pulposus.

Elastin, another fibrous component of the ECM, forms the elastic fibers including an elastin core surrounded by fibrillin-rich microfibrils [28]. Elastic fibers allow recoil of stretched or deformed ECM in skin, lung, or arteries [29]. Extension of the elastic fibers is very high; they are stretched more than twice their length before rupture occurs [30]. In arteries, stretched elastin stores energy to smooth out blood flow pulsation and favor blood perfusion [10]. Also, the glycoprotein fibronectin may form fibrils acting as an extracellular mechano-regulator by mediating cell adhesion and migration in the ECM and functioning as "biological glue" [31].

Basement membrane containing laminin, type IV collagen, nidogens, PGs, and growth factors physically separates epithelia, endothelia, fat, nerve, and muscle cells from their underlying connective tissues but also connects these cells via integrins to fibrils of ECM. Type V fibrils as well as type VII collagen anchoring fibrils seem to play a crucial role in connecting

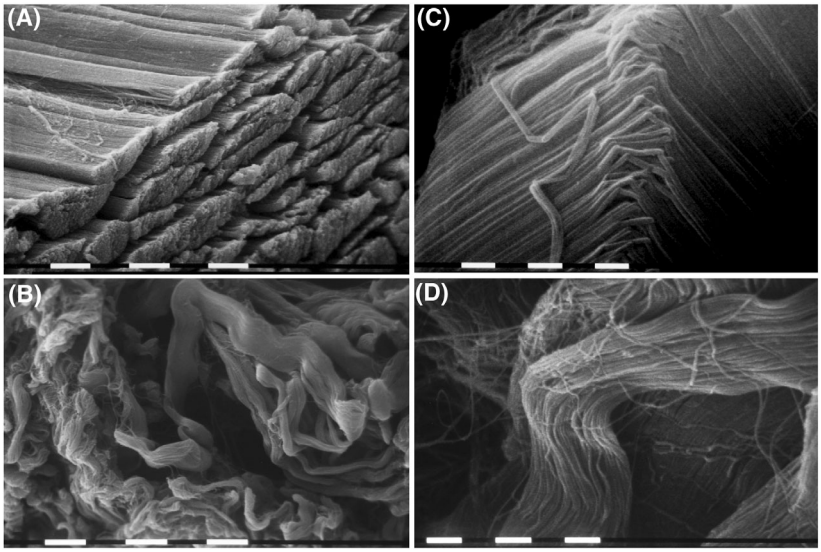

Fig. 2. SEM of a rabbit Achilles tendon with surrounding sheaths, which correspond to dense connective tissues whose ECM is rich in collagen fibers. (A) In the rabbit Achilles tendon, the densely packed, parallel, and straight collagen fibers include large fibrils of type I collagen. (B) Sheath of a rabbit Achilles tendon: wavy collagen fibers are composed of thin and flexible type III collagen fibrils. Among the fibers, wide spaces appear like empty spaces, but they contained water linked to GAGs and PGs before dehydration during the sample preparation for SEM (White bar = 10 μm). (C) In tendon, the large and stiff type I collagen fibrils assembling in a fiber develop fibrillar crimps when they change their course (White bar = 1 μm). (D) In tendon sheath, the thin and very flexible type III collagen fibrils assembling in a wavy fiber do not show fibrillar crimps when they bend (White bar = 1 μm). ECM, extracellular matrix; GAGs, glycosaminoglycans; PGs, proteoglycans; SEM, scanning electron microscopy.

subepithelial ECM components to the lamina densa of BM [22,32]. BM connected to fibrils of the reticular dermis via integrins can be deformed by repeated mechanical stresses during body movement, touch, and tissue growth.

A temporal mechanical adaptation of ECM (Fig. 2) occurs in wound healing, where collagen plays a critical mechanical role, as demonstrated by a variable ratio of type I and III collagen and the newly formed collagen fibers arranging parallel to the BM in the scar [33]. When tendons and ligaments are subjected to abnormal compression, they adapt to new mechanical stimuli by developing shock-absorbing fibrocartilage [34]. Similarly, in a bone fracture, the repairing tissue adapts to sequentially develop different ECMs (i.e., hematoma, soft callus of fibrocartilage, hard callus of trabecular bone), which ensure the most rapid union of bone ends and fast recovery [35].

Biomechanical roles of ECM in regulating cancer cell phenotype, inflammatory infiltration, and response to chemotherapy

All epithelia are separated from the underlying stroma by the BM, but a continuous dynamic interplay between epithelial cells and different stromal cells occurs (Fig. 1) [36]. Cell-matrix adhesion complexes, including the focal adhesion (FA) complex, mediated by matrix kinases and cytoskeletal proteins such as integrins, p130Cas, zyxin, talin, vinculin, Src, and focal adhesion kinase (FAK), allow epithelial cells to convert mechanical stimuli from the environment into electrical or chemical signals that stimulate various aspects of biochemical responses [37]. For instance, integrin-mediated adhesions initiate force transmission across plasma membranes by exerting mechanical cues on cells, while talin and vinculin facilitate mechanical forces by linking integrins to the actin cytoskeleton [38,39]. As a result of force transmission, integrin conformational changes occur, resulting in activation of FA signaling, which plays a key role in transducing mechanical signals. FA complexes such as FAK, paxillin, or vinculin, undergo further alterations and recruit more signaling molecules. FAK activation triggers a cascade of phosphorylation events that activate downstream effectors implicated in several cell functions like adhesion, migration, and gene expression [40]. Except for these "primary" mechanosensors, emerging evidence suggests the involvement of alternative ones, such as G-protein-coupled receptors (GPCRs), cell surface glycocalyx components, and ion channels, in

detecting and transducing mechanical signals [41]. Thus, multiple mechanosensing mechanisms could coexist, acting in parallel or interplaying with each other to coordinate cellular responses.

Moreover, changes in composition and array of ECM activate mechanosensing and mechanotransduction, which trigger signaling pathways affecting cancer development [42]. Simultaneously, a direct connection between collagen fibrils and the lamina densa of BM through type V collagen, supports a mechanical crosstalk between ECM and epithelial cells [22]. Different chemical and structural arrays of ECM in the tumor microenvironment (TME) can affect the growth and fate of the tumor by opposing but also driving individual or collective cancer cell invasion [43].

Solid tumors become invasive when cancer cells breach the BM and invade the subepithelial meshwork of collagen, GAGs, and PGs, which represents the first ECM barrier. Once cancer cells have invaded the subepithelial ECM, they can easily penetrate a second ECM barrier, including the endothelial BM and plasma or lymph, in which cancer cells flow to colonize distant organs. When epithelial cancer cells cross BM and encounter a reticular collagen network, they find a new, unknown, physical microenvironment which induces the development of epithelial-to-mesenchymal transition (EMT) markers and an elongated-mesenchymal phenotype which suggest increased aggressiveness (Fig. 3).

Peri-tumoral ECMs activate and regulate cascades of chemical signals but also mechanically control tumor progression and invasion by promoting biomechanical resistance, which affects cancer cell phenotypes and behavior. A denser and stiffer peri-tumoral ECM induces cancer cells to assume migrating phenotypes, offers rigid sites of focal adhesions for cells, favors growth of cytoplasmic protrusions, and has the capability to develop high-traction forces [36,44,45].

Cancer cells penetrate connective tissues by collective or individual invasion. In collective or multicellular invasion, grouped cells follow a leader cell (Fig. 4A). Cells invading ECM can display mainly two different migration and invasion modes: mesenchymal-like and amoeboid-like movement. A third alternative migration mechanism via water permeation of cancer cells has also

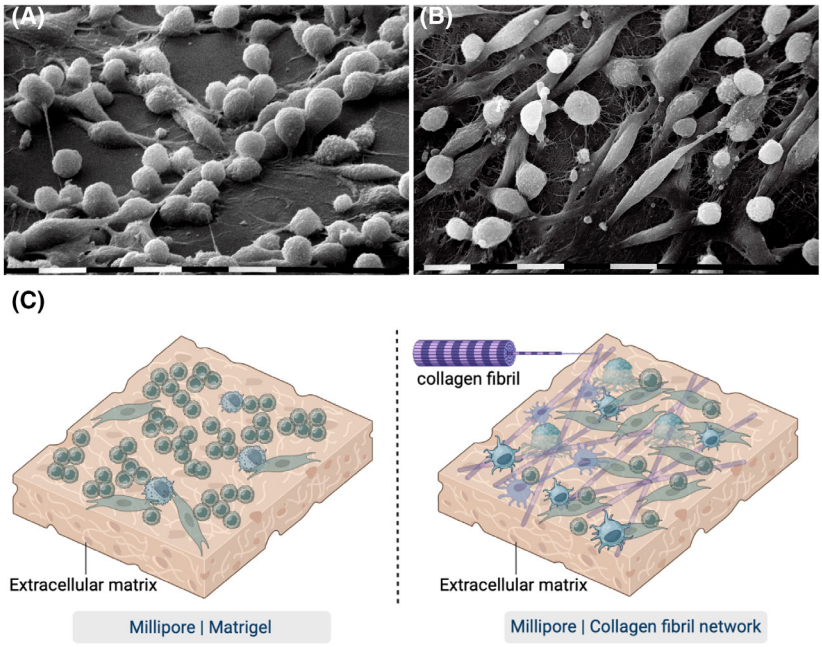

Fig. 3. Collagen, the major component of ECM, affects colorectal cancer cell phenotype. (A) SEM picture of LoVo colorectal cancer cells cultivated on a Millipore filter covered by Matrigel mimicking the BM. Almost all the cells show a globular shape (White bar = 10 μm). (B) SEM image of LoVo cancer cells growing on a Millipore filter covered by a reticular collagen fibril network. Many cells develop a morphological epithelial-to-mesenchymal transition phenotype characterized by elongated mesenchymal shapes. Filopodia and lamellipodia of the elongated shaped cells strongly adhere to the collagen fibril layer (White bar = 10 μm). (C) Different matrix substrates affect cell shape, as alterations in the ECM composition can occur, such as excessive deposition of ECM-degrading enzymes, enabling invasion and metastasis. The ability of cancer cells to sense and respond to changes in the ECM microenvironment as a result of cell adaptation involves dynamic cell-matrix interactions that guide abnormal cell behavior and phenotype, leading to tissue dysfunction. Created with BioRender.com. BM, basement membrane; ECM, extracellular matrix; SEM, scanning electron microscopy.

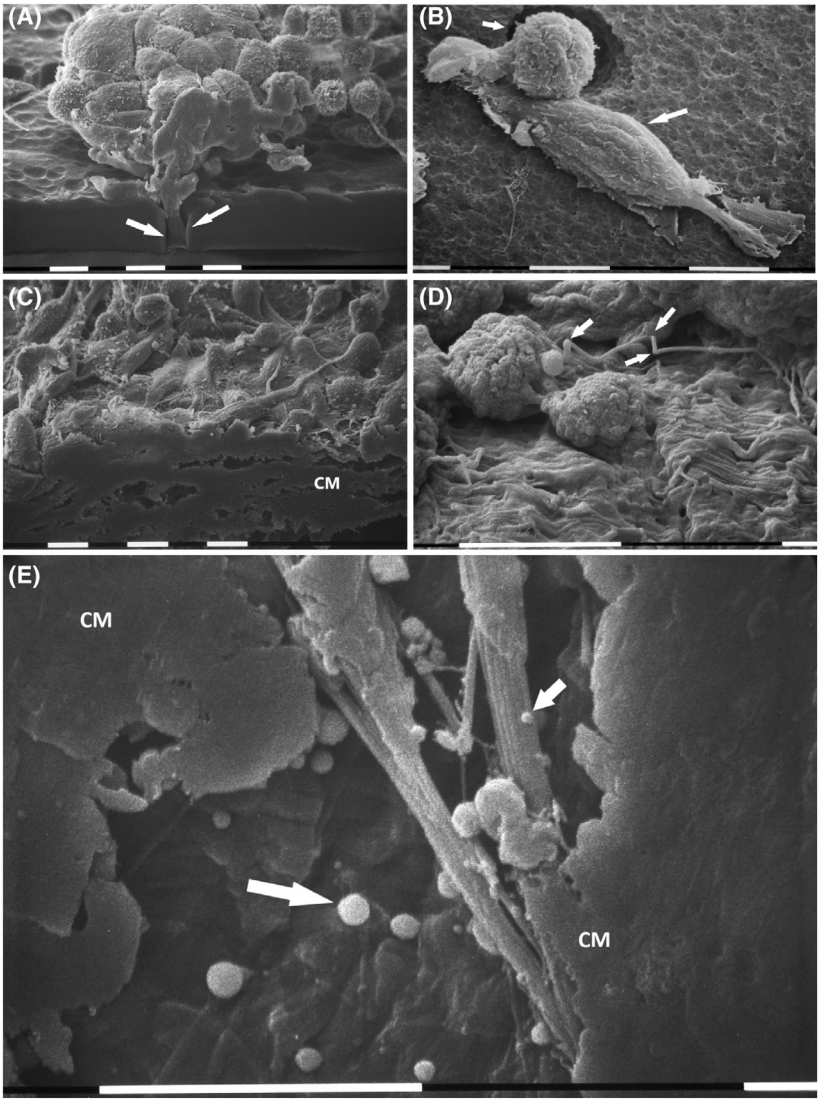

Fig. 4. SEM pictures of 3D breast cancer cell cultures showing different modalities of cell invasion. (A) In a cross section of a Millipore filter, grouped invasive globular MDA-MB-231 breast cancer cells showing tight cell–cell contacts are driven by their leader cell, which is passing through a filter pore (arrows) whose diameter (8 μm) allows the nucleus to squeeze and completely cross the filter (White Bar = 10 μm). (B) An elongated mesenchymal MCF-7 breast cancer cell (long arrow) and an ameboid migrating globular one (short arrow) are individually migrating through a pore of a Millipore filter (White bar = 10 μm). (C) MDA-MB-231 breast cancer cells cultivated on a CM composed of parallel and densely packed type I collagen fibrils. Breast cancer cells developing both elongated-mesenchymal and globular phenotypes are not able to penetrate the compact collagen membrane barrier (White bar = 10 μm). (D) At higher magnification, the breast cancer cells are not able to invade the densely packed, large, and stiff type I collagen fibrils. Fibrillar crimps are recognizable (arrows) in regions where fibrils bend. (E) MDA-MB-231 breast cancer cells develop long filopodia and shed both microvesicles (long arrow) and exosomes (short arrow) which find free small spaces and penetrate the thickness of the dense CM (White bar = 10 μm). 3D, three-dimensional; CM, collagen membrane; SEM, scanning electron microscopy.

been proposed [46]. Mechanical properties like microporosity, density, or stiffness of collagen meshworks represent a physical constraint to nucleus deformability so that ECM can regulate cancer cell invasion mode [47]. The mesenchymal-like invasion is associated to elongated phenotypes which displays a slower speed because cells need high adherence to ECM and local matrix metalloproteinases (MMPs) released by filopodia or invadopodia to create microtracks in the TME [48]. Differently, the ameboid movement is a relative protease independent and fast movement which requires lower adhesion to the TME and is adopted by rounded and globular cells that find wide spaces in the ECM (Fig. 4B). The mesenchymal-like migration, associated with most aggressive phenotypes and metastasis requires an EMT, which, following the mesenchymal motility, is characterized by a loss of cell–cell adhesions (E-cadherin decrease) and apico-basal polarization. Despite this classification, an EMT is not always associated with clinical metastasis because a hybrid state with both epithelial and mesenchymal markers or matrix effectors has been recently discovered in metastatic cancers, and most tumors develop metastasis by clustering cancer cells showing normal E-cadherin expression with cell–cell junctions [43]. A confining microenvironment also induces an alternative migration mechanism via water permeation, which consists of an inflow of water and ions at the cell's leading edge and a corresponding outflow of water and ions at the trailing edge. Interestingly, the reverse migration direction occurs when hypotonically shocked at the leading edges or hypertonically shocked at the trailing edges [46].

Extracellular matrices regulate solid tumor development by first opposing but then favoring cancer cells phenotype and invasive behavior. A tumor-associated collagen signature classification (TACS) of tumor matrix has been proposed. At first, around breast cancer masses a deposition of type I collagen fibrils, or desmoplasia, occurs (TACS-I) to mechanically oppose cancer invasion. Subsequently, the same fibrils bundle to form fibers that run parallel to the tumor surface and oppose cancer cell invasion and metastasis (TACS-II) (Fig. 4C,D). Cancer cell invasion of TME occurs when the peri-tumoral fibers follow a radial alignment, creating spaces for cancer cells invasion (TACS-III) [8,49]. However, the local mechanical properties of single fibrils and their array seem to primarily regulate cancer cell behavior: an increase of fibril diameter and lysyl oxidase-mediated crosslinking have been described as being related to advanced tumor stages [50]. The stiff type I collagen fibrils, forming straight and radially oriented fibers, favor the adhesion of cancer cells and create highways for invasion. ECM plays double opposite mechanical roles in tumor progression: a different orientation of stiff collagen fibrils or fibers prevent or favor cancer cell invasion [51].

Although TACS-II collagen fibers represent an efficient barrier for cell penetration, plasma membrane-derived particles like microvesicles and exosomes secreted by cancer cells can penetrate very small pores of TME, and transmitting biological information activates cancer-associated fibroblasts (CAFs) (Fig. 4E). Activated CAFs and tumor-associated macrophages can change the ECM microenvironment by secreting MMPs to degrade ECM and deposit radially aligned fibers, which form a stiff and confining TME and enhance cancer cell invasion [52–55]. Tightly packed, circular collagen fibers (TACS-II) may also oppose the penetration of immune cells into the tumor, as leukocytes must break down the BM and ECM meshwork by releasing MMPs and heparanase to reach the tumor mass [36,56]. TACS-II type I collagen fibers seem to also contribute to cancer chemoresistance by limiting drug penetration. Differently, the more flexible type III collagen fibrils can suppress primary murine breast tumor growth and metastasis and improve the clinical outcome in breast cancer patients [57].

High deposition of HA in the TME of many tumors regulates tumor progression by modulating hydration and osmotic balance in the TME [58–60]. An increase in the hydraulic resistance in TME, related to the secretion of HA linking water, develops a larger FA complex at the leading edge of the cell and promotes the development of a thicker actomyosin cortex and longer actin filaments [61]. When cancer cells invade interfiber spaces, they find resistance, which contrasts their penetration. However, cancer cells can sense the increased hydraulic pressure and switch their migration behavior (plasticity) by developing an alternative mechanism via water permeation, including the uptake and discharge of water [61].

Thanks to its hygroscopic properties, HA improves viscoelasticity and lubrication in connective tissues. Overexpressing of the HA receptor (CD44) on mesenchymal stem cell membranes improves their homing potential towards the inflammatory site [62]. Melanoma cells penetrating trails along muscle or collagen fibers must squeeze through small pores or channels [63]. Cancer cells develop a protective pericellular HA coating, which could mechanically help cancer cell invasion by reducing friction with TME components [64]. HA is a marker indicating poor prognosis in cancer patients as it increases cancer cell growth and motility and acts as a biological barrier to drug delivery in solid tumors [65]. Increased HA contributes to the fluidity of the ECM. Viscoelastic properties of ECM guide tissue proliferation in space and time; changes in viscosity in TME regulate invasive cytoplasmic protrusions and infiltrative aggressiveness in glioblastoma, breast, and liver cancers [66–68]. Hence, the role of fluid mechanics and the transduction of fluid mechanical signals in tumors and TME will become crucial in cancer research.

Also, some PGs regulate cancer progression by playing mechanical roles in the ECM of solid tumors. Both decorin and lumican are potential anti-tumor therapeutic molecules in melanoma and breast or pancreatic cancer. Decorin, lumican, and biglycan bind to type I collagen fibrils. Biglycan inhibits cancer cell proliferation and extends long-term survival in pancreatic or bladder cancer patients [69,70]. Decorin inhibits collagen fibrillogenesis, whereas lumican controls the size of fibrils [71]. These PGs might contribute to compacting fibrils into dense fibers that arranged parallel to the tumor surface, oppose cancer cell invasion.

Conclusions

Every human or other mammal species holds genetically printed ECM macromolecular networks, contributing to the uniqueness of everyone. ECMs form the bioscaffolds of our body and adapt to the environmental variables influencing growth and the final adult body shape in mammals. ECMs regulate our lives by controlling the diffusion of infections and inflammations, sensing and adapting to external stimuli and gravity from the surrounding habitat, and in the case of cancer, interplaying

with cancer cells and regulating tumor invasion and drug resistance. The biomechanical properties of ECM are negatively affected by aging, but the successful applications of new ECM medical devices as well as genetic therapeutic procedures will allow us to modify the altered ECMs to control many diseases and cancers or attenuate aging decline in the near future.

Acknowledgments

This research did not receive any specific grants from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

MF and NK contributed to conceptualization, literature review, writing, and editing the manuscript; ZP contributed to literature review, writing, editing the manuscript and figure preparation; NSM conducted literature review and manuscript editing; and NK contributed to conceptualization, editing, and supervision of the manuscript preparation. All authors read and approved the final manuscript.

References

1 Tu Y & Rappel W-J (2018) Adaptation in living systems. *Annu Rev Condens Matter Phys* **9**, 183–205.
2 Theocharis AD, Manou D & Karamanos NK (2019) The extracellular matrix as a multitasking player in disease. *FEBS J* **286**, 2830–2869.
3 Karamanos NK, Theocharis AD, Piperigkou Z, Manou D, Passi A, Skandalis SS, Vynios DH, Orian-Rousseau V, Ricard-Blum S, Schmelzer CEH *et al.* (2021) A guide to the composition and functions of the extracellular matrix. *FEBS J* **288**, 6850–6912.
4 Kyriakopoulou K, Koutsakis C, Piperigkou Z & Karamanos NK (2023) Recreating the extracellular matrix: novel 3D cell culture platforms in cancer research. *FEBS J* doi: 10.1111/febs.16778
5 Shin J-W, Kwon S-H, Choi J-Y, Na J-I, Huh C-H, Choi H-R & Park K-C (2019) Molecular mechanisms of dermal aging and antiaging approaches. *Int J Mol Sci* **20**, 2126.
6 Atakan MM, Koşar ŞN, Güzel Y, Tin HT & Yan X (2021) The role of exercise, diet, and cytokines in preventing obesity and improving adipose tissue. *Nutrients* **13**, 1459.
7 Iranmanesh B, Khalili M, Mohammadi S, Amiri R & Aflatoonian M (2022) Employing hyaluronic acid-based mesotherapy for facial rejuvenation. *J Cosmet Dermatol* **21**, 6605–6618.
8 Franchi M, Masola V, Bellin G, Onisto M, Karamanos K & Piperigkou Z (2019) Collagen fiber array of peritumoral stroma influences epithelial-to-mesenchymal transition and invasive potential of mammary cancer cells. *J Clin Med* **8**, 213.
9 Anil U, Markus DH, Hurley ET, Manjunath AK, Alaia MJ, Campbell KA, Jazrawi LM & Strauss EJ (2021) The efficacy of intra-articular injections in the treatment of knee osteoarthritis: a network meta-analysis of randomized controlled trials. *Knee* **32**, 173–182.
10 Scott JE (2003) Elasticity in extracellular matrix 'shape modules' of tendon, cartilage, etc. a sliding proteoglycan-filament model. *J Physiol* **553**, 335–343.
11 Liu Z, Lin W, Fan Y, Kampf N, Wang Y & Klein J (2020) Effects of hyaluronan molecular weight on the lubrication of cartilage-emulating boundary layers. *Biomacromolecules* **21**, 4345–4354.
12 Roig-Rosello E & Rousselle P (2020) The human epidermal basement membrane: a shaped and cell instructive platform that aging slowly alters. *Biomolecules* **10**, 1607.
13 Pratt RL (2021) Hyaluronan and the fascial frontier. *Int J Mol Sci* **22**, 6845.
14 Mutsaers SE (2002) Mesothelial cells: their structure, function and role in serosal repair. *Respirology* **7**, 171–191.
15 Porta C, Sironi C, Bodega F & Agostoni E (2016) Pleural lubrication. *Lubricants* **4**, 15.
16 Shen ZL, Dodge MR, Kahn H, Ballarini R & Eppell SJ (2010) In vitro fracture testing of submicron diameter collagen fibril specimens. *Biophys J* **99**, 1986–1995.
17 Ricard-Blum S (2011) The collagen family. *Cold Spring Harb Perspect Biol* **3**, a004978.
18 Bella J & Hulmes DJS (2017) Fibrillar collagens. *Subcell Biochem* **82**, 457–490.
19 Hulmes DJS (2002) Building collagen molecules, fibrils, and Suprafibrillar structures. *J Struct Biol* **137**, 2–10.
20 Franchi M, Ottani V, Stagni R & Ruggeri A (2010) Tendon and ligament fibrillar crimps give rise to left-handed helices of collagen fibrils in both planar and helical crimps. *J Anat* **216**, 301–309.
21 Asgari M, Latifi N, Heris HK, Vali H & Mongeau L (2017) In vitro fibrillogenesis of tropocollagen type III in collagen type I affects its relative fibrillar topology and mechanics. *Sci Rep* **7**, 1392.
22 Adachi E, Hopkinson I & Hayashi T (1997) Basement-membrane stromal relationships: interactions between collagen fibrils and the lamina densa. *Int Rev Cytol* **173**, 73–156.
23 Franchi M, Raspanti M, Dell'Orbo C, Quaranta M, De Pasquale V, Ottani V & Ruggeri A (2008) Different

crimp patterns in collagen fibrils relate to the subfibrillar arrangement. *Connect Tissue Res* **49**, 85–91.
24 Franchi M, Trirè A, Quaranta M, Orsini E & Ottani V (2007) Collagen structure of tendon relates to function. *Scientific World Journal* **7**, 404–420.
25 Kuivaniemi H & Tromp G (2019) Type III collagen (COL3A1): gene and protein structure, tissue distribution, and associated diseases. *Gene* **707**, 151–171.
26 Ottani V, Raspanti M & Ruggeri A (2001) Collagen structure and functional implications. *Micron* **32**, 251–260.
27 Franchi M, Fini M, Quaranta M, De Pasquale V, Raspanti M, Giavaresi G, Ottani V & Ruggeri A (2007) Crimp morphology in relaxed and stretched rat Achilles tendon. *J Anat* **210**, 1–7.
28 Kielty CM, Sherratt MJ & Shuttleworth CA (2002) Elastic fibres. *J Cell Sci* **115**, 2817–2828.
29 Ushiki T (2002) Collagen fibers, reticular fibers and elastic fibers. A comprehensive understanding from a morphological viewpoint. *Arch Histol Cytol* **65**, 109–126.
30 Gosline J, Lillie M, Carrington E, Guerette P, Ortlepp C & Savage K (2002) Elastic proteins: biological roles and mechanical properties. *Philos Trans R Soc Lond B Biol Sci* **357**, 121–132.
31 Zollinger AJ & Smith ML (2017) Fibronectin, the extracellular glue. *Matrix Biol* **60–61**, 27–37.
32 Chung HJ & Uitto J (2010) Type VII collagen: the anchoring fibril protein at fault in dystrophic epidermolysis bullosa. *Dermatol Clin* **28**, 93–105.
33 Mathew-Steiner SS, Roy S & Sen CK (2021) Collagen in wound healing. *Bioengineering* **8**, 63.
34 Benjamin M & Ralphs JR (1998) Fibrocartilage in tendons and ligaments – an adaptation to compressive load. *J Anat* **193**, 481–494.
35 Holmes D (2017) Closing the gap. *Nature* **550**, S194–S195.
36 Karamanos NK, Piperigkou Z, Passi A, Götte M, Rousselle P & Vlodavsky I (2021) Extracellular matrix-based cancer targeting. *Trends Mol Med* **27**, 1000–1013.
37 Petridou NI, Spiró Z & Heisenberg C-P (2017) Multiscale force sensing in development. *Nat Cell Biol* **19**, 581–588.
38 Saunders RM, Holt MR, Jennings L, Sutton DH, Barsukov IL, Bobkov A, Liddington RC, Adamson EA, Dunn GA & Critchley DR (2006) Role of vinculin in regulating focal adhesion turnover. *Eur J Cell Biol* **85**, 487–500.
39 Yao M, Goult BT, Chen H, Cong P, Sheetz MP & Yan J (2014) Mechanical activation of vinculin binding to Talin locks Talin in an unfolded conformation. *Sci Rep* **4**, 4610.
40 Parsons JT (2003) Focal adhesion kinase: the first ten years. *J Cell Sci* **116**, 1409–1416.
41 Eyckmans J, Boudou T, Yu X & Chen CS (2011) A Hitchhiker's guide to mechanobiology. *Dev Cell* **21**, 35–47.
42 Urbanczyk M, Layland SL & Schenke-Layland K (2020) The role of extracellular matrix in biomechanics and its impact on bioengineering of cells and 3D tissues. *Matrix Biol* **85–86**, 1–14.
43 Jolly MK, Ware KE, Gilja S, Somarelli JA & Levine H (2017) EMT and MET: necessary or permissive for metastasis? *Mol Oncol* **11**, 755–769.
44 Brábek J, Mierke CT, Rösel D, Veselý P & Fabry B (2010) The role of the tissue microenvironment in the regulation of cancer cell motility and invasion. *Cell Commun. Signal.* **8**, 22.
45 Acerbi I, Cassereau L, Dean I, Shi Q, Au A, Park C, Chen YY, Liphardt J, Hwang ES & Weaver VM (2015) Human breast cancer invasion and aggression correlates with ECM stiffening and immune cell infiltration. *Integr Biol* **7**, 1120–1134.
46 Stroka KM, Jiang H, Chen S-H, Tong Z, Wirtz D, Sun SX & Konstantopoulos K (2014) Water permeation drives tumor cell migration in confined microenvironments. *Cell* **157**, 611–623.
47 Wolf K, te Lindert M, Krause M, Alexander S, te Riet J, Willis AL, Hoffman RM, Figdor CG, Weiss SJ & Friedl P (2013) Physical limits of cell migration: control by ECM space and nuclear deformation and tuning by proteolysis and traction force. *J Cell Biol* **201**, 1069–1084.
48 Karamanou K, Franchi M, Vynios D & Brézillon S (2020) Epithelial-to-mesenchymal transition and invadopodia markers in breast cancer: lumican a key regulator. *Semin Cancer Biol* **62**, 125–133.
49 Provenzano PP, Inman DR, Eliceiri KW, Knittel JG, Yan L, Rueden CT, White JG & Keely PJ (2008) Collagen density promotes mammary tumor initiation and progression. *BMC Med* **6**, 11.
50 Sapudom J, Kalbitzer L, Wu X, Martin S, Kroy K & Pompe T (2019) Fibril bending stiffness of 3D collagen matrices instructs spreading and clustering of invasive and non-invasive breast cancer cells. *Biomaterials* **193**, 47–57.
51 Xu S, Xu H, Wang W, Li S, Li H, Li T, Zhang W, Yu X & Liu L (2019) The role of collagen in cancer: from bench to bedside. *J Transl Med* **17**, 309.
52 Liao Z, Tan ZW, Zhu P & Tan NS (2019) Cancer-associated fibroblasts in tumor microenvironment – accomplices in tumor malignancy. *Cell Immunol* **343**, 103729.
53 Yoshida GJ (2020) Regulation of heterogeneous cancer-associated fibroblasts: the molecular pathology of activated signaling pathways. *J Exp Clin Cancer Res* **39**, 112.
54 Biffi G & Tuveson DA (2021) Diversity and biology of cancer-associated fibroblasts. *Physiol Rev* **101**, 147–176.

55 Nurmik M, Ullmann P, Rodriguez F, Haan S & Letellier E (2020) In search of definitions: cancer-associated fibroblasts and their markers. *Int J Cancer* **146**, 895–905.
56 Piperigkou Z, Kyriakopoulou K, Koutsakis C, Mastronikolis S & Karamanos NK (2021) Key matrix remodeling enzymes: functions and targeting in cancer. *Cancers (Basel)* **13**, 1441.
57 Brisson BK, Mauldin EA, Lei W, Vogel LK, Power AM, Lo A, Dopkin D, Khanna C, Wells RG, Puré E *et al.* (2015) Type III collagen directs stromal organization and limits metastasis in a murine model of breast cancer. *Am J Pathol* **185**, 1471–1486.
58 McCarthy JB, El-Ashry D & Turley EA (2018) Hyaluronan, cancer-associated fibroblasts and the tumor microenvironment in malignant progression. *Front Cell Dev Biol* **6**, 48.
59 Tavianatou AG, Caon I, Franchi M, Piperigkou Z, Galesso D & Karamanos NK (2019) Hyaluronan: molecular size-dependent signaling and biological functions in inflammation and cancer. *FEBS J* **286**, 2883–2908.
60 Karalis T & Skandalis SS (2022) Hyaluronan network: a driving force in cancer progression. *Am J Physiol Cell Physiol* **323**, C145–C158.
61 Zhao R, Afthinos A, Zhu T, Mistriotis P, Li Y, Serra SA, Zhang Y, Yankaskas CL, He S, Valverde MA *et al.* (2019) Cell sensing and decision-making in confinement: the role of TRPM7 in a tug of war between hydraulic pressure and cross-sectional area. *Sci Adv* **5**, eaaw7243.
62 Corradetti B, Taraballi F, Martinez JO, Minardi S, Basu N, Bauza G, Evangelopoulos M, Powell S, Corbo C & Tasciotti E (2017) Hyaluronic acid coatings as a simple and efficient approach to improve MSC homing toward the site of inflammation. *Sci Rep* **7**, 7991.
63 Weigelin B, Bakker G-J & Friedl P (2012) Intravital third harmonic generation microscopy of collective melanoma cell invasion. *Dermatol Int* **1**, 32–43.
64 Rilla K, Siiskonen H, Tammi M & Tammi R (2014) Hyaluronan-coated extracellular vesicles—a novel link between hyaluronan and cancer. *Adv Cancer Res* **123**, 121–148.
65 Voutouri C & Stylianopoulos T (2018) Accumulation of mechanical forces in tumors is related to hyaluronan content and tissue stiffness. *PLoS One* **13**, e0193801.
66 Elosegui-Artola A, Gupta A, Najibi AJ, Seo BR, Garry R, Tringides CM, de Lázaro I, Darnell M, Gu W, Zhou Q *et al.* (2023) Matrix viscoelasticity controls spatiotemporal tissue organization. *Nat Mater* **22**, 117–127.
67 Streitberger K-J, Lilaj L, Schrank F, Braun J, Hoffmann K-T, Reiss-Zimmermann M, Käs JA & Sack I (2020) How tissue fluidity influences brain tumor progression. *Proc Natl Acad Sci USA* **117**, 128–134.
68 Shahryari M, Tzschätzsch H, Guo J, Marticorena Garcia SR, Böning G, Fehrenbach U, Stencel L, Asbach P, Hamm B, Käs JA *et al.* (2019) Tomoelastography distinguishes noninvasively between benign and malignant liver lesions. *Cancer Res* **79**, 5704–5710.
69 Karamanos NK, Piperigkou Z, Theocharis AD, Watanabe H, Franchi M, Baud S, Brézillon S, Götte M, Passi A, Vigetti D *et al.* (2018) Proteoglycan chemical diversity drives multifunctional cell regulation and therapeutics. *Chem Rev* **118**, 9152–9232.
70 Diehl V, Huber LS, Trebicka J, Wygrecka M, Iozzo RV & Schaefer L (2021) The role of Decorin and Biglycan signaling in tumorigenesis. *Front Oncol* **11**, 801801.
71 Neame PJ, Kay CJ, McQuillan DJ, Beales MP & Hassell JR (2000) Independent modulation of collagen fibrillogenesis by decorin and lumican. *Cell Mol Life Sci* **57**, 859–863.
72 Iozzo RV, Theocharis AD, Neill T & Karamanos NK (2020) Complexity of matrix phenotypes. *Matrix Biol Plus* **6–7**, 100038.