

Review

Tissue architecture in tumor initiation and progression

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The 3D architecture of tissues bearing tumors impacts on the mechanical microenvironment of cancer, the accessibility of stromal cells, and the routes of invasion. A myriad of intrinsic and extrinsic forces exerted by the cancer cells, the host tissue, and the molecular and cellular microenvironment modulate the morphology of the tumor and its malignant potential through mechanical, biochemical, genetic, and epigenetic cues. Recent studies have investigated how tissue architecture influences cancer biology from tumor initiation and progression to distant metastatic seeding and response to therapy. With a focus on carcinoma, the most common type of cancer, this review discusses the latest discoveries on how tumor architecture is built and how tissue morphology affects the biology and progression of cancer cells.

3D architecture conditions tumor biology

Tissue architecture is dependent on tensional homeostasis that is necessary for organ function [1]. When tumors arise and grow, they alter the organized morphology of tissues, creating aberrant tensional forces and inducing changes in the mechanical microenvironment. The 3D morphology of the resulting lesion and the associated aberrant mechanics influence the biology of cancer cells and surrounding tissues. Recently, several groups have focused on studying the etiology of different tumor architectures, their evolution throughout progression, and their prognostic value [2–4]. This review discusses the recent literature evaluating the relevance of tissue architecture in tumor biology.

Architectural and mechanical heterogeneity of primary tumors

The 3D architecture of tumor host tissues and malignant lesions is driven by mechanical traits and is a source of mechanical and functional intratumor heterogeneity. Diverse architectural properties of tissues, including their geometry, confinement, and fluidity, exert different mechanical stimuli on cancer cells within a tumor [5].

Tumorigenesis disrupts physiological architecture

The disruption of tissue architecture at the onset of tumorigenesis is conditioned by the molecular drivers of transformation. *Bona fide* cancer drivers such as RAS oncogenes (*HRAS*, *NRAS*, and *KRAS*) cause loss of tensional homeostasis and reorganize tissues by altering local actomyosin contractility. In non-malignant mammary MCF-10A cells, stress fibers are localized in the apical region and contribute to the stiffness of the cell. In malignant mammary cells (MCF-7 and MDA-MB-231), stress fibers localize basally [6]. Upon transformation with oncogenic *HRAS*, non-malignant MCF-10A mammary cells lose their epithelial monolayer organization and aggregate into 3D structures *in vitro*. This occurs by reduction of cellular adhesion and decreased traction forces to the substrate, together with disrupted cytoskeletal polarization [7].

Highlights

Interplay between cellular, molecular, and mechanical factors shapes tumor morphology.

Tumor architecture has genetic, epigenetic, and phenotypic effects on cancer cells.

Primary tumor architecture informs cancer progression.

Cytoskeletal architecture remodels before metastatic invasion.

Carcinoma cells invade as single cells upon epithelial-to-mesenchymal transition or as epithelial clusters.

The architecture and topography of the tumor impacts on therapy delivery and efficacy.

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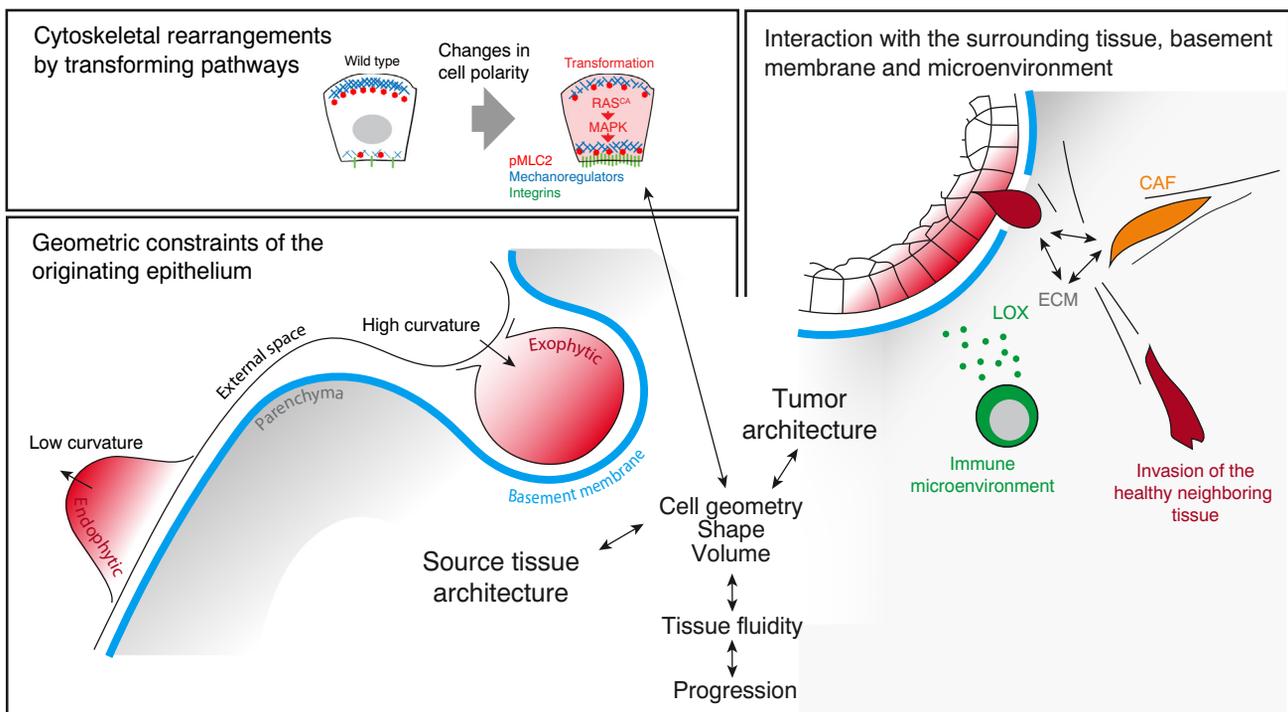
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Epithelial cells of the lung airways, hepatic ducts, and exocrine pancreas present a physiological apical polarization of actin and phosphorylated myosin. Upon transformation, constitutively active KRAS triggers the inhibition of phosphatases that maintain this actomyosin polarity, and leads to the basal accumulation of actomyosin cortex-related components including integrins and mechanoregulators [2]. Thus, the local loss of cytoskeletal polarity in transformed cells contributes to the malignant cellular morphologies that in turn result in the characteristic architecture of early lesions (Figure 1).

Intratumor mechano-architectural heterogeneity

Throughout tumor progression, cell–cell interactions and tissue fluidity determine the individual cellular geometry [8]. In MCF10A spheroids, the cytoplasmic and nuclear volume of cells varies between the core and periphery [9], and different force magnitude levels are at play in these two regions, suggesting that local confinements throughout a tumor contribute to intratumor cellular heterogeneity. In the tumor margins, confinement by the extracellular matrix (ECM) plays a key role in cancer progression. Cancer cell confinement together with reduced cell–cell and cell–ECM adhesions regulate tumor unjamming transitions (increase of tissue fluidity) and promote invasion [10]. Conversely, an increase in collagen concentration provokes tissue jamming and prevents invasion [11]. Under artificial confinement *in vitro*, HRAS^{V12} transformed cells present a proliferation advantage. Although they are softer in intermitotic periods, their rigidity increases during mitosis, surpassing that of healthy cells, and thereby allows correct chromosome segregation without mitotic arrest [12]. Hence the mechanical heterogeneity driven by the tumor composition and architecture plays an important role in dictating cancer cell shape and competitive proliferation.



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Figure 1. Architects of tumor morphology. Oncogene-driven cytoskeletal rearrangements, geometric constraints of the tissue of origin, and the cellular and molecular microenvironment shape the architecture of cancer cells and tumors. Abbreviations: CAF, cancer-associated fibroblast; ECM, extracellular matrix; LOX, lysyl oxidase; pMLC2, phospho-myosin light chain 2; RAS^{CA}, constitutively active RAS.

The different local forces created by the tumor architecture have further consequences on the biology and behavior of cancer cells. In morphologically identical spherical colonies of breast cancer cells, traction stresses distribute stochastically, and a single cell can alter the organization and mechanics at the multicellular level [13]. Conversely, mouse and human cancer cell lines cultured in a variety of matrix shapes show tension at the matrix–media interface of curved geometrical traits. Increased local tension induces the expression of cancer stem cell markers and modulates cell shape, adhesion, and signaling [14]. Tumor growth and shape, conditioned by mechanical tension and spatial restrictions among other factors, affect the diversification of genetic subclones in clear cell renal carcinoma, demonstrating crosstalk between cellular and architectural intratumor heterogeneity that impacts on cancer evolution [15]. The use of spatial transcriptomics and single-cell RNA sequencing in human pancreatic ductal adenocarcinoma (PDAC) allowed the correlation of transcriptomic signatures and stromal enrichment with spatially isolated architectures within a tumor [16]. The aberrant tissue architecture upon transformation alone could contribute to heterogeneity and malignancy through chromosome instability. Native tissue architecture and integrin function are necessary for proper chromosome segregation [17], and cancer-induced disruption of epithelial architecture may lead to chromosome instability.

Intertumor mechano-architectural heterogeneity

Tumors of similar etiology or cell of origin often present different morphologies from early tumorigenesis that later affect their progression. The drivers of these different morphologies are starting to be elucidated, and, as in normal tissue developmental processes, these may depend on the organization and geometry of the naïve epithelium before transformation [18]. In the pancreas, the heterogeneous geometry of the pancreatic duct determines early morphogenesis of PDAC precursor lesions. Transformation in ducts with a diameter of 17 μm or above leads to the formation of endophytic lesions that grow into the duct lumen. The higher curvature of narrower ducts forces early tumors to grow exophytically into the surrounding parenchyma. Through their higher exposure to the non-cancerous tissue environment, exophytic lesions interact with protumorigenic cancer-associated fibroblasts (CAFs) more efficiently and display increased tumor cell dissemination [2]. Strikingly, the actomyosin cortical perturbations are similar among cells in both lesion types, but lead to different tissue morphological outcomes owing to the disparate geometries of large- and small-diameter ducts and the cells within (Figure 1).

In the skin, non-invasive basal cell carcinoma (BCC) forms bud-like lesions, whereas invasive squamous cell carcinoma (SCC) forms folds. This key architectural difference is explained by the assembly and stiffness of the basement membrane (BM). BCCs accelerate the assembly of the BM, creating a softer BM that is more resilient to tension. SCCs decrease BM assembly and exert higher tensional forces on it, facilitating rupture and invasion beyond [3].

Thus, the spatial position of the tumor-originating cell in the normal tissue epithelium influences key aspects of lesion biology through an interplay between cell behavior and external geometric cues (Figure 1). Although driver mutations modulate cancer cell behavior and shape, local forces exerted by cancer cells and the geometry of the tissue before transformation condition tumor morphology, and the tumor-adjacent molecular and cellular microenvironment further crucially shapes tumor morphology.

The tumor microenvironment modulates its architecture

The tumor microenvironment is the ensemble of ECM molecules and non-transformed cells that surround cancer cells. Cancer cells secrete matrix modifiers [19] and exert forces [20,21] to expand in restricted spaces and break through mechanical barriers. The architecture of ECM fibers and the cellular microenvironment can physically facilitate or restrict invasion. The biophysical

characteristics of the tumor stroma have important implications in tumor morphology and cancer cell behavior (Figure 1). A stiffer stroma can foster invasion, which in part may come from the promotion of cancer cell interaction with the surrounding endothelium [22]. On the other hand, high ECM deposition stiffens the stroma and reduces pore sizes, leading to confinement and reduced tissue fluidity which may limit cancer cell proliferation. For example, PDAC lesions grow slower in their stiffer acellular primary site than as metastatic lesions in the liver, with reduced stroma [23].

ECM architecture and cancer

The ECM is an acellular array of macromolecules that function as a scaffold for maintaining the architecture of the tissue. Tumors have aberrant ECMs because tumor cells, CAFs, and other cells of the tumor microenvironment secrete components that alter the physicochemical characteristics of the matrix. The ECM is viscoelastic and it can also undergo irreversible deformations [24]. Forces exerted by tumor growth alone can cause breaking of weak crosslinks and untangling of fibers [25]. Cancer cells can modulate the architecture of the ECM by exerting pulsating forces and contracting against collagen fibers, inducing anisotropy [20,21]. Similarly, CAFs create forces that align fibers, creating anisotropic paths that facilitate cancer cell migration [26].

The BM is a thin sheet of ECM that provides essential structural support to epithelial, mesothelial, and endothelial tissues, and constitutes a physical barrier for immune infiltration and tumor invasion. The BM is permeable to nutrients and is hyperelastic. Upon experimental pressure application, the BM was discovered to have a non-linear stiffening behavior, making it resistant to instability, breaching, or softening under mechanical stress [27]. BM stiffness is a key determinant of distant metastasis. Netrin-4 induces openings of laminin node complexes and softens the BM. Although this creates pores in the BM, the softening increases its resilience to growing tumors and reduces metastasis. The netrin-4/laminin ratio determines the stiffness of the mammary gland BM. A higher ratio results in lower stiffness and decreased invasion [28]. Recent evidence shows that the relationship between BM and tumors is more complex than was previously anticipated because tumors can produce their own BM [29].

CAFs induce mesenchymal traits and invasion

In vitro, CAFs can drive the invasion of single cells or collective migration by directly interacting with cancer cells through N-cadherin/E-cadherin heterotypic adhesions. CAFs exert pulling forces on the cancer cells away from 3D spheroids, inducing invasion [30]. In addition, CAFs can alter the integrity of the BM by exerting physical forces, making it permissive for tumor cell invasion, as shown in coculture experiments with colon cancer cell lines [31]. Conversely, adipose stromal fibroblasts stiffen the ECM by deposition of fiber proteins such as collagen and fibronectin [32].

CAFs induce epithelial-to-mesenchymal transition (EMT) and proliferation of PDAC cells through TGF- β secretion. This leads to a heterogeneous composition of tumor cell types (proliferative, EMT, neither or both), allowing the classification in eight architecture 'units' that can coexist within the same tumor [33]. CAFs also aid breast cancer growth in an architecture-dependent manner via secretion of IL-6, which induces local hepcidin expression and iron retention that are characteristic of breast cancer. Interestingly, this mechanism has only been observed *in vivo* and in organoids, and not in 2D culture, suggesting that the 3D architecture of the transformed epithelium is required [34].

Spatial interactions between tumors and healthy epithelium

Epithelial malignancies involve mechanical crosstalk between the expanding lesion and the surrounding healthy cells. Local transformation by KRAS^{V12} in *Xenopus laevis* embryos leads to actomyosin hypercontractility, creating a radial anisotropic tension around the lesions and inducing

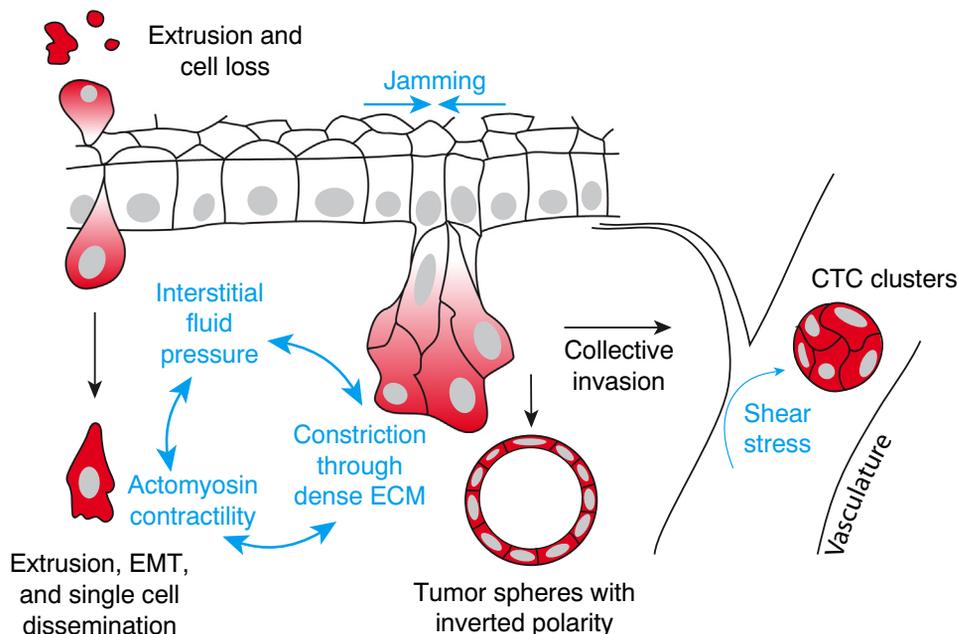
division of contiguous wild-type cells [35]. In mouse skin, tissue straining through self-inflating gels boosts cell proliferation in the absence of tumors [36]. Hence the tension exerted by an expanding tumor affects the tissue shape and can induce proliferation of surrounding healthy cells.

Extrusion of transformed cells is an epithelial defense mechanism against cancer that involves increased lateral interfacial actomyosin contractility [37] (Figure 2). However, cancer-induced stiffening of the surrounding ECM drives reorganization of the cytoskeleton of healthy cells including perinuclear localization of filamin that impedes extrusion [38].

The basal myoepithelium of glandular organs such as the mammary gland and prostate impairs the preinvasive-to-invasive transition of transformed luminal cells. Genetic perturbations that affect the function of the myoepithelium increase the risk of invasive carcinomas [39]. Live imaging of mammary tumor 3D cultures has revealed that the myoepithelium not only functions as a physical barrier against invasive phenotypes of cancer cells but also dynamically impairs invasion by interacting with cells undergoing EMT, exerting pulling forces and returning them to the luminal region upon escape [40]. The mechanisms inducing the forces that myoepithelial cells exert on cancer cells of the luminal epithelial layer remain to be investigated. Most recently, morphometric quantifications comparing the myoepithelium of patients with ductal carcinoma *in situ* have shown that progressors have a thicker continuous myoepithelium [41], perhaps in response to aggressive luminal phenotypes.

Mechanical interaction between tumor and immune cells

The recent success of immunotherapy against cancer has focused the attention of researchers on the immune microenvironment. Different immune cells play distinct pro- and antitumorigenic functions. Inflammation induced by immune cells can change the biophysical properties of a



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Figure 2. Invasive architectures. Invasive routes of carcinomas as single cells that abandon an epithelial architecture or as collective multicellular epithelial structures. Forces modulating invasion are indicated in blue. Abbreviations: CTC, circulating tumor cell; ECM, extracellular matrix; EMT, epithelial-to-mesenchymal transition.

tumor. Furthermore, by modulating the architecture of the ECM, immune cells impact on the architecture and invasive routes of tumors.

Natural killer (NK) cells interact with B16 melanoma cells through Ncr1 receptor–Ncr1 ligand interaction. This induces IFN- γ secretion by NK cells which promotes tumor cells to express the ECM protein fibronectin 1. Increased fibronectin 1 builds a benign architecture of the primary tumor that impedes metastasis [42]. At the cellular level the aberrant cytoskeletal architecture of cancer cells can be detected by cytotoxic cells. Myocardin-related transcription factors can enhance the rigidity of the cytoskeleton of cancer cells and their invasiveness in the absence of immune cells. However, cytotoxic T lymphocytes and NK cells efficiently detect and eliminate cancer cells with increased rigidity [43]. Consistently, inducing stiffening of the cancer cell plasma membrane by depleting cholesterol enhances T cell cytotoxicity [44].

Macrophages create crosslinking in the ECM by secreting lysyl oxidase (LOX) enzymes that stiffen the stroma and induce cell invasion and poor prognosis in patients with aggressive breast cancer [45]. In zebrafish, macrophages and neutrophils were shown to span breaches in the BM to access tumors of the epidermis. The cells have protumorigenic activity, and might aid invasion by creating breaches in the BM [46].

Chemotherapy has been shown to affect cancer cell behavior by modulating the activity of immune cells. After paclitaxel chemotherapy, CD8⁺ T cells have shown to remodel the ECM of lungs via LOX expression and the deposition of collagen and laminin, aiding the metastatic seeding of breast cancer cells [47].

These recent discoveries manifest the cell- and context-dependent role of the immune microenvironment in enhancing or impairing tumorigenesis and invasion by modifying mechanical traits.

Tumor morphology and invasion

Cancer cells acquire invasive traits throughout primary tumor evolution and metastasize through the lymphatic and blood vasculature to distant organs. In this section we differentiate between two types of carcinoma invasion based on tissue and cell architecture. Upon EMT, cancer cells undergo total remodeling of their cytoskeletal architecture and can abandon the tissue of origin as single cells [48]. Epithelial cells or cells that undergo partial EMT can invade tissues and vasculature as clusters, partially preserving their epithelial organization (Figure 2).

EMT and single-cell invasion

EMT is induced by oncogenes that remodel the cytoskeleton (as discussed in the preceding text) and by the physical properties of the tumor such as interstitial fluid pressure [49] and forces exerted by the stroma. A higher ECM stiffness correlates with the invasive phenotype of cells of different cancer types. Non-malignant MCF10A breast cells behave like invasive cancer cells in stiff matrices [50]. In endometrial cancer cells, EGF induces EpCAM proteolysis, nuclear translocation of its intracellular region, and interaction with LEF1. These molecules act as transcription coactivators and upregulate the key EMT regulators TWIST1, ZEB1, and SNAI1. Atomic force microscopy (AFM) experiments revealed that cells treated with EGF are both softer and less adhesive as a result of EMT and loss of membrane EpCAM, respectively [51]. BAR proteins sense and generate membrane curvature and are inhibited physiologically in epithelial cells [52]. The plasma membrane tension is maintained by membrane-to-cortex attachment proteins ezrin, radixin, and moesin (ERM) in healthy epithelia and in early carcinomas. Upon EMT, these protein families are dysregulated, the tension of the plasma membrane decreases, and the cell undergoes deformation, thereby increasing its disseminating capacity [53].

Although apical cell extrusion is a defense mechanism of epithelia, transformed cells can hijack this mechanism to facilitate invasion. In zebrafish epidermis KRAS^{V12} mutation induces basal extrusion, facilitating invasion beyond the epithelium [54].

Single cancer cell migration through confined spaces increases energy demand, and cells migrate towards the area of lower confinement because of the lower energy cost [55]. Immune cells such as dendritic cells also choose the path of least resistance [56]. The nucleus constitutes the limiting structure for migrating cells to move through narrow spaces. *In vitro*, the cytoplasm and plasma membrane of invasive cancer cells passing through constriction channels can bend with ease, but spaces narrower than nuclei lead to cell obstruction in the absence of matrix degradation. Nuclei sense the degree of confinement and communicate with the actomyosin cortex through stretch-sensitive proteins in the nuclear envelope [57,58]. Compared to the cytoplasm, deforming the nucleus requires higher cellular forces and time [59]. Experimentally, 10–20 nN exerted by AFM is sufficient to rupture the nucleus of U2OS osteosarcoma cells [60]. Passing through narrow constrictions (2–5 μm) *in vitro* can lead to nuclear envelope rupture, cytoplasmic translocation of nuclear DNA, and activation of cGAS-mediated inflammatory cues [61]. Although holes in the nuclear envelope are quickly repaired, these ruptures and nuclear compression alone can induce double-strand breaks in the DNA [62] which may contribute to mutagenesis and genetic instability of invasive cells. Consistent with these discoveries, markers of DNA damage and nuclear envelope rupture are enriched in the invasive margins of mouse and human mammary tumors [63]. In addition to DNA damage, compression of the nucleus has epigenetic consequences. In a recent preprint, confined migration of fibrosarcoma and breast cancer cell lines was shown to induce the formation of heterochromatin that was associated with an increased invasive phenotype [64].

The apicobasal polarity inherent to epithelia limits EMT. In breast and colorectal cancer (CRC) organoids, the PAR complex, an atypical PKC that maintains cell polarity, phosphorylates and targets the EMT factor SNAIL1 for degradation [65]. However, lack of EMT or partial transition does not necessarily restrict invasion and metastasis, and epithelial clusters can develop mechanisms to invade beyond the primary tumor site.

Multicellular structures of collective invasion

In carcinoma, cancer cells can migrate collectively, thereby preserving epithelial cell–cell interactions and providing a protective architecture that facilitates distant metastasis (Figure 2). RAB5A-mediated internalization of EGFR triggers MAPK hyperactivation and downstream actin nucleation that causes unjamming and collective migration in breast cancer spheroids. Similarly, downregulation of E-cadherin and p120 catenin promotes epithelial fluidization, thus favoring tumor migration [10]. CRC cells spontaneously form tumor spheres of inverted polarity, with an outward apical pole and basal ezrin. Apical budding triggered by non-canonical TGF-β signaling and downstream ROCK and myosin II activity allows migration and metastatic seeding in the peritoneum [66]. Multicellular cancer structures can reach the vasculature, and cells migrate through the bloodstream as circulating tumor cell (CTC) clusters of epithelial architecture.

Tumor shape and mechanics during metastatic invasion

Cells of a primary tumor invade distant organs through the lymphatic and blood vasculature. CTCs enter the draining lymphatic vasculature where the low-velocity laminar flow created by low-amplitude pulsations does not exert high shear stress on CTCs. Cells arrive in lymph nodes where they can proliferate and form tumors. Cells may enter the venous blood circulation at the primary tumor or at the lymph nodes. In large vessels, CTCs will encounter turbulent flow and strong shear forces. In narrow capillaries and during extravasation, cancer cells experience

mechanical constrictions. Surviving cells can exit from the circulation and proliferate in secondary organs with biological and physicochemical characteristics distinct from the organ of origin [67,68] (Figure 3).

Once the invasive cancer cells reach the draining lymph nodes through the lymphatic vasculature, a necessary first step in successful lymph node colonization is metabolic rewiring towards fatty acid oxidation. Interestingly, this metabolic adaptation occurs via YAP, a mechanosensory transcriptional regulator, suggesting that the differential mechanical landscape of the lymph node

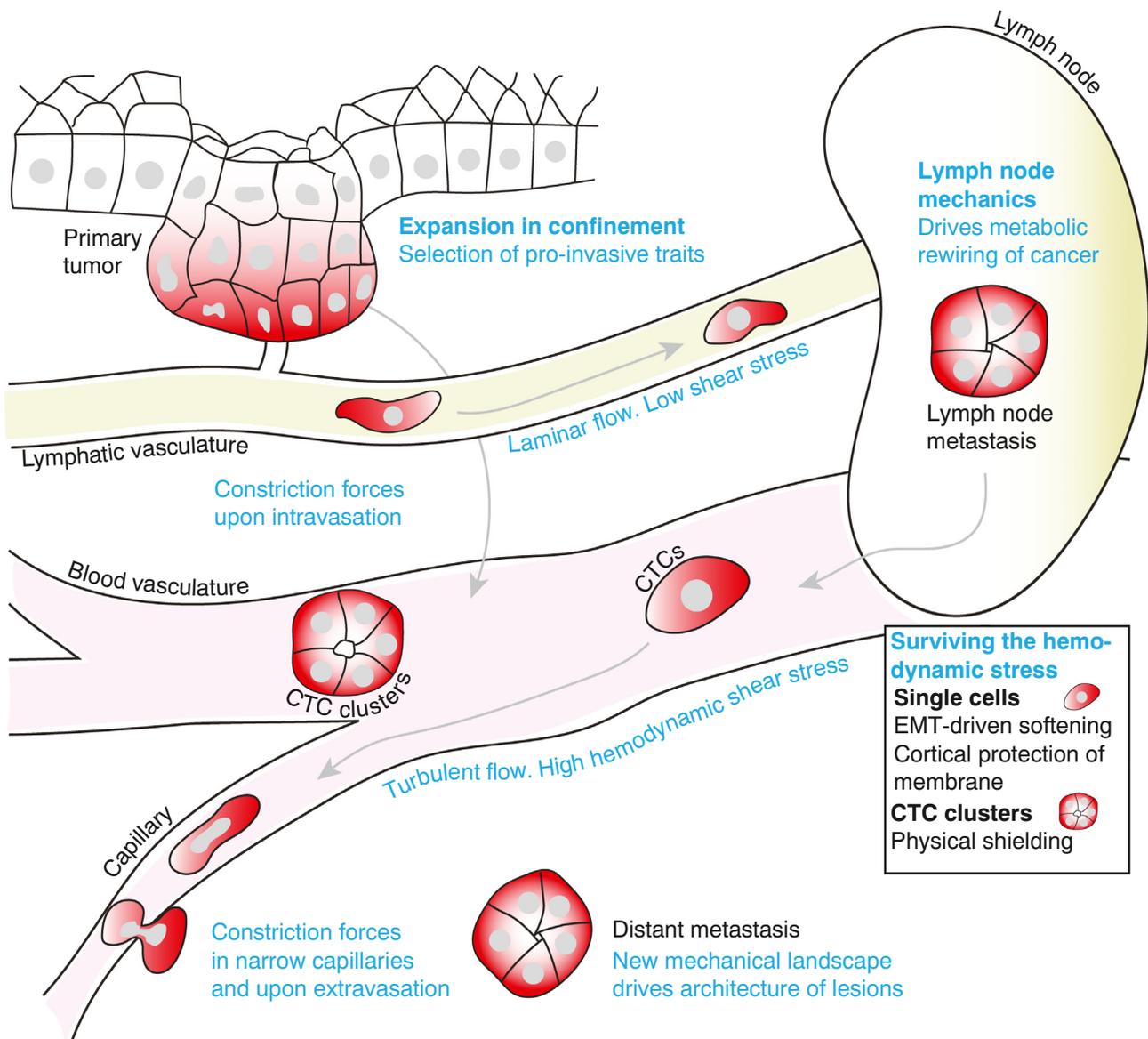


Figure 3. Mechanical forces during tumorigenesis and progression. From oncogenic transformation to the successful establishment of distant metastases, cancer cells experience a myriad of different mechanical forces that lead to the selection of the most resilient, deformable, and motile cells. Forces can induce genetic, epigenetic, and morphological changes in cancer cells throughout tumor progression. Abbreviations: CTCs, circulating tumor cells; EMT, epithelial-to-mesenchymal transition.

environment triggers this shift [69]. However, the link between lymph node mechanics and rewiring via YAP remains to be identified.

When cancer cells enter the blood flow, most die as a result of hemodynamic shear stress. For example, most breast cancer cells in suspension exposed to hemodynamic shear flow are eliminated. Those that survive present EMT features such as elongated morphology and low stiffness associated with reduced F-actin assembly [70]. Healthy prostate cells are efficiently killed by the extreme fluid shear stress in the circulation, but, upon PTEN and P53 deletion, transformed cells can resist this mechanical stress via RhoA–actomyosin cortical protection of plasma membrane integrity [71]. Homotypic and heterotypic clustering of CTCs can protect them from shear stress-induced cell death in the circulation. CTCs interact with each other [72], as well as with blood cells such as neutrophils [73]. Platelets may protect CTCs from venous and arterial-like shear stress, as shown *in vitro* for ovarian cancer cells [74]. These interactions might cushion the shear stress in circulation, but how mechanical forces affect CTCs of different sizes and cellular composition has not been explored. Although it was originally believed that CTC clusters would be jammed in capillaries and hence never reach arteries, it has since been demonstrated that clusters of up to 20 cells can pass through the capillary bed [75]. In thin capillaries and during extravasation, cancer cells experience constrictive forces that could trigger further selection, DNA damage, and epigenetic changes. Cells forming part of the CTC cluster architecture present hypomethylated chromatin at the binding sites of the embryonic transcription factors OCT4, SOX2, NANOG, and SIN3A. Disruption of the CTC cluster architecture into single cells by Na⁺/K⁺ ATPase and tight-junction inhibitors changes the chromatin methylation profile, and limits stem cell features and metastasis [76]. The link between multicellular and chromatin architecture and translational activity [77,78] might be explained by mechanical crosstalk between the cytoskeleton and nucleoskeleton. The linker of nucleoskeleton and cytoskeleton (LINC) complex connects these dynamic architectural cortexes [79]. Nuclear mechanics are influenced by cytoskeletal forces [80] and vice versa [81].

Cancer cells can produce extracellular vesicles that prime the premetastatic niche by influencing its ECM architecture and mechanics. Upon exposure to taxol chemotherapy, breast cancer cells secrete extracellular vesicles that increase the elasticity of the lung mechanostucture, thus facilitating metastatic seeding [82]. Once cancer cells arrive in a secondary organ, they colonize by competing with pericytes and adhering to the perivascular niche through L1CAM. This triggers mechanotransduction effectors such as YAP and MRTF which enable metastasis outgrowth [83], indicating that the mechanostucture of invasive sites conditions successful metastasis (Figure 3).

Concluding remarks and future perspectives

The study of tissue and tumor architecture and its consequences for cancer cell biology and progression have benefited from the development of techniques to study tissue transformation in 3D. Although spheroids and organoids are widely used in biomedical research, the recent development of epithelial assembloids containing a complex layered microenvironment recapitulating whole organs [84] holds promise for a more rigorous study of tissue architecture and mechanics. In this study, muscle and stroma surrounding luminal epithelium recapitulated the architecture of a healthy bladder, and, upon transformation, the lineage identity of tumor cells was preserved. *Ex vivo*, tissue clearing and fluorescence 3D imaging allows the characterization of tissue and tumor architecture with unprecedented resolution [85]. Using tissue clearing of prostate resections, a staging method based on 3D architecture of prostate lesions has demonstrated prognostic value [4]. In CRC, tissue architecture informs on Src activity because Src induces ezrin which changes the morphology of cells and multicellular structures, informing on prognosis [86].

Outstanding questions

How do other components of the tumor microenvironment (nerves, endothelium, other immune cells) shape tumor architecture?

Does clustering of cells in collective invasion cushion and protect them from mechanical stress?

Can benign tumor morphologies be promoted by pharmacologically targeting architecture drivers?

The topographical presentation of tumors is an important consideration for therapeutic options. Sheet-like lesions are difficult to efficiently resect surgically. The use of topical hydrogels containing nanoparticles with small RNAs is agnostic to tissue topography and can be used to treat sheet-like lesions [87]. Therapeutic efficiency is further influenced by tumor composition and morphology, as well as by their underlying physical properties. The density and integrity of the vascular network, as well as interstitial fluid pressure, are crucial for the efficient delivery of anticancer therapies [88]. Architectural tumor compartmentalization leads to regional molecular and functional fluctuations that shape treatment response, as is apparent in skin BCC where the basal cells in direct contact with the BM (presenting low Notch signaling) and suprabasal cells (high in Notch) respond differently to the hedgehog inhibitor vismodegib that is widely used in BCC treatment [89]. The increased stiffness upon tumor development can be exploited in creative treatment avenues for efficient drug delivery [90]. Altering tumor stiffness [91] can help the efficacy of immuno- and chemotherapies [92] in a tumor-specific manner. Targeting the ECM architecture could restrict tumor invasion, but needs to aim at ECM normalization rather than at ablation so as to provide the controlled environment that regulates cell behavior and naturally limits proliferation and invasion. Therapeutic ECM interference could also facilitate immune cell infiltration, boosting the effect of immunotherapy. Stiffness increases the expression of the immune checkpoint PD-L1 in lung cancer, inducing immune evasion [93]. Using LOX inhibitors, preclinical tumor stiffness was reduced, increasing T cell infiltration and the response to anti-PD-L1 immune checkpoint blockade treatment [94].

These examples illustrate the importance of tumor and stroma architecture in cancer cell behavior. Further investigation on the etiology and consequences of different tumor morphologies will expand our understanding of fundamental and clinical cancer biology (see [Outstanding questions](#)).

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Declaration of interests

The authors declare no conflicts of interest.

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