“Say Yes to the Stress”: the perfectly tailored mechanical environment for tumor metastasis

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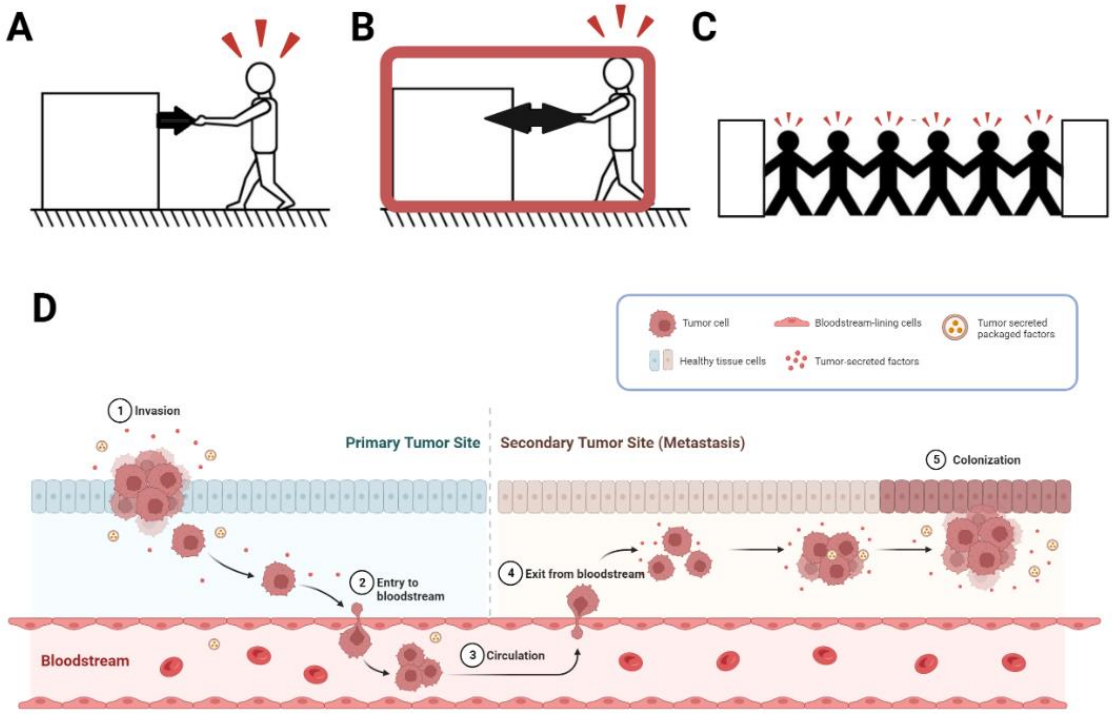
Layman's Summary

The second largest medical burden in society is cancer, which is a category of illnesses having the potential to spread throughout the body and includes unchecked multiplication of cells. Cancer can prevent healthy tissues from working normally and become fatal if untreated and allowed to spread and grow in multiple organs, called metastasis.

The process of metastasis happens in multiple steps. First, cells from the tumor must break away from the primary tumor and make their way through the supportive tissue surrounding them. Tumor cells must reach the bloodstream, survive in circulation and exit at a second location. There, tumor cells must establish themselves in a new tissue. Whether this secondary site fits their needs depends on the primary origin of the cancer cell and the cancer cell's ability to change the environment around them.

During the multiple steps of metastasis, starting at the outgrowth of the primary tumor and ending at the outgrowth at the secondary site, cells are affected strongly by mechanical forces. Cells can be pulled and pushed, but they can also generate pulling and pushing forces to affect other cells around them. Sensing these forces sends signals on the inside of tumor cells. These signals can lead to changes in behavior related to movement, survival and/or cell division. For cancer, these responses in behavior support their existence, growth and spread in the body. The forces that cells experience is linked to the way they sense this and also to the inputs received surrounding supportive tissue and other cell types present. Tumor cells located at a primary site are even able to change mechanical aspects of a tissue at the secondary site before they physically arrive there. This is accomplished by shedding proteins or instructions to make them, into small packages that travel through the bloodstream and are delivered in a secondary spot, where the tumor cells eventually reach as well.



Mechanical forces on (tumor) cells and their journey of metastasis A) Cells can exert pulling forces on their surroundings, like supportive tissue, and feel this tension. B) Cells can experience and feel compression by being in confined space. C) Cells can experience forces together, by being attached to their surroundings and each other. D) Several steps of metastasis are affected by mechanical forces from detaching and migrating from the primary tumor site, to entering and exiting the bloodstream, and establishing at a secondary location Figure created with www.biorender.com

Improving our understanding of how cancer spreads will allow for improved clinical interventions, as we currently diagnose metastasis at a stage in which the secondary tumor is relatively large, e.g. visible to our detection methods.

In this review, we discuss several ways that tumor cells are affected by mechanical inputs from their environment and how they respond to this, in several steps of the metastatic journey. An important outstanding question that remains, is the way in which the secondary site contributes to the outgrowth of cells from a primary tumor, particularly in the context of mechanical inputs. With better understanding of why a tumor cell will grow out at a different location might provide insights. From this, we might also learn why specific tumors favor certain locations to form another tumor, and allow us to predict or monitor the occurrence so we can intervene.

List of abbreviations

AGE – advanced glycation end product
BM – basement membrane
CAF – cancer associated fibroblasts
CD – cluster of differentiation
COPD – chronic obstructive pulmonary disorder
CTC – circulating tumor cell
ECM – extracellular matrix
EMT – epithelial-mesenchymal transition
ER – endoplasmic reticulum
EV – extracellular vesicle
FA – focal adhesion
FAK – focal adhesion kinase
HNSCC – head and neck small squamous cell carcinoma
IPF – idiopathic pulmonary fibrosis
LOX – lysyl oxidase
miRNA – micro-RNA
MMP – matrix metalloprotease
MSC – mesenchymal stem cell
N - Newton
NET – neutrophil extracellular trap
RAS – renin-angiotensin system
ROCK – Rho-associated protein kinase
VEGF – vascular endothelial growth factor
YAP – yes-associated protein

Glossary

Amoeboid movement: moving or changing shape by protoplasmic movement, and therefore resembling an amoeba.

Angiogenesis: a physiological process that occurs during the early stages of forming of vasculature in which new blood vessels are created from pre-existing ones.

Compliance: The volume change that takes place for every unit change in the system's pressure. Compliance can be defined as the simplicity with which an elastic structure extends. Hence, compliance is essentially a measurement of a system's elastic resistance.

Dysplasia: refers to the abnormal growth of cells, observed histopathologically. Cells are no longer in a regular, organized pattern of cell shape and arrangement. This stage precedes malignant progression, a carcinoma in situ.

Extra-/Intravasation: extravasation in cancer refers to the cancer cells that exit from circulation into a secondary organ during metastasis. Intravasation refers to the exit of a tissue into circulation.

Filopodium: protrusive structures created by migrating and invading cells. Filopodia are formed at the leading edge of migratory cells.

Haptotaxis: the migration of cells along an adhesion gradient in the direction of substrate adherence.

Hippo-pathway: this pathways regulates organ size though close regulation of proliferation and apoptosis.

Invadopodium: a protrusive structure created by migrating and invading cells. Invadopodia are observed on the ventral side of the cell membrane, in regards to a uni-directional movement. It involves F-actin-rich matrix-degrading structures.

Ischemic reperfusion injury: the paradoxical worsening of cellular dysfunction and mortality after the restoration of blood flow to previously ischemic tissue, due to oxygen radicals.

Laminar flow: this type of flow occurs when a fluid flows between parallel layers within a tube with no disruption of the parallel layers. The forces and directionality remain straight and there are no cross currents. Opposite of laminar is turbulent flow, which is chaotic and diffusive. This turbulence can be induced by changes in flow velocity or pressure.

Micro-/macrometastasis: micrometastases are too small to be identified with conventional medical imaging tools, whereas macrometastases can be seen in these scans.

Neo-epitope: areas of antigens produced by altering the original antigen, frequently through pathologic events. It is an antigen that the immune system has not encountered before.

Rheology: a part of physics that studies the flow and deformation of materials, both liquids and solids.

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Abstract

The main cause of cancer lethality is metastasis. Throughout cancer progression cells are affected by mechanical forces. Forces like tension, compression and shear of fluid flow can be generated and sensed by cells (mechanosensing), and can biochemically be transduced (mechanotransduction), leading to alterations in cellular fate and behavior. In tumors, changes can occur in how cells sense forces in the environment, or the environment can change. Tumor cells are affected by changes in extracellular matrix (ECM) composition and mechanical strength, of which these changes can also be induced by the tumor itself or tumor-recruited stromal cells. Mechanical forces and sensing of them are involved in many steps of metastasis, from the local environment of the primary tumor, to dissemination through circulation, re-entry into tissues and colonization of secondary sites. In this review we discuss our current understanding of the contribution of mechanosensing and mechanotransduction at several steps of the metastatic cascade, including mechanical changes prior to colonization caused by cancer, various pathologies and aging.

Introduction

Metastasis is a process by which cancer cells disperse from their primary site of tumorigenesis and disseminate to a different part of the body. It remains the major cause of morbidity and mortality in cancer patients. Before colonization at secondary sites, cancer cells undergo a complicated cascade, starting with invasion of the primary tumor into surrounding tissue and into the circulation. Tumors that survive in the circulation can form an initial seeding at distant organs, followed by their outgrowth and colonization to thrive at secondary sites (Welch & Hurst, 2019).

The metastatic cascade is influenced by many factors present in the tumor microenvironment. Increased tumor cell contractility, the growth of the expanding tumor mass, and modifications to the extracellular matrix (ECM) contribute to changes in the tumor microenvironment. Mechanobiology studies cellular responses to stress, strain and forces, as well as the cell-generated contributions to forces. Cellular responses are linked to the ECM’s mechanical properties like stiffness and adhesiveness, but can also be influenced by the way tumor cells sense these properties. This is of relevance because probing the mechanics of the environment influences cellular behavior such as motility or survival, and consequently tissue organization and function. In cancer, this can drive cells to become metastatic.

Receptor-mediated mechanosensing is the binding of cell surface receptors to their ligands on the surfaces of nearby cells or the ECM. By generating cell-intrinsic tensile forces, the resistance of surrounding tissue is translated to intracellular protein conformational changes, allowing for further signaling. When numerous mechanical stresses are applied to cells, a process called mechanotransduction activates downstream signaling pathways. Consequently, mechano-activated signaling pathways can be oncogenic during overactivation, thereby promoting the growth and aggressivity of a tumor (Northcott et al., 2018).

In addition to determinants of primary cancer progression, mechanical sensing and forces are involved at secondary sites of tumors. The location of a metastasis must be a favorable environment to accommodate to the demands of the tumor cells, often referred to as the "seed/soil hypothesis' (S. Paget, 1889; S. J. Paget, 1888). Many cancers show patterns of non-random distant metastasis called organotropism, arguably an inherent trait of both the origin and nature of the primary cancer, as well as the characteristics of the site of metastasis. A framework that can predict further steps of the metastatic cascade and particularly the location of metastases, is desired. The role that mechanosensing fulfills in cells by responding to the environment, therefore may regulate favoritism of certain tissues during the later stages of metastasis.

In this review, we describe the role of mechanobiology in progression of solid primary tumors and its dissemination. We also discuss mechanical interactions contributing to outgrowth at a secondary site. This also includes modifications to tissues by tumor-derived factors prior to the physical presence of the tumor cells. We continue with discussion on the mechanobiological role of pathologies that can similarly establish a tumor-promoting niche. Finally, we reflect on future perspectives of methods for investigation of mechano-regulated steps in metastasis.

General Mechanisms of Metastasis

In their seminal framework, Hanahan and Weinberg described the several "hallmarks of cancer", in which they described multiple intrinsic aspects of cancer, such as angiogenesis, immune evasion and resisting cell death (Hanahan & Weinberg, 2000). This work was later complimented with hallmarks such as acquiring phenotypic plasticity and activating invasion and metastasis. These hallmarks are strongly influenced by mechanical sensing of the environment and their relationship will be discussed in this review (Hanahan, 2022; Hanahan & Weinberg, 2011a).

To allow for migration, cancer cells must disrupt the basement membrane (BM) surrounding the tumor, as well as degrade and modulate the ECM. They do this by releasing matrix degrading enzymes, including matrix metalloproteases (MMPs), heparanase, hyaluronase, which contribute to ECM turnover (Welch & Hurst, 2019).

After breaking through the BM, tumor cells can become more motile, for which they undergo cytoskeletal reorganization: cell migration is a multistep mechanical process involving a number of well-coordinated processes, starting with the leading edge protruding (lamellipodium), with smaller transient protrusions within this edge (filopodia) and more stable, protrusions (invadopodia) localized underneath the cell body that possess substrate degradation properties (**Figure 1A**). Mechanical contractile forces as a result of actin and myosin interaction (**Figure 1B**), propel the cellular body in the direction of the leading edge through ECM attachment using focal adhesions (FAs). FAs are connected to the actin cytoskeleton and attaches the cell to the ECM substrate at the front of movement. Inversely, their disassembly takes place on the retracting end.

As the cell type of origin of the cancer is often stationary and not a motile cell type, this movement is caused by autocrine signaling and/or sensing of the surrounding matrix (Welch & Hurst, 2019). As invasive epithelial cells drastically change cell shape

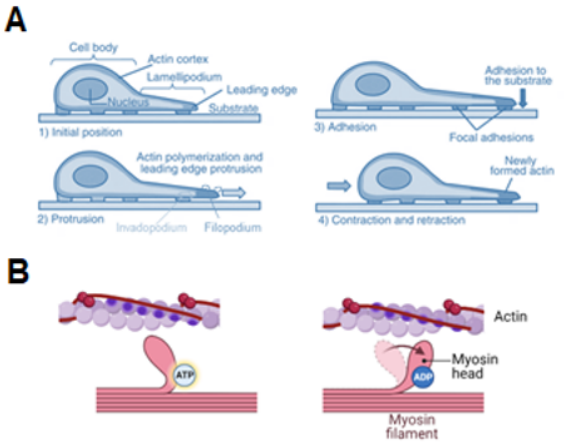

Figure 1 | Cellular migration through attachment and contraction. A) Through extending at the leading edge, cells can attach to the ECM in different modalities. Contractile force combined with attachment at the leading edge, pulls the cellular body forwards. B) Myosin heads flip through ATP hydrolyzation to move actin filaments closer together, thereby generating contractile forces.

ECM: extracellular matrix

Figure adapted from (Okeyo et al., 2015) and created with www.biorender.com

and lack polarity, it is thought that they acquire a more mesenchymal phenotype. This transition, referred to as epithelial-mesenchymal transition (EMT), is characterized in cancer cells by the loss of the adherens junctions component E-cadherin and the upregulation of specific cytoskeletal components such as vimentin, which acts as an intracellular scaffold for the formation of invadopodia during cancer cell migration (Their, 2002). Apart from single-cell migration, cancer cells can also migrate collectively. This does not require full EMT and its mechanocontrol is more dependent on varied adhesion protein distributions in leader and follower cells within a collective forefront, than a single-cell's phenotypic and mechanical profile (Khalil & de Rooij, 2019; Yang et al., 2019).

It should be emphasized that invasion of tissues is necessary but not yet sufficient for metastasis to take place, as the metastatic cascade involves several steps that tumor cells must overcome before forming a distant secondary tumor. Progressing from invasion of surrounding tissue, tumor cells must next enter the vasculature and survive in the circulation, a process which is highly inefficient: the number of circulating tumor cells (CTCs) by far exceeds the amount of metastases formed (Nagrath et al., 2007). This lack of survival can be attributed to characteristics such as CTCs not being adept to the strong shear forces in the bloodstream, but also being intercepted by surveilling immune cells (Follain et al., 2020; Mohme et al., 2016). To extravasate, cells must arrest and adhere to the vessel wall. Cells can arrest upon encountering a physical occlusion, e.g. the diameter of the tumor cell exceeds the diameter of the vessel. Additionally, arrest is mediated by the amount of adhesive forces relative to the forces pushing CTCs through the vessels. Adhesion occurs through the expression of adhesion molecules and ligands by both cancer cells and endothelial cells. For instance, lectins are upregulated during inflammation and induce endothelial adhesion (Follain et al., 2020).

Upon entering the stroma of organs at distant sites, initial seeding must transition into successful colonization. Extravasated cells may never grow out or stay dormant for an extended period of time, varying from months to years. Whether tumor cells remain dormant or switch to outgrowth, can be dependent on multiple factors in the metastatic niche. Tumor cells influence this niche proximally and distally, thus upon arrival of tumor cells as well as prior to metastasis to the tissue.

Preceding metastasis, modification of a tissue can make it more mechanically favorable for tumor cells to establish a distant secondary tumor at a certain location. Cancers can prepare the niche of metastasis prior to arrival, as theorized

earlier by (Kaplan et al., 2006). To achieve remodeling at a distance, tumors secrete factors can be delivered to specific tissues. This includes proteins or RNAs by extracellular vesicles (EVs), allow the cells and surrounding tissue found in the pre-metastatic niche to be more receptive for colonization, for instance by modifying the architecture of the ECM leading to differential mechanical sensing of a tissue (Peinado et al., 2017).

The role of mechanical forces in cancer progression and dissemination

Cells experience mechanical forces from their external environment and neighboring cells that they are attached to. These forces are influenced and generated by both cells and their surroundings, such as fluid flow. Forces lead to (deformation) stress in cells through cell-cell and cell-matrix adhesions. For instance, a cell can be stretched when it is attached to rigid ECM on one side and its neighboring cells contract on the other side. Next to tensile forces, are two other types of mechanical stress that can act on cells: compressive and shear, as summarized in **Figure 2**.

Three types of mechanical forces affect cells

Cells can experience compressive forces perpendicular to their surface. In cancer, compressive stress is elevated through proliferation of the cancer cells within a confined space, as well as an increase in interstitial fluid pressure. Poorly formed vasculature through tumor-mediated angiogenesis leads to leaky vessels contributing to an increase in interstitial fluid pressure within the tumor (Northcott et al., 2018). Additionally, increased deposition of proteoglycans with water-binding properties, such as hyaluronan, causes swelling and further increase of the internal pressure of a tumor (Nia et al., 2020). The difference in pressure within the tumor tissue and adjacent healthy tissue generates flow outwards of the tumor, thereby facilitating dissemination. When tumor cells are adjacent to fluid flow like in blood or lymph, they encounter another sort of stress: shear. This type of stress is caused by the movement of fluid, such as blood. The amount of shear is influenced by the viscosity of a fluid and the amount of flow. Cancer cells show increased resistance to shear stress, e.g. fatal levels for non-transformed cells. Depending on the magnitude, it can have tumor-promoting effects, e.g. resting levels of blood pressure/speed thus hemodynamic shear (S. Ma et al., 2018; Qin et al., 2019). But higher amount of shear can also induce cell death, e.g. exercise levels of hemodynamic shear lead to cell death) (Regmi et al., 2017).

Lastly, cells can experience tensile forces, e.g. stretching. These forces are intracellularly generated and imposed on cells by cell-cell adhesions, as well as adhering to the ECM.

Experiencing mechanical forces can induce or alter intracellular signaling leading to a cellular response. In cancer, altered mechanosignaling leads to responses in survival, invasion and proliferation (Provenzano & Keely, 2011).

Sensing of forces leads to intracellular responses

To relay signals of the combinatorial effects of stress, several types of mechanosensors are present on and in cells. They are influenced by the extent, orientation and dynamics of mechanical forces through time. Mechanosensors act via molecular interactions or protein activity as a result of force-induced conformational changes. For example, the mechanosensor Piezo1 is an ion-channel found on the cell membrane as

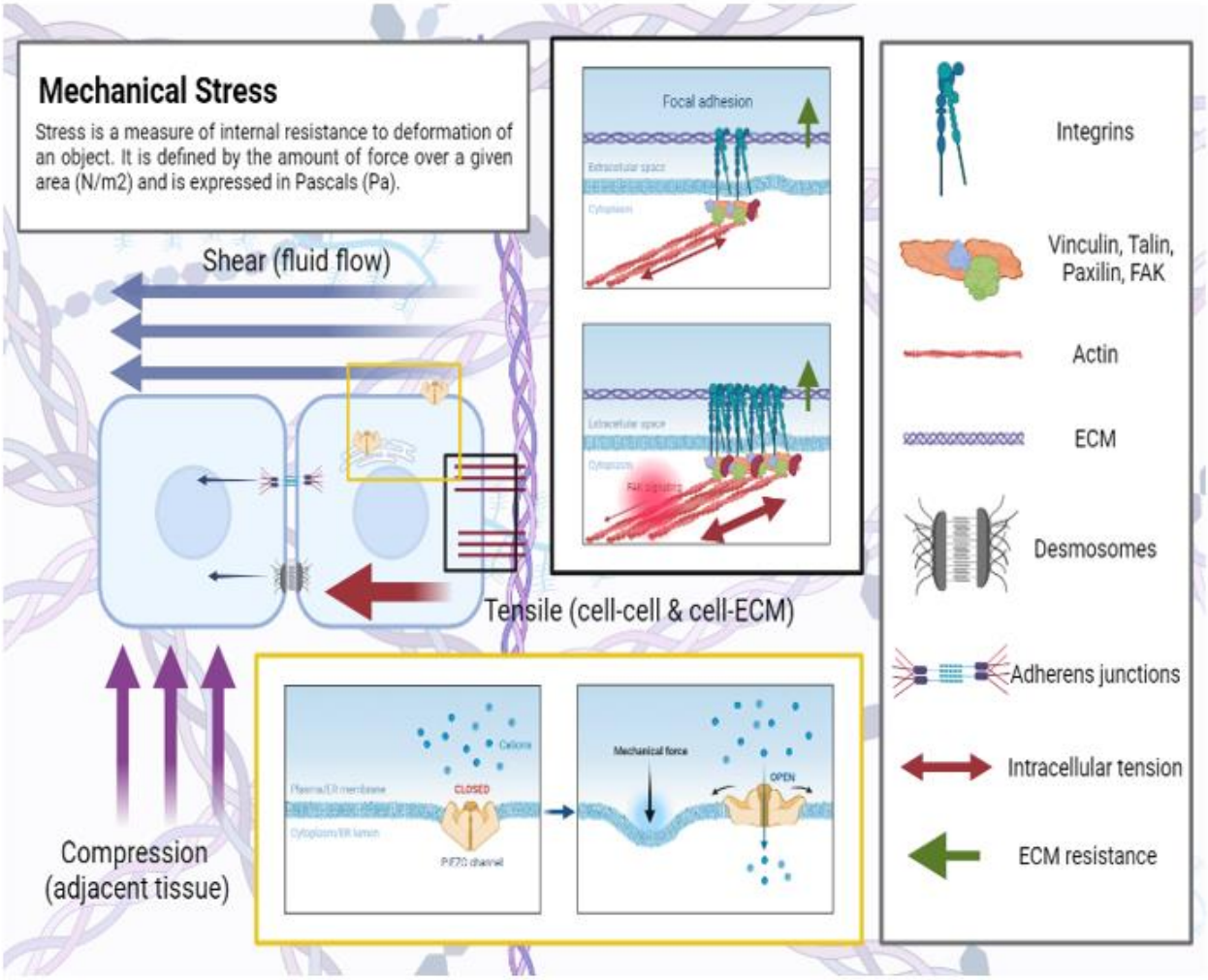

Figure 2 | Mechanical stress in and on cells. There are three types of stress that can occur upon induction of forces: tensile (stretch), compressive and shear. Intracellular tension is generated by actomyosin tractional forces which is resisted by binding to other cells or the ECM. Binding to the ECM occurs through focal adhesion proteins with cluster in maturation, a force induced process. Various mechanosensors are found on and in cells that respond to mechanical cues resulting in intracellular signaling. Figure adapted from (Northcott et al., 2018).

ECM: extracellular matrix

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well as the endoplasmic reticulum (ER) of various tissue types (**Figure 2**).

Piezo1 responds to compressive and tensile forces. Upon its activation by mechanical stress, the channel opens and allows for Ca^{2+} influx to the cytosol, resulting in cell signaling. Piezo1 senses compressive and tensile forces in epithelial cells and induces cell division and extrusion, respectively. To further illustrate, tensile forces are generated through cellular actomyosin contracting, which is counteracted by resistance of neighboring stroma or cells. For example, cells can be attached to the ECM through FAs, and is formed by the oligomerization of integrins and other associated proteins connected to actomyosin. Next to providing contraction and movement, FAs also act as sensors. Depending on the buildup of tension, generated through ECM-mediated resistance to deformation, a conformational shift in the adhesion plaque protein talin, exposes cryptic hydrophobic binding sites

for other proteins to associate for further downstream signaling (Swaminathan & Gloerich, 2021) (**Figure 3**).

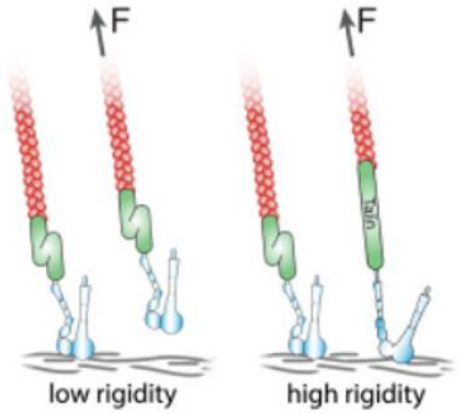

Figure 3 | Stiffness sensing by focal adhesions. Tensile force is generated through actomyosin contraction. Depending on the rigidity, and thus counteracting force of the ECM, the force is translated to a conformational shift in the FA protein talin. This exposes hydrophobic binding sites in talin for further downstream signaling.

ECM: extracellular matrix; FA: focal adhesion

Figure adapted from (Swaminathan & Gloerich, 2021) and created with www.biorender.com

Yes-associated protein (YAP), part of the Hippo-pathway, is regulated by mechanosensors. As a result of stretching forces, YAP is released from sequestration by adherens junctions and phosphorylated by downstream effectors of FAs, leading to its ability to exert its effector functions in the nucleus, like inducing survival and proliferation. The link between YAP/TAZ and mechanical forces is notably interesting as data from mouse models indicates it is dispensable for homeostatic self-renewal of several tissues, whilst often required for cancer cell survival and proliferation (Moya & Halder, 2019). This elevates its potential for cancer drug target development as selectively inhibiting YAP might mitigate treatment side effects compared to cytostatic agents.

Classes of mechanosensors and combinations of forces result in a distinct response and biological outcomes. The ability to discriminate between these different parameters will be determined by the specific mechanisms of force transduction in individual mechanosensors, as well as their organization within the cell, further excellently reviewed by (Swaminathan & Gloerich, 2021).

Cellular responses to mechanical signaling affect cellular fate and phenotypic plasticity

Mechanical forces affect intracellular signaling and responses to this can affect cellular differentiation and phenotype. For instance, cellular apical/basal polarity is regulated by adhesion of epithelial cells to the BM through integrins (Zuk et al., 1998). This adhesion suppresses oncogenic signaling (Goulas et al., 2012). Tissue stiffness sensing can dictate cell fate, as demonstrated in mesenchymal stem cells (MSCs). MSC differentiation into osteocytes, myocytes and neurons can be achieved by altering the stiffness of the collagen culturing matrices. Differentiation into aforementioned cell types happens when the stiffness of the ECM resembles that of its native tissue (Engler et al., 2006). Similarly to sensing stiffness, other mechanical forces can influence cell fate, for instance tensile and compressive forces can bring cells into a more stem-like state for regeneration (Moya & Halder, 2019).

Cells generate forces to affect their surroundings

Next to sensing tissue properties and responding to this, cells also generate forces to impose them on other cells. For instance, cancer associated fibroblasts (CAFs) in mice have been shown to encapsulate tumors and show contractile behavior, thereby compressing colorectal cancer metastases leading to their outgrowth (Barbazan et al., 2021). The impact of aberrant mechanical forces

generated by tumor growth not only affects tumor cells but also phenotype of healthy tissue. To illustrate, expanding tumors apply pressure to healthy cells around them and it has been shown that by applying such stress to intestinal crypts will induce dysplasia in healthy epithelium. Interestingly, in this particular *in vivo* system it was not possible to apply symmetrical forces. This is due to the cylindrical shape of the crypts and their orientation in different planes throughout the organism. It's feasible that combinatorial amounts of tension and compression activate oncogenic signaling in the healthy tissue (Fernandez-Sanchez et al., 2015).

Regulation of the extracellular matrix further drives tumor progression at the primary site

The ECM plays an important role in promoting invasion and is actively remodeled by tumor cells leading to a reciprocal relationship.

During invasion cancer cells become motile and show haptotactic behavior, which is the directional movement of cells in response to substrates of adhesion, including what is present in the ECM. For instance, pancreatic stellate cells promote cancer cells to show haptotactic behavior towards larger concentrations of type I collagen (Lu et al., 2014). The same holds true for human breast cancer cells that frequently migrate in a direction towards areas with greater fibronectin concentrations (Oudin et al., 2016). To add, the role of ECM components is not limited to the absolute concentration, but especially spatial architecture. Migration of breast cancer cells occurs over progressively thicker and linearized collagen fibers (**Figure 4A**), allowing for effective directional migration independent of speed (Riching et al., 2014). This is influenced by collagen fibril diameter and pore-size within collagen structures (Sapudom et al., 2015). Migration and invasion contributes through cell-based activity, whereas cells also enter circulation through tumor-established flow patterns directed outwards and into circulation. In fact, intra-tumoral interstitial fluid pressure was more than nine times greater in pancreatic ductal adenocarcinoma than the pressure evaluated in matching healthy tissue (Provenzano et al., 2012). Adjacent flow can promote breast cancer cell invasion (Huang et al., 2015). To add to this finding, cancer cells also migrate against the flow when this is not outwards of tumors, along ECM substrates. As tumors continuously release CTCs, is tempting to speculate that the magnitude in contribution of active displacement (e.g. migration through substrate adhesion) is greater than that of passive displacement. It should be emphasized that cell release to circulation often occurs in mid- to late-stage tumors, and that the timing of intravasation to circulation can vary depending on the type of solid cancer.

Changes of mechanical sensing can occur both through alterations in cancer cells in how they sense, as well as what is available in the environment to sense. For instance, tumor cells can diversify integrin expression to become anchorage independent or promote tumor stemness (Desgrosellier et al., 2014; Sapudom et al., 2015), but what is mechanically sensed is altered by additional modulation of the ECM. Cancer cells remodel the ECM by secreting MMPs, or activating stromal cells to do so. For instance, pro-angiogenic signaling to endothelial cells leads to endothelial cell-driven destruction of the BM, an important early step in cancer dissemination (Kai et al., 2019; Neve et al., 2014). Increased turnover by MMPs has been shown to modulate contact guidance

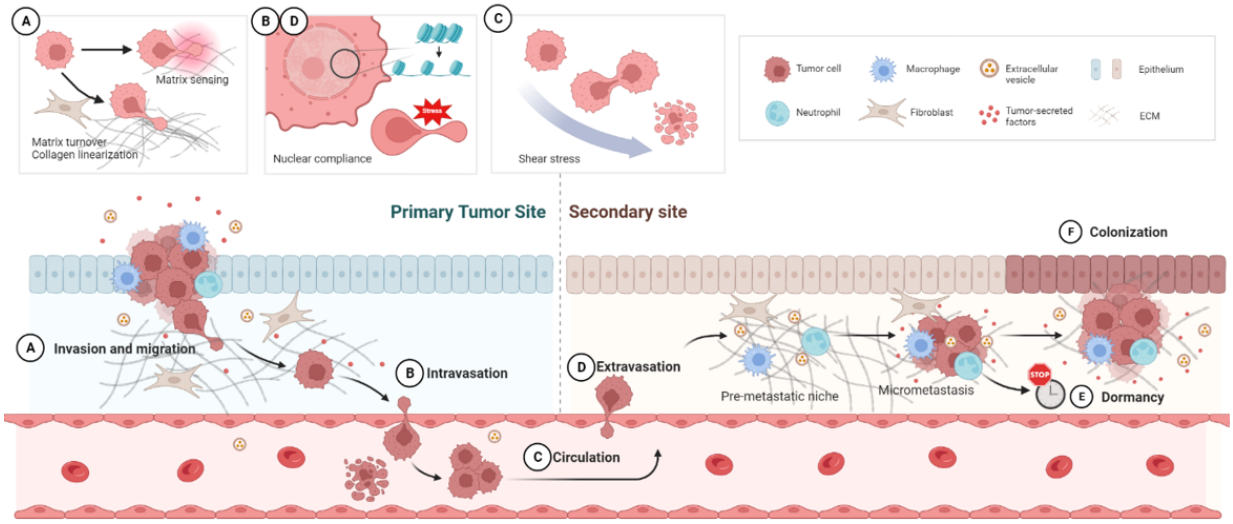

Figure 4 | Mechanobiology regulates steps of metastatic cascade. Multiple steps of metastasis, from progression to dissemination, to colonization are regulated by cell mechanics. During invasion and migration from the primary tumor, changes occur in the sensing, composition and structure of the ECM. Entry and exit to circulation is tightly regulated by nuclear softening and compliance, for non-destructive diapedesis to take place. When entering a secondary tissue, this has been modulated prior to arrival to a pre-metastatic niche. After seeding, outgrowth to full macrometastasis takes place if cells are not kept dormant.

ECM: extracellular matrix

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efficiently in 2D and 3D planes, also known as migration directionality (**Figure 4A**) (J. Wang et al., 2019).

In addition to elevated turnover, present ECM components are modified which alters global material rheology. Changes in stiffness and compliance therefore alters sensing of the environment by cells. Lysyl oxidase (LOX) enzyme crosslinks components in the ECM, thereby promoting tissue stiffening. LOX is one of five secreted copper-dependent enzymes whose known purpose is to remodel the ECM. This is accomplished by the oxidative deamination of peptidyl lysine residues in collagen and elastin to enable covalent cross-linking, perceived as crucial for creating and maintaining structural integrity of the ECM in regards to its tensile strength (Smith-Mungo et al., 1998). This crosslinking promotes PI3K/AKT signaling, integrin activation, FA maturation, and tumor cell invasion in the tumor microenvironment through sensing LOX-induced stiffness. A role for LOX in cancer progression at primary sites has been described in breast, colorectal, prostate, gastric, pancreatic cancer, renal clear cell carcinoma, head and neck squamous cell carcinoma (HNSCC), oral and oropharyngeal SCC, melanoma, as well as basal and squamous cell skin carcinoma (T. H. Wang et al., 2016). LOX inhibitors can vice versa prevent the stiffening of the ECM, the establishment of FAs, and metastasis that is caused by high LOX expression in breast cancer (Levental et al., 2009; Rubashkin et al., 2014). In cancer, stiffness promotes tumor growth. Computational modeling has found that in order to keep growing, the stiffness of a solid tumor must be >1.5 times the stiffness of surrounding tissue to allow for expansion (Voutouri et al., 2014).

As the tumor grows and stiffens, this provides a feed-forward loop of stress induction in present stromal cells. Through this feed-forward loop, stiffness is further increased by deposition of collagen, which can be attributed to tumor cells and fibroblasts (Northcott et al., 2018). The deposition of collagen to induce stiffening,

is structurally complemented by fibroblasts. As previously discussed, the orientation of collagen I fibers forming a more mesh-like framework change to a linear formation. This aids in cancer progression by allowing for directional migration, and requires fibroblasts (Riching et al., 2014). The mechanisms of linearization of matrix proteins point to a reciprocal role for fibroblasts: under stretch, normal tissue-associated fibroblasts deposit linear matrix structure, unlike their unstretched counterparts producing mesh-like matrix. In a study from Ao and colleagues, stretch led to linear alignment of fibronectin, which caused directed migration of human HNSCC cells (Ao et al., 2015). As the fibroblasts phenotypically resembled cancer-associated fibroblasts (CAFs) eventually through the process of stretching, this illustrates the mechanical reciprocity of tumor and stromal cells in cancer progression.

Dissemination through circulation: entering and exiting requires mechanical adaptations.

Following tumor progression at the primary site and invasion of local tissue, which is strongly controlled by mechanical cues, cells encounter many more forces during entry and exit of-, and presence in circulation.

Entry into the circulation, either hematic or lymphatic, is both an active and a passive act. Cells can migrate actively along ECM substrates and endothelial cells of the vasculature, as well as passively be displaced through natural flow patterns, which are outwards of tumors and into circulation. Invasion and especially extravasation is dependent on migratory forces generated in cells as well as cellular compliance to move through dense matrices and openings, including the space between endothelial cells of the vasculature. As tumors grow, vasculature is heavily compressed and diffusion distances become greater, leaving parts of tumors hypoxic and deprived of nutrients. This induces angiogenesis, an established cancer hallmark involving vascular endothelial growth factor (VEGF) release. VEGF is known to activate macrophages that aid in the extravasation of tumor cells into the blood by creating nanotubes for tumor cells to squeeze through and activate RhoA GTPase signaling by contact (Harney et al., 2015; Roh-Johnson et al., 2014).

Cellular compliance is needed for intra- and extravasation to squeeze through endothelial cells to enter vessels and survive the shear forces in circulation. Cellular compliance is related to both cortical tension and nuclear compliance, albeit nuclear compliance forms the greatest bottleneck (**Figure 4B**) (Mekhdjian et al., 2017). To squeeze through tight spaces, nuclei must be sufficiently malleable, but retain enough resistance to strong shear stress CTCs encounter entering, exiting and being in circulation (Moose et al., 2020). For instance, nuclear lamins increase nuclear stiffness, and their removal makes cancer cells more susceptible to rupturing under shear pressures *in vitro*. Severe depletion of lamin A and C impairs survival under shear stress (Mitchell et al., 2015). As shown in glioma and adenocarcinoma cell lines, the stoichiometry of lamins co-determines compliant potential, and in addition to this compliance increases metastatic capacity in breast cancer (Harada et al., 2014; Bell et al., 2022). Next to intrinsic nuclear compliance, the nucleus can also be softened through mechanoregulation. This is achieved through changing spatial density of chromatin through Piezo1 activation. As demonstrated in cancer cell lines, Piezo1 on the ER is involved in signaling affecting the reduction of

heterochromatin (Nava et al., 2020). Interestingly, this is co-dependent on the stiffness of the nuclear envelope determined by its lamin composition. As both nucleus and ER are connected together, its combined stiffness of both organelles could affect the ER-docked Piezo1 activation threshold. The genomic damage that is not lethal, arising from nuclear mechanical stress may be advantageous to tumor progression, as genomic instability is an established hallmark of cancer (Hanahan & Weinberg, 2011b). Migration through very narrow pores (~ 3 µm) leads to DNA damage which is incorrectly repaired as a result of squeezing-dependent mis-localization of repair factors, as shown in osteosarcoma (Irianto et al., 2017). However, if genomic instability as a result of nuclear stress is a driving event caused by migration and extra-/intravasation, rather than a passing adaptation, remains unclear.

As tumor cells enter circulation, they must survive the shear forces when circulating and then extravasate into a secondary tissue. Shear forces acting on tumor cells can promote survival or lead to cell death depending on the magnitude and the cancer cells' intrinsic properties of sensing these forces (**Figure 4C**) (Follain et al., 2020). Shear stress is sensed and can then induce F-actin polymerization and increased cortical stiffness via ROCK-mediated signaling (Moose et al., 2020). Inhibiting this route impairs the survival of non-circulating cancer cells in mice models of experimental breast- and prostate cancer (Liu et al., 2009; Schackmann et al., 2011).

The amount of shear forces can vary greatly throughout circulation and can affect the viability of tumor cells as well as location of extravasation. When considering hemodynamic physiology, larger arterial vessels induce pressure waves as a function of heart rhythm resulting in timely varying amounts of flow (Qiu et al., 2019). Therefore, oscillatory shear stress is applied to the contents of blood, including tumor cells. Not much is known about the effects of cyclic strain on CTCs caused by shear forces in circulation. One study found that laminar flow, not oscillatory shear stress, to be causative of cell death (Lien et al., 2013). However, the applied frequency of 1Hz, a 1 second cycle, does not correctly represent cardiac physiology, as resting heart rates are general 65 to 100 bpm, depending on sex and fitness (Regitz-Zagrosek & Kararigas, 2017).

The shedding of CTCs is very inefficient and many do not survive circulation. Next, the local flow profile and endothelial environment coordinates extravasation into secondary tissue. A favorable flow rate, amount of shear and vasculature architecture are necessary for cancer cells to arrest and induce extravasation (Follain et al., 2020). To induce physical adhesion, CTCs can mimic neutrophils and bind to the vascular endothelium through upregulation of leukocyte-specific adhesion molecules like selectins (Burdick et al., 2003; Burdick & Konstantopoulos, 2004; Läubli & Borsig, 2010). Next to adhesive and flow properties, vasculature architecture is thought to be a determinant of organotropism. Using cell lines from breast cancer, from brain- and bone marrow-tropic subclones, it was demonstrated that CTCs preferably arrest and extravasate in tortuous vs linear capillaries (Paul et al., 2019). Interestingly, many methods in modelling the biophysical adhesive dynamics include velocity and shape of vasculature (e.g. diameter) as linear parameters (Paddillaya et al., 2019). Studies modelling fluid dynamics in microfluidics systems of cancer indeed make use of linear capillaries, although

other pathologies such as thrombosis, have been studied in tortuous systems (Luna et al., n.d.; Y. H. V. Ma et al., 2018). Furthermore capillary architecture in the liver and kidney is fenestrated and has openings of comparable size to leukocytes and CTC nuclei (Aird, 2007; Hao et al., 2018). This may facilitate organ specific metastasis in the liver, whereas metastases to the kidney are rare (mostly case studies), arguably due to the higher shear stress of ultrafiltration, the glomerular BM as well as podocyte function (Kriz & Lemley, 2017).

Tumor cell extravasation is classically seen as an endpoint in the route from entering circulation to extravasation, before forming a secondary tumor (**Figure 4D**). However, CTCs are able to extravasate and re-enter systemic circulation, under control of mechanical cues by constitutively expressed YAP (Benjamin et al., 2020).

Contributions of mechanobiology in the formation of secondary tumors

The environment at a secondary site of tumor cells must be favorable and support survival and proliferation for outgrowth. After extravasation and initial seeding at a secondary tissue, cancers can stay dormant for a period of time as a micrometastasis, e.g. too small to pick up with diagnostic imaging tools, varying from weeks to years. Dormancy is an ambiguous step of metastasis, relating to cells that are not yet proliferating after extravasation. Although not proliferating, they can survive in a quiescent state for an unlimited amount of time and/or until 'awakened'. In time, alterations of the tumor microenvironment lead to a switch to proliferation. Along with the environment, primary tumor cell identity also affects whether dormancy is sustained or the cells have potential for outgrowth to a macrometastasis. The switch from dormancy to outgrowth will not occur in all secondary tumor sites and can be influenced by many mechanosensing factors within the tumor microenvironment. Altered sensing takes place when for instance stromal cells in the environment change the composition and structure of the ECM, thereby switching cells from a quiescent to proliferative state. (**Figure 5**).

Tumor cell dormancy is regulated locally in a niche

Dormancy can be sustained for a longer period of time through tumor cell mechanosensing of the ECM at the secondary site. The composition of the ECM at a secondary tumor site can be linked to intrinsic homeostatic composition, and can also be modified by the tumor cells at the secondary location, thereby the tumor cells support their own dormancy. In a breast cancer mouse model, tumor-derived collagen type III kept disseminated tumors dormant, as it disrupts signaling through the collagen receptor DDR1 and downstream STAT1 (di Martino et al., 2022). Collagen type III is produced by fibroblasts in granulation tissue during wound healing, which is later replaced by the longer and stronger collagen I. A decreased ratio of collagen I over III is associated with a loss in tensile strength (**Figure 5A**) (Nielsen & Karsdal, 2016). Collagen III is degraded by MMP-9 to neo-epitope C3m (Barascuk et al., 2010). Degradation of collagen III might lead to an increase in tissue stiffness due to stoichiometry changes. The neo-epitope C3m has been extensively studied in the context of fibrosis and can be found in the serum of patients with chronic obstructive pulmonary disorder (COPD) and idiopathic pulmonary fibrosis (IPF) (Leeming et al., 2012). In several types of breast cancer, serum C3m is predictive of metastasis, and is present in the serum of stage IV

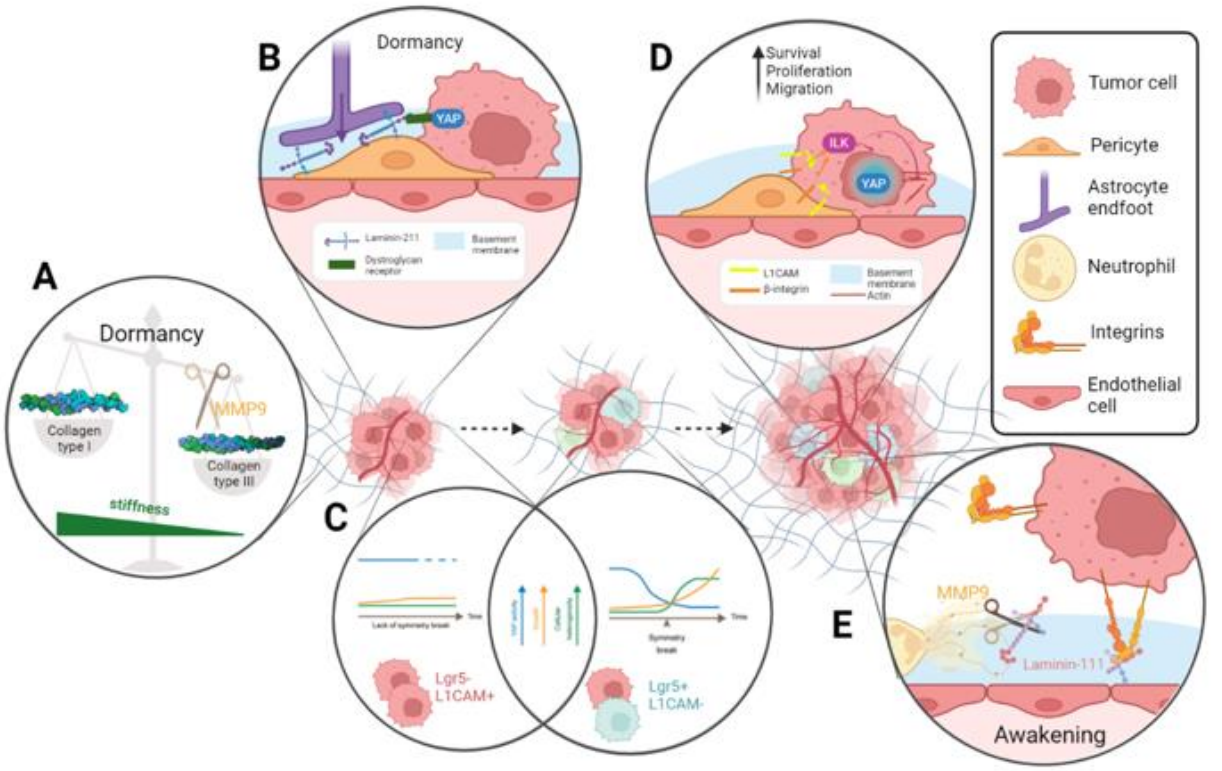

Figure 5 | Dormancy and outgrowth is coordinated by mechanical cues. Keeping cells dormant is tightly regulated by ECM composition. A) For instance collagen III keeps breast cancer cells dormant. Increased type III collagen over collagen type I is associated with decrease in stiffness. B) In brain metastases, astrocytes produce laminin-211 that will bind to tumor cells' dystroglycan receptor, therefore retaining it and preventing activation and nuclear localization. C) Cancer stem cells play a role in self-organization, which can be required for micrometastases to progress to macrometastases. Establishment of this cellular plasticity is under control of mechanosignaling. D) Tumor cells can show pericyte-like behavior, spreading around capillaries. Interaction with pericytes and BM leads to ILK-induced migration. E) NETosis releases MMPs that can cleave ECM products, exposing binding epitopes for integrins leading to awakening and subsequent outgrowth.

ECM: extracellular matrix; ILK: integrin-linked kinase; BM: basement membrane; NET: neutrophil extracellular traps; MMP: matrix metalloprotease

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colorectal cancer patients (Kehlet et al., 2016; Willumsen et al., 2017).

As well as altering global mechanical properties, MMP-9 can change smaller-scale mechanical signaling. MMP-9 is released during NETosis, a process in which neutrophils shoot out extracellular traps (NET). NETs are scaffolds of chromatin, dressed in proteolytic enzymes, projected by neutrophils leading to their cell death. MMP-9, is capable of exposing an epitope of laminin-111 by cleaving it and thereby waking up disseminated cancer cells through integrin activation and FAK/ERK/MLCK/YAP signaling (**Figure 5E**) (Albrengues et al., 2018). Taken together, MMP-9 plays a role in awakening tumor cells at secondary sites from dormancy by altering global mechanical properties, as well as intracellular signaling. Therefore, C3m, or other cleavage products of MMP-9, may be a useful biomarker of a switch from micro- to macrometastasis although this requires further investigation.

Intrinsic tissue characteristics also contribute to dormancy. In disseminated breast cancer, astrocyte end feet inhibit

pericytic behavior of tumor cells through depositing laminin-211 (**Figure 5B**). Pericytic localization of disseminated tumor cells provides access to oxygen, nutrients and a supportive BM to bind to. Deposition of laminin-211 by the astrocyte end feet drives quiescence in the disseminated breast cancer cells as it signals through the dystroglycan receptor. Through this signaling, YAP associates intracellularly, thereby sequestering it from the nucleus and preventing its pro-metastatic functions (Dai et al., 2022). To note, contributors to dormancy that do not lead to outgrowth can still positively impact cancer cells by keeping them in a quiescent state through pro-survival signaling, thereby preventing cell death.

The success of cancer colonization depends partially on its ability to self-assemble ECM components if not favorable at the location of dissemination. This is necessary for pro-survival signaling at a secondary site. To illustrate, in a breast cancer model placed in secondary site-mimicking ECM, tumor cells self-assembled fibronectin to promote survival and quiescence, by inducing ROCK-generated intracellular tension (Barney et al., 2020).

Growing out from micrometastases to macrometastases is controlled by mechanical cues.

During outgrowth, tumor cells can produce several cell divisions but still halt in growth. Further outgrowth is highly controlled by mechanical cues. For instance, in colorectal cancer metastasis, the ability for tumor cells to self-organize epithelium at a multicellular stage is a key step in outgrowth from a micrometastasis to micrometastasis. In both colorectal homeostasis and -cancer, this requires phenotypic plasticity such as organization of (cancer) stem cells, from differentiated cells. In homeostasis, this is frequently triggered by external cues such as mechanical signaling. Colorectal cancer cells disseminating to the liver require this phenotypic plasticity to become macrometastases (**Figure 5C**). Short lived, early YAP activation is essential for the emergence of cancer stem cells, epithelial self-organization and macrometastasis formation (Heinz et al., 2022). YAP is strongly regulated by various mechanical cues, suggesting that YAP attenuation following its brief activation is controlled by mechanical changes at secondary sites, such as compression of cells by surrounding tissue (Fernandez-Sanchez et al., 2015). Although YAP is considered an excellent target for drug development since it is thought to be redundant for many homeostatic processes, these findings illustrate how caution should be taken due to the adverse tumor-promoting effects it can have to attenuate its signaling after prior activation.

Conversely, constant YAP activation can sustain other types disseminated cancer cells growth at secondary sites such as in lung, bone and brain. L1CAM, an E-selectin ligand, was identified to sustain spreading throughout the perivascular niche in multiple organs. L1CAM triggers β1-ILK signaling leading to YAP nuclear localization. ILK signaling activated PAK1/2, which promotes filopodia-like protrusions associated with invasion and metastatic colonization (**Figure 5D**) (Er et al., 2018).

Tumor growth induces plasticity in surrounding non-tumorigenic tissue

The presence of tumor cells and their growth can exert forces on tissue in the environment. Dysplasia in colorectal crypts can be induced by consistent ectopic application of strain. By releasing β-catenin from E-cadherin cellular adhesions, it

translocates to the nucleus and enables TCF/β-catenin target genes (Fernandez-Sanchez et al., 2015). Although this study is aimed at elucidating mechanisms at the site of primary tumor outgrowth, it can conceptually be applied to secondary sites. The support of outgrowth of micrometastases to macrometastases can be controlled by how the micrometastasis applies strain to surrounding tissue and the responsiveness to this strain by surrounding cells, or lack thereof. However, our knowledge surrounding how mammalian tissues generally respond to mechanic stimuli is centered around methods applying external stress. Therefore, it is still unknown how non-tumor can cells respond to various mechanical signals and to what extent mechanoresponses are caused by physiological forces that are either spontaneously generated or derived from external factors like cancer at a secondary site.

Organotropism in metastasis

In the late 19th century, Sir James Paget introduced the emerging hypothesis that the locations of the secondary lesions might not be random (S. J. Paget, 1888). In support of this theory and the "seed/soil" concept, the theory was further established by Stephen Paget when he noticed that the locations of secondary lesions were not random for more than 900 patients with breast and uterine cancer. He hypothesized that considerations of the inherent characteristics of the tumor, the "seed', and of the organ environment, the "soil', were partly responsible for organ selectivity (S. Paget, 1889). Simply put, metastatic outgrowth only occurs when the seed and the environment of the newly formed soil are compatible. The wealth of information from clinical data and murine models supports the occurrence of non-random metastasis (Obenauf & Massagué, 2015). Other theories, like patterning of circulation, also influence tropism. For instance, the hepatic portal system drains the blood from the colon and proximal rectum, whereas the distal rectum's blood travels to the lung. This vascular arrangement is consistent with colorectal cancer's preference for liver metastasis, with lung serving as its secondary metastatic location (Riihimäki, Hemminki, Sundquist, & Hemminki, 2016; Riihimäki, Hemminki, Sundquist, Sundquist, et al., 2016). The notion remains that both theories co-exist (Lu et al., 2019). Apart from circulation directing organotropic metastasis, an unanswered question remains whether tissue-intrinsic mechanical aspects contribute to this distribution, as links between "seed' and "soil' are required for more accurate clinical projections of metastasis. For instance, BM stiffness of certain tissue correlates to metastasis formation in secondary organs (Reuten et al., 2021). Following the framework of cancer cells sensing and responding to their environment which can be favorable for their progression, it puts forward the idea that bother "preparing" and "probing' the "soil' for a mechanically favorable location of metastasis may take place.

The secretome of the tumor educates the tissue for a favorable environment

Tumor identity is closely linked to the metastatic niche as certain disseminated tumor cells emerge in specific secondary sites, following the "seed/soil' hypothesis.
It is important to consider that prior to tumor cells entering tissue, or circulating in any way, they can modulate distant tissues creating a favorable environment for metastasis. This includes changes to mechanical properties of tissues directly or indirectly through recruitment of stromal cells.

Extracellular vesicles as carriers of mechanical alterations

An important contributor to the distant modulation of tissue is EVs. They are crucial for intercellular communication as they transmit cytosolic and membrane proteins, lipids, and RNA between cells (**Figure 6A**). Highly stable, they can persist in systemic circulation until directed uptake takes place, which is influenced by both proteins on the surface of the EV and target cell (Doyle & Wang, 2019). EVs can distally alter tissue mechanics by directly interacting with the ECM or through the action of stromal cells and induce secondary tumors (**Figure 6B**). Next to distal alterations of tissue mechanics, EVs influence organotropism of tumor metastasis.

Lysyl oxidase induces tissue stiffening and recruits stromal cells at metastatic sites

As EVs can contain ECM-modifying-enzymes, they can deliver changes in tissue mechanics and structure at a secondary site. LOX delivered in EVs has a prominent role at regulating tissue stiffness at a distance (Levental et al., 2009; Rubashkin et al., 2014; Smith-Mungo et al., 1998). In primary tumor sites, the stiffness of the tumor should be >1.5 times the stiffness of surrounding tissue (Voutouri et al., 2014). For a secondary site, this may imply that disseminated tumor cells seek

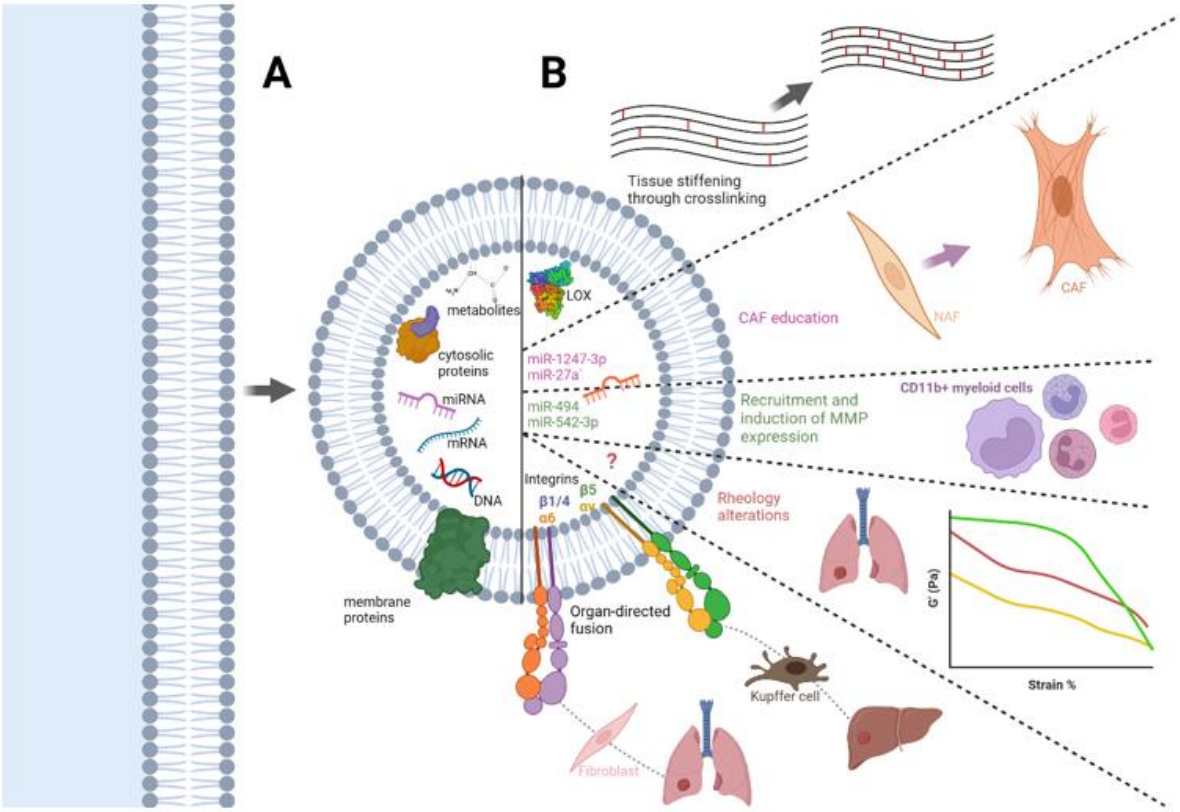

Figure 6 | Extracellular vesicles alter tissue mechanics to facilitate metastasis. A) Extracellular vesicles are released from the plasma membrane by tumors and can contain various types of cargo. B) Fusion of extracellular vesicles with stromal cells at secondary sites can recruit them to alter mechanical characteristics of the tissue.

LOX: lysyl oxidase; miRNA/miR: micro-RNA; CAF: cancer-associated fibroblast; NAF: normal-tissue associated fibroblast; MMP: matrix metalloprotease; CD: cluster of differentiation; G': elastic modulus; Pa: Pascals

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out a tissue of fitting stiffness, of which LOX is an important contributor locally and at a distance. Not much is known about global tissue stiffness and how this affects organotropism. However, LOX is described not only as a paracrine acting molecule, e.g. histopathological analysis of primary tumor, its presence in circulation in the form of EVs and their distant effects have been described, in HNSCC and breast cancer respectively (Erler et al., 2009; Sanada et al., 2020). Through its enzymatic activity, LOX contributes to tissue stiffening and can regulate tropism together with stromal cells. The role of LOX is dictating tropism was described in lung-metastasizing breast cancer releasing EVs with LOX, thereby crosslinking collagen IV in the lung, following recruitment of CD11b+ myeloid cells and subsequent bone marrow-derived cells. These immune players alter the ECM in the lung through MMP activity, allowing for directed metastasis (Erler et al., 2009).

Extracellular vesicles reprogram stromal cells to induce a pro-metastatic niche

EVs can contain non-protein factors influencing stromal cells like metabolites or nucleic acids, e.g. micro-RNAs (miRNAs). miRNAs are small non-coding RNAs regulate both post-transcriptional and post-translational gene expression. A cell's expression profile can change drastically as a result of a single miRNA's ability to affect multiple mRNA targets. Several miRNAs have been found to be able to remodel the ECM by inducing expression of MMPs and adhesion molecules, and lead to a pro-metastatic inflammation response, as reviewed by (Alečković & Kang, 2015).

Stromal cells, often recipients of miRNAs, play an important role in altering tissue mechanics. Through EVs, their fate at secondary sites can be redirected, which may facilitate organ-specific metastasis. CAFs play a critical role in primary tumors by contributing to excessive matrix remodeling, matrix stiffening, inflammation and tumor migration, driving tumor progression and metastasis (Asif et al., 2021; Zhao et al., 2021). These processes can also occur at secondary sites prior to metastasis. Before differentiating into CAFs, fibroblasts at secondary sites are educated by EVs derived from the primary cancer (Fang et al., 2018; J. Wang et al., 2018). The presence of CAFs, and many of their functions are correlated to secondary metastasis, for instance shown for lung-metastasizing liver cancer (J. Wang et al., 2018). In addition to fate-switching fibroblasts, EVs derived from orthotopic melanoma and breast tumors can induce phenotypic switching of distant pericytes and vascular smooth muscle cells in mice to de-differentiate and produce ECM with a fibronectin-rich environment leading to increased metastasis (Murgai et al., 2017).

Extracellular vesicles change global tissue mechanics

Next to specific stromal cells being involved in creating a pre-metastatic niche, EVs can change global mechanical rheology through various strain modalities of a tissue. This leads to altered sensing of the environment, and for some tumors favorability of a tissue for the formation of a secondary tumor. Breast cancer-derived EVs stiffened lung ECM through increased expression of ECM proteins and -modifying enzymes (Barenholz-Cohen et al., 2020). Interestingly, the tumor cells of which the EVs were derived, show lung tropism. Hypothetically, this could be linked to multiple ECM changes leading to distinct changes in rheology specifically to the lung, as control liver tissue showed significant changes albeit in lower order of magnitudes compared to lung.

In observation of EVs leading to global mechanical changes in specific

tissues, investigation does not always include through what mechanisms, or if EVs specifically localize to certain tissues to exert their functions at a secondary site. The observations of tissue-tropism could be due to both tissue-intrinsic characteristics combined with EV composition, as well as what tissues EVs target due to their composition. The nature of EVs plays an important role in their directed targeting, and therefore possibly an additional role in organotropism. Human lung, liver, and brain-tropic tumor cells release exosomes that preferentially fuse with native cells of their tropic destiny. This could be observed for lung fibroblasts and epithelial cells, liver Kupffer cells, and brain endothelial cells. In the organotropic metastatic niche, these EVs mediated the contact between tumor cells and organ cells. EVs isolated from patients and the composition of integrins on these EVs correlated to metastasis location (Hoshino et al., 2015). A tissue-specific inflammation response could also be observed, in lung Kupffer cells compared to lung endothelial cells. However, the mechanism of targeting through the expressed integrins on the EVs remains unclear (Hoshino et al., 2015).

Horizontal transfer of protein activity by extracellular vesicles

Next to priming a pre-metastatic niche, tumor-derived EVs from a primary site could possibly promote metastasis to a metastatic site after initial seeding. Tumor-derived EVs can horizontally transfer functionality. Uptake of EVs can lead to recipient cells acquiring the functionality of the protein on the EV. For EVs derived from various prostate cancer cell lines, transfer from tumorigenic to non-tumorigenic cells led to an increase in recipient cell adhesion and migration (Singh et al., 2016).

Pathologies and aging: mechanical alterations with links to cancer

Various pathologies and aging are associated with increased risk of cancer, as well as increased risk of cancer. Many cancer patients also present with co-morbidities (Sarfati et al., 2016; Tu et al., 2018). When addressing tissue elements and their potential contribution to metastasis, this is often described in a physiological context and/or a niche influenced by tumor cells. Understanding tissue mechanics their relationship to formation of secondary tumors goes beyond this and requires there be accounting for confounding factor such as aging and pathologies. In efforts to predict all stages of metastasis including the likelihood of a secondary outgrowth at a certain location, this highlights our need to understand how other pathologies affect the mechanobiology linked to cancer metastasis.

Fibrosis

A commonly linked change in mechanical composition to cancer is fibrosis. Fibrosis occurs through excessive collagen deposition by fibroblasts under conditions of chronic inflammation (**Figure 7A**). Chronic inflammation is linked to increased risk of cancer initiation and fibrosis to cancer progression and metastasis (Piersma et al., 2020). Increased stiffness of tissue due to increased collagen crosslinking by LOX can form a pre-metastatic niche. LOX expression has been described thoroughly in atherosclerosis, as well as in glaucoma to be increased as a result of oxidative stress (Chen et al., 2002; Xiao et al., 2016). Oxidative stress can also be a result of traumatic fibrosis such as in ischemic reperfusion injury, leads to increased LOX expression (Jiménez-Castro et al., 2019). This may have implications for clinical choices in resecting

tumors, if systemic therapies are available as well. Furthermore, many perturbations of ECM regulation in fibrosis have been described for the progression of primary tumors and the association with metastasis, not the establishment of a secondary tumor (Chandler et al., 2019). Still, the fibrotic environment is a risk factor on its own for the development of metastases in the liver from colorectal cancer, irrespective of its disease etiology, e.g. non-alcoholic steatohepatitis (Kondo et al., 2016).

Hypertension

Increased blood pressure affects mechanically regulated pathways that have implications in cancer.

Fibroblasts isolated from liver metastases, which express phosphorylated myosin light chain, collagen-1, and SMA, are highly contractile and increase the local stiffness surrounding the tumor, favoring angiogenesis and metastatic growth (Shen et al., 2020). To overcome the negative impacts of these contractile fibroblasts, drugs targeting the renin-angiotensin

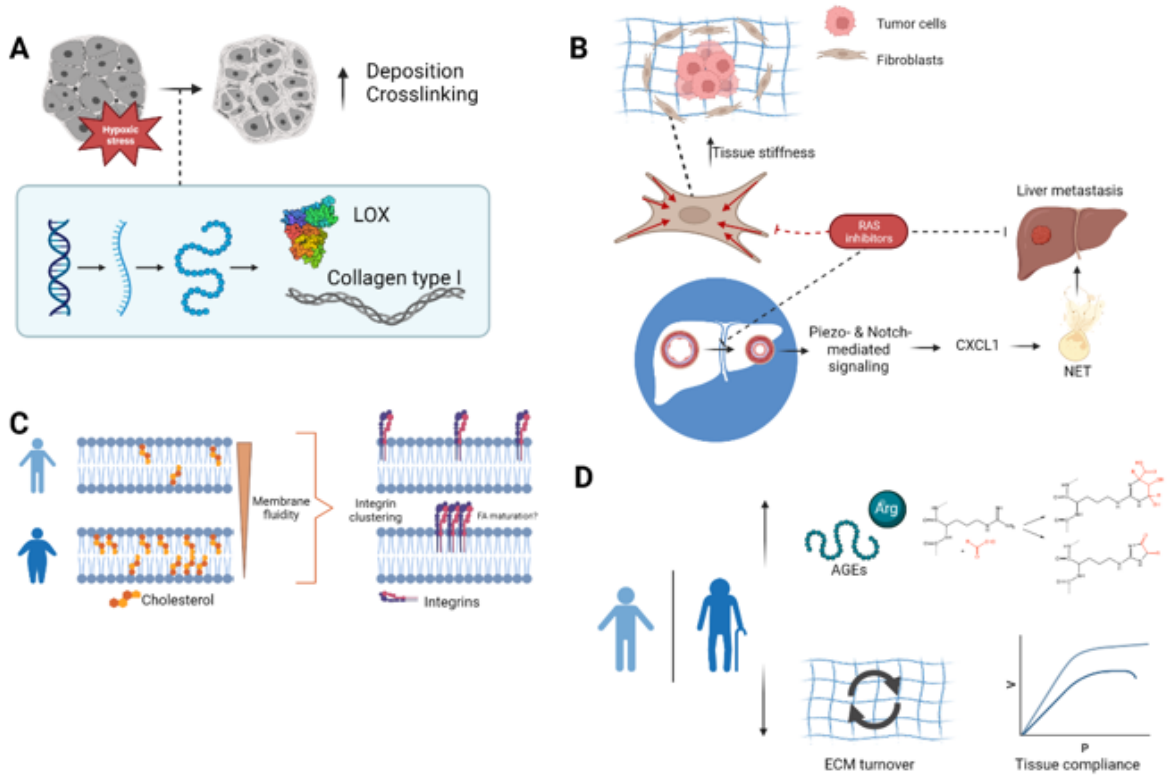

Figure 7 | Pathologies and aging affect tissue mechanics. A) Fibrosis of tissues is associated with increased deposition and crosslinking of collagen. B) Fibroblasts can increase tissue stiffness by showing contractile behavior, which is reversible through RAS inhibitors. RAS inhibitors are used to treat hypertension, a pathology that increases shear stress. An increase in shear leads liver endothelial cells to express CXCL1, a known activator of NETosis. Liver metastasis dependent on NETosis can be prevented through administration of RAS inhibitors. C) Obese persons show increased cholesterol in plasma membranes of cells, which has been shown to increase integrin clustering. These membrane dynamics may contribute to focal adhesion maturation. D) Aging is associated with AGE formation, decreased ECM turnover and compliance. These factors are all associated with tumor progression

LOX: lysyl oxidase; RAS: renin-angiotensin system; NET: neutrophil extracellular trap; AGE: advanced glycation end products; ECM: extracellular matrix; V: volume; P: pressure (mmHg)

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system (RAS) were employed, which are primarily used to lower blood pressure. Hypertension may be an additional contributor to supporting metastatic outgrowth (**Figure 7B**). Under hypertensive conditions, endothelial cells of the liver respond to increased shear stress through a combination of Piezo and Notch signaling, leading to CXCL1 expression. CXCL1 activates neutrophils to undergo NETosis which was shown to awaken dormant breast cancer cells in the lungs (Albrengues et al., 2018). Consistent with these findings, previous findings of a different breast cancer cell line, it has also been observed that NETosis is required for breast cancer to form liver metastases which can be reversed with RAS inhibitors, indicating that there could be a role for hypertension in increasing hepatic metastases (Park et al., 2016; Shen et al., 2020).

Obesity

Increase in adipose tissue and obesity is correlated to cancer initiation and progression, most often described in the context of inflammation (de Pergola & Silvestris, 2013; Quail & Dannenberg, 2019). For obesity, higher levels of membrane cholesterol can influence membrane dynamics (**Figure 7C**). Earlier reports mentioning increased cholesterol levels report more clustering of integrins, altered signaling, as well as increased cell adhesion and tumorogenic potential (Gopalakrishna et al., 2000; Kaur et al., 2004). Indeed, upon addition of cholesterol oligomerization of integrins can be observed, a characteristic of FA maturation (Ge et al., 2018).

Aging

Through natural aging, tissues undergo distinct changes in mechanical properties, such as stiffening and loss of elasticity. During aging, tissues show increased deposition of ECM proteins and abrogated posttranslational modification (typically nonenzymatic covalent cross-linking, advanced glycation end products (AGEs), which decreases the mechanical compliance of the tissue. Several features of fibrosis can be found, but to a lesser extent. One could hypothesize that due to aging, not only cancer initiation and progression occurs more frequently, but also the potential for metastasis (**Figure 7D**) AGEs play a temporal role in cancer progression, as they inhibit onset of invasion but support it if cells show invasion prior to their presence (Staneva et al., 2018). Partly supporting aging as mechanical risk factor is the example of decreased breast cancer risk in postpartum women. Decreased collagen I linearization, tissue stiffness and decreased tumor cell invasion could be observed for postpartum samples (Maller et al., 2013). Although not controlled for age, active remodeling may reset tissue architecture beyond the passive age-linked crosslinking. In the lung, aging is associated with an increase in elastic moduli in vascular and parenchymal compartments, as well as increased deposition of fibronectin and collagen I (Sicard et al., 2018). Many changes take place in tissues during aging, leading to altered ECM mechanics (Phillip et al., 2015). These age-related phenotypes are important to consider in timing experiments when working with *in vivo* models to more accurately represent patient populations.

Pathologies come with distinct biochemical alterations that change small-scale cellular dynamics and thus, cellular behavior (Quail & Dannenberg, 2019).

Discussion and future perspectives

The importance of mechanobiology is governing cancer progression and metastasis has increased during the past

15 years. As metastasis to secondary locations renders cancer lethal, many efforts have been directed to understanding mechanisms regulating this, throughout the metastatic cascade. More recently studies are emerging focusing on the latter stages, e.g. dormancy and colonization and the different mechanisms that take place during this, compared to more local processes at the primary site.

Perspectives on methods

Studying cancer progression at primary sites with *in vitro* systems has been aided by the development of organoids. These multicellular 3D culture systems, embedded in an ECM scaffold, more accurately recapitulate cellular heterogeneity of patient tumors, which is not as accurately reflected in mouse models. However ECM components of the culturing scaffold are mouse-derived and cannot always be modulated with similar human ECM factors (Drost & Clevers, 2018). Moreover, using these systems it remains difficult to discriminate between mechanisms occurring at a primary site and at a site of metastasis. *In vivo* models allow for full-system and tumor microenvironment study and with the development of intravital imaging, also improved means to study this over time (Entenberg et al., 2022).

Improved human *in vitro* microfluidics systems are being developed, which could more accurately represent the biology and physiology of humans, especially shear forces (Carvalho et al., 2022; Pradhan et al., 2018). These developments improve on recapitulating mechanical forces found in humans that are exceptionally different in mice. Resting heart rates and wall shear rates in mice exceed 10-fold that of humans (Panteleev et al., 2021). Apart from the correct magnitude of strain, the frequency applied should aim to be similar to human physiology. In a human organ-on-chip model of the lung, cyclic strain similar to resting respiration rates, was linked to tumor cell dormancy as well as resistance to a tyrosine kinase inhibitor (Hassell et al., 2017). Use of microfluidics devices can aid in our understanding of organotropism. In a model with four equal branching points to different types, tumor cells circulated for a prolonged period of time to interrogate preferential tissue of colonization. Using this device, circulation patterns would no longer play a role, limiting the tropism to stromal interactions (Aleman & Skardal, 2019). This is interesting, as such devices can also contain biomimetic scaffolds receptive of chemical modification to continually change tissue mechanics (Skardal et al., 2016). Finally, *in vitro* systems should validate the mechanical rheology of system to ensure it sufficiently biomimetic of *in vivo* tissue, for instance by comparing scaffolds to decellularized ECM from *in vivo* samples. Stroma act like composite material, e.g. showing non-linear mechanical responses, and depends heavily on composition and respective orientation of both ECM and cells (Song et al., 2021). Therefore, including stoichiometry of ECM components in a culture system may lack. The non-destructive methods to measure mechanical properties, like rheometers or atomic force microscopy can work in favor of multiplexing to study for instance protein composition.

Not unlike other scientific fields, sex differences are understudied and poorly included in methodology, similarly in cancer studies and pre-clinical models (Haupt et al., 2021). Indeed, many studies referenced in this review fail to mention the sex of their animals in methodology. Of relevance, distinct sex differences in ECM composition and rheology are present in the brain of mice (Batzdorf et al., 2022). To illustrate,

the cerebral cortex of male mice contains twice as much laminin as their female counterparts, whereas laminin was previously identified as a key dormancy regulator in brain-metastatic breast cancer (Dai et al., 2022). Furthermore, liver-tropic metastasis of a highly metastatic pancreatic cancer in under control om TIMP-1, an overall inhibitor of MMPs, whose expression differs between the sexes (Hermann et al., 2021)

Concluding remarks

It is evident that mechanobiology plays an important role in the metastatic cascade, of which of interest the latter phases of dormancy and colonization. Mechanical signals may also play a role in regulating tissue-specificity of metastasis in ways that are matched in tumor identity and tissue characteristics. For instance, supporting dormancy in the brain by astrocytes their deposition of laminin-211 is closely linked to this specific BM component, as well as the tumor cells' dependability on YAP for further growth (Dai et al., 2022). EVs can preferentially fuse with cells that are located in sites of secondary metastasis (Hoshino et al., 2015). The EV composition and nature is dictated by tumor identity, whereas its receival and integration is linked to specific tissues. It is necessary to note that this review discusses mechanobiology in solid tumors as a whole, with specific examples, although for certain cancer types there might be other dominant mechanisms, such as lack of immunological activity in 'cold' and 'hot' tumors (Bonaventura et al., 2019).

Nonetheless, a prominent function for mechanical forces remain in tumor metastasis. Further studies are necessary to learn more about the late stages of metastasis, for which innovative *in vitro* methods should be used.

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