Essays in Biochemistry (2022) **66** 359–369
https://doi.org/10.1042/EBC20220014

PORTLAND PRESS

Review Article

Biomechanics of cancer stem cells

Xi Chen[1,2,3], Kai Tang[1,3], Xueyi Li[3], Cunyu Zhang[1,2,3], Ying Xin[3], Keming Li[3] and Youhua Tan[1,2,3]

[1]Shenzhen Research Institute, The Hong Kong Polytechnic University, Shenzhen, China; [2]Research Institute of Smart Ageing, The Hong Kong Polytechnic University, Hong Kong, China; [3]Department of Biomedical Engineering, The Hong Kong Polytechnic University, Hong Kong, China

Correspondence: Youhua Tan (youhua.tan@polyu.edu.hk)

Cancer stem cells (CSCs) have been believed to be one driving force for tumor progression and drug resistance. Despite the significance of biochemical signaling in malignancy, highly malignant tumor cells or CSCs exhibit lower cellular stiffness than weakly malignant cells or non-CSCs, which are softer than their healthy counterparts, suggesting the inverse correlation between cell stiffness and malignancy. Recent years have witnessed the rapid accumulation of evidence illustrating the reciprocity between cell cytoskeleton/mechanics and CSC functions and the potential of cellular stiffness for specific targeting of CSCs. However, a systematic understanding of tumor cell mechanics and their role in CSCs and tumor progression is still lacking. The present review summarizes the recent progress in the alterations of tumor cell cytoskeleton and stiffness at different stages of tumor progression and recapitulates the relationship between cellular stiffness and CSC functions. The altered cell mechanics may mediate the mechanoadaptive responses that possibly empower CSCs to survive and thrive during metastasis. Furthermore, we highlight the possible impact of tumor cell mechanics on CSC malignancy, which may potentiate low cell stiffness as a mechanical marker for CSC targeting.

Introduction

Multiple types of solid tumors have been believed to be organized as a hierarchy [1,2], in which CSCs are at the apex. CSCs hold the abilities to self-renew and differentiate into other cancer cells, and have been proposed to drive tumorigenesis and metastasis [2]. Current mainstream anticancer treatments include chemotherapy and radiotherapy, which primarily target proliferating tumor cells, while CSCs are quiescent in unperturbed tumors, and can thus survive and potentially lead to tumor relapse [3]. Therefore, CSCs play a key role in drug resistance and tumor recurrence. Understanding the mechanisms underlying CSC's high malignancy is essential for the development of effective therapeutic strategies.

CSCs are believed to arise either through the transformation of normal tissue stem cells into cancerous states or through the acquisition of additional mutations in a subset of tumor cells [2,3]. A number of biochemical signaling are involved in CSC's properties and functions, such as Wnt, Notch, and Hedgehog pathways [4]. In addition to these factors, accumulating evidence has demonstrated that mechanical cues play important roles in CSC functions [5,6]. The mechanical properties of the tumor microenvironment are essential in maintaining CSC's stemness and malignancy [6,7], while CSCs exhibit unique cellular mechanics, which are related to malignancy. In the present review, we focus on the characteristic cytoskeleton and mechanical properties of CSCs and the link between cell mechanics and their stemness, and highlight the potential of cell stiffness as a mechanical target for the development of new mechanomedicine for cancer therapy.

Received: 02 June 2022
Revised: 27 July 2022
Accepted: 02 August 2022

Version of Record published:
16 September 2022

Cytoskeletal alterations during tumor progression

Eukaryotic cells mainly contain three types of cytoskeletal elements: actin microfilaments, intermediate filaments, and microtubules, which are associated with cytoskeleton-binding proteins. For example,

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nonmuscle myosin II pulls on actin filaments to produce contractile force, which can remodel actin cytoskeleton and form actin bundles [8]. Actinin crosslinks actin filaments, regulates the length and tension of stress fibers [9], and is necessary for focal adhesion maturation [10]. Arp2/3 binds to the side of actin filaments and promotes the addition of single actin monomers to existing actin filaments, which enhance cell stiffness [11]. Filamins bind to cortical actin filaments, dynamically regulate the cross-linking of actin filaments, and stabilize the 3D structure of actin webs [8]. These cytoskeletal proteins significantly change in cancer, which accompanies tumor progression [6–8].

First, actin filaments change dynamically from tumor initiation to metastatic colonization and considerably influence tumor cell stiffness [12]. For example, Src-mediated transformation of normal breast epithelial cells leads to transient increase in stress fibers, Ena/VASP-like, and cellular stiffness, which facilitates breast tumor growth during the premalignant stage [13]. F-actin in normal epithelial cells is dramatically decreased when they are transformed from normal to malignant and metastatic state [14]. Epithelial–mesenchymal transition (EMT) is important for metastasis and usually involves actin cytoskeleton remodeling, morphological change, and cell softening [15]. Highly metastatic cancer cells acquire a mesenchymal phenotype and exhibit less dense and less organized stress fibers [16]. Actin cytoskeleton is critical for the formation of lamellipodia and invadopodia and thus essential in tumor cell migration and invasion. When tumor cells migrate through confined space, the stress fiber network undergoes remodeling, which may lead to cell softening [17]. Our recent work shows that after intravasation, circulating tumor cells (CTCs) experience fluid shear stress in the vasculature, which decreases F-actin assembly and enhances chemoresistance [18]. Breast cancer cells with bone metastatic tropism exhibit more F-actin than tumor cells with brain metastatic tropism [19], suggesting an association between tumor cell mechanics and metastatic organotropism.

Furthermore, overexpressing SLC7A11 in glioblastoma cells redistributes F-actin from the cell membrane to the cytoplasm and enhances the stemness and chemoresistance [20]. It will be interesting to explore whether disrupting polymerized F-actin can rescue the effect of SLC7A11 on malignancy. Compared with non-CSCs, pancreatic CSCs exhibit lower levels of actin polymerization and higher Ezrin (one cytoskeletal regulator). Inhibiting Ezrin induces actin polymerization but decreases CSC frequency [21]. Lung and breast CSCs show lower F-actin compared with conventional tumor cells [22,23]. Lovastatin suppresses the formation of pseudopodia, redistributes F-actin from the cytoplasm to the nuclear or perinuclear area, and inhibits EMT, thereby reducing liver metastasis and drug resistance of breast CSCs [24]. Interestingly, polyploidal giant cancer cells exhibit high self-renewal ability and increased length and width of actin stress fibers [25].

Second, intermediate filaments extend from the nuclear surroundings to the plasma membrane to form an elaborate network, interact with actin filaments and microtubules, and influence cellular mechanics. Previous studies have implicated the role of intermediate filaments in cancer initiation and progression. For example, oncogene-induced cell transformation up-regulates the intermediate filament vimentin, increases its width and entanglement, and redistributes it to the perinuclear area, which elevates cellular stiffness and cell invasion via up-regulation of histone deacetylase 6 [26]. Breast CSCs up-regulate vimentin, inhibition of which abolishes SHIP2-induced increase in the stemness and tumorigenicity [27]. Keratins, the most abundant intermediate filaments in epithelial cells, are up-regulated in multiple types of cancer and have been utilized for cancer diagnosis [12]. As a major intermediate filament protein, cytokeratin 18 negatively regulates EMT and stemness of cancer cells [28]. However, silencing cytokeratin 17 reduces the stemness and tumorigenicity of cervical cancer cells, while overexpressing it facilitates lymphatic metastasis [29]. Furthermore, intermediate filaments maintain and regulate intracellular mechanical homeostasis of actin filaments [30], and are critical for cell migration, invasion [26], and division. These findings suggest the correlation between intermediate filaments and malignancy.

Third, microtubules are highly dynamic cytoskeletal structures, and distribute from the nucleus to the plasma membrane [8]. Two tumor-suppressor genes, HIC1 and RassF1A, mediate the transformation of MSCs, which reduces tubulin expression and cell stiffness and disorganizes F-actin [31]. Epithelial cells should maintain the balance between the outward-directed microtubule force and inward-directed force of the actin cytoskeleton [32]. Loss of CRMP2 remodels the microtubule network and induces an EMT and stem-like phenotype in prostate cancer [33]. Elevated matrix stiffness facilitates microtubule glutamylation and stabilization and promotes breast cancer cell invasion [34]. Breast CSCs hold larger numbers of tubulin-based microtentacles, which are important in the reattachment of suspended tumor cells and the subsequent metastatic colonization [35]. Glioblastoma cells that are resistant to microtubule-targeting agents exhibit reduced α- and β-tubulin expressions and enhanced stemness [36].

Fourth, cytoskeleton-related proteins regulate cytoskeletal networks and structures and are involved in tumor progression. For example, myosins are up-regulated in many types of cancer and critical in tumor cell migration and invasion [37]. Melanoma cells that are resistant to MAPK inhibitors show high myosin II activity, inhibition of which resensitizes these resistant cells to targeted therapies [38]. α-Actinin-4 (ACTN4) is overexpressed in cervical cancer and facilitates tumor formation through Snail-mediated EMT [39]. Cervical CSCs exhibit enhanced levels of ACTN4,

Essays in Biochemistry (2022) **66** 359–369
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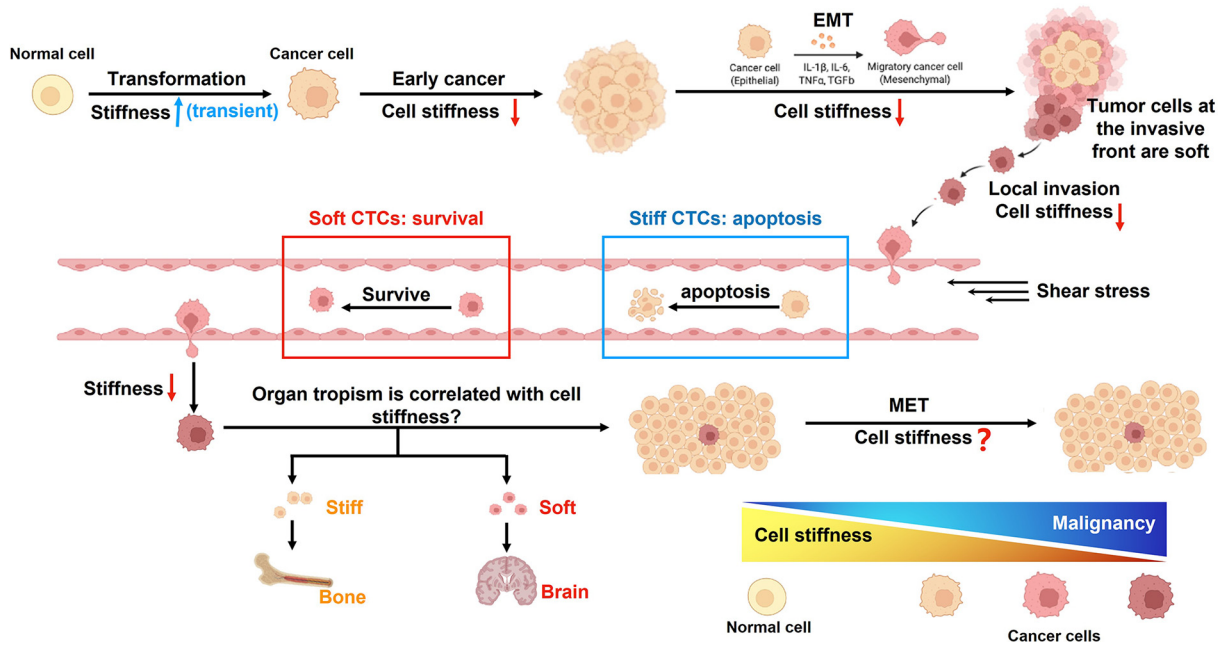

Figure 1. The dynamic alterations of cancer cell stiffness during the metastatic process

which enhance the CSC properties and chemoresistance probably through EMT [40]. Arp2/3 mediates the migration of colorectal cancer cells and facilitates the growth of liver metastases [41]. In addition, Arp2/3 is required for the activation of Notch signaling and the maintenance of glioma CSCs [42]. Colorectal CSCs express high levels of Filamin A, inhibition of which suppresses their stemness [43].

In summary, the cytoskeletal filaments and their binding proteins alter significantly during tumor progression, which influences not only tumor cell functions but also their mechanical properties. For example, disruption of F-actin, microtubule, and intermediate filament decreases cell stiffness by 90, 60, and 40%, respectively [44,45]. Pharmacologically activating myosin IIB and IIC elevates the cortical tension of pancreatic cancer cells and increases their stiffness [46]. Inhibition of α-actinin reduces cell stiffness from 6 to 2 kPa in glioblastoma cells [47]. Loss of Arp2/3 softens pancreatic cancer cells from 3.5 to 2 kPa [48]. Depletion of Filamin A reduces the storage modulus of human melanoma cells from 350 to 170 Pa. Silencing Formin 1 reduces the stiffness and mobility of glioblastoma cells [49]. Therefore, alterations in cytoskeleton-related proteins during tumor progression may induce changes in cell mechanics [8].

Tumor cell stiffness at various metastatic stages

Metastasis is a force journey of tumor cells, in which they not only experience different types of mechanical cues but also passively and/or actively alter their own cytoskeleton and cellular mechanics to adapt to distinct tumor microenvironments at different metastatic stages (see Figure 1) [50]. Starting from tumor initiation, the cytoskeleton remodeling mediated by Rif/mDia2 and integrin-linked kinase/beta-parvin/cofilin signaling is necessary for primary tumor formation and metastatic outgrowth [51]. Transient-enhanced cell stiffness is associated with malignant transformation [26,51] and promotes the proliferation of preinvasive breast cancer cells [13]. The core of the breast tumor is much softer than the tumor periphery, which is likely due to the fact that there are more soft tumor cells in the core while more stiff collagen at the periphery [52]. In local invasion, tumor cells in the invasive front of a tumor organoid are softer than the cells in the core [53]. During the migration of non-small lung cancer cells, the leader cells up-regulate the mesenchymal markers Snail and Vimentin, and are much softer and less adhesive than the follower cells [54]. Cancer cells with high invasiveness are five times softer than the cells with low metastatic potential [55]. In 3D environments, the stiffness of head and neck cancer cells is negatively correlated with EMT phenotype and migration ability [56]. Tumor cells become softer during the migration through a confined space [17]. In the vasculature, CTCs that survive in blood shear flow exhibit reduced cell stiffness [18,57]. During the intravasation and extravasation process, tumor cells need to transmigrate the endothelium, during which tumor cells and their nuclei become softened [58,59]. The low stiffness of CTCs facilitates the extravasation and tumor metastasis [59].

Essays in Biochemistry (2022) **66** 359–369
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MDA-MB-231 cells that metastasize to the lung are much softer and more migratory than parental tumor cells and the CTCs derived from parental tumor cells [60]. In addition, breast cancer cells that exhibit bone metastasis tropism are stiffer than the tumor cells with brain metastasis tropism [19]. To form secondary tumors at a metastatic site, cancer cells may undergo mesenchymal–epithelial transition (MET) to promote proliferation. However, the alteration of cell stiffness during this process remains unknown. A study that mimics the progression of ovarian cancer from early to late aggressive cancer stages shows that cell stiffness decreases progressively and that CSCs are at least 46% softer than cancer cells at all stages [61].

Many previous findings have shown that disseminating tumor cells have reduced cellular stiffness, which could be explained by two possible mechanisms. First, the cells in the primary tumor are mechanically heterogeneous. Soft tumor cells have the advantage to detach from the tumor mass and invade into the surrounding stroma for dissemination. This suggests that the low stiffness of invasive cancer cells could be the consequence of an active selection process, which is worthy of further investigation. Second, both soft and stiff tumor cells in the primary tumor can disseminate and invade. When they penetrate the dense tumor stroma or transmigrate the tight endothelium, disseminating tumor cells need to soften their cytoskeleton in order to acquire high deformability for successful passage [17,58]. Furthermore, these two mechanisms can also function synergistically during tumor metastasis.

The relationship between cellular stiffness and CSC functions

The mechanical signatures of cancer cells are different from their normal counterparts. In particular, cancer cells are often softer than normal cells in various types of cancer possibly because of the loss of cytoskeletal elements and the associated proteins and cytoskeleton remodelling [6–8]. For example, metastatic tumor cells from patients with lung, breast, and pancreas cancer show ∼70% lower stiffness than the corresponding benign cells [62]. Metastatic ovarian cancer cells are softer than less metastatic cells and cell stiffness can grade their metastatic potential [55]. Soft subpopulation of cancer cells are more metastatic and stiffening these cells suppresses their invasive ability [53]. These findings suggest that the stiffness of tumor cells is inversely correlated with their invasiveness. Nevertheless, several studies report that tumor cells are stiffer than normal cells in liver cancer [63], breast cancer [64], and leukemia [65]. Note that all the stiffness measurements are conducted *in vitro*, which lack many essential factors in the *in vivo* microenvironment. Therefore, the measured stiffness may not necessarily represent their actual mechanical phenotypes within unperturbed tumors. Recently, cell mechanics have been measured *in vivo* by combining particle tracking microrheology and intravital microscopy. The acquired stiffness is higher for tumor cells in the xenografts than those cultured on 2D substrates or in tumor spheroids [66].

CSCs account for a very small fraction (e.g., 0.1–10%) within a tumor and exhibit unique cell mechanics [7]. For example, our previous work shows that melanoma CSCs selected by soft fibrin exhibit much lower stiffness (∼50 Pa) than unselected cells (∼200 Pa) [67] and that CSC differentiation accompanies with the increase in cellular stiffness [68]. Interestingly, melanoma CSCs do not change but non-CSCs elevate their own cellular stiffness on the substrates with increasing rigidity due to the down-regulation of Cdc42. Overexpression of Cdc42 in CSCs restores this cell stiffening response. However, the influence of Cdc42 on CSC functions is still not clear. Our recent study shows that the stiffness of breast CSCs (∼2 kPa) is only half of parental cancer cells (∼4 kPa) [22]. Compared with less tumorigenic cells (∼1200 Pa), colorectal CSCs show much lower stiffness (∼300 Pa), which is regulated by the reduced expression of capping protein-inhibiting regulator of actin dynamics (CRAD) [69]. Ovarian CSC-enriched populations show cell stiffness of 320 Pa, which is 46, 61, and 72% lower than the stiffness of tumor cells at the late, intermediate, and non-malignant stage, respectively [61]. The stiffness of liver CSCs is ∼580 Pa, which is lower than that of non-CSCs (730 Pa) [70]. On the other hand, recent evidence shows that the sorted soft breast tumor cells exhibit higher stemness and tumor formation ability than the stiff cells [71,72]. Microfluidics-sorted soft K562 cells show enhanced chemoresistant ability [73]. Soft melanoma CSCs have the enhanced ability to escape from the destruction induced by cytolytic T cells compared with stiff non-CSCs [74]. Taken together, all these findings demonstrate the close association between low cell stiffness and stemness.

The cross-talk between cytoskeleton-mediated cell mechanics and CSC stemness

Accumulating results show the correlation between CSC's low stiffness and malignancy, such as metastasis, stemness, survival, and drug resistance, as summarized in Table 1. It is known that Wnt, Notch, and Hedgehog signaling are involved in tumor cell stemness and CSC properties [80] and inextricably linked to cell mechanics through

Table 1 The correlation between cell stiffness and CSC functions

CSC function	Cytoskeleton and cell stiffness	Relationship between cell stiffness and CSC functions	References
Tumorigenicity	Soft (0.2 kPa) and stiff tumor cells (1 kPa) are sorted by the microfluidic chip.	Soft cells are highly tumorigenic and metastatic by up-regulating the Wnt-BCL9L pathway.	[72]
	Fibrin-selected colorectal cancer cells (0.3 kPa) are much softer than control cells (1.2 kPa).	Soft fibrin-selected colorectal cancer cells exhibit higher stemness, tumorigenicity, and metastatic potential than control cells via CRAD.	[69]
Metastasis	Breast CSCs display increased deformability and reduced nuclear stiffness.	Soft CSCs exhibit enhanced invasion in restricted 3D microenvironments via the up-regulated myosin IIB-mediated nuclear translocation.	[75]
	CSCs (<2 kPa) are more deformable and contractile than non-CSCs (3 kPa) in breast cancer and melanoma.	Soft CSCs are more metastatic partially due to ROCK-mediated contractility and ECM degradation capability.	[76]
	ALDH+ breast CSCs exhibit higher cell deformability and lower traction force and adhesion strength than ALDH− non-CSCs.	Soft breast CSCs are more invasive, migratory, and tumorigenic than stiff non-CSCs.	[77]
	Salinomycin increases the stiffness of liver CSCs from 0.5 to 1 kPa and actin filaments.	Salinomycin inhibits the migration and invasion of liver CSCs by increasing cell stiffness and F-actin and phosphorylating FAK and ERK1/2 pathways.	[78]
Stemness	Pancreatic CSCs express higher levels of Ezrin (membrane-actin linker) and lower levels of F-actin.	Ezrin promotes self-renewal and tumorigenicity in pancreatic cancer via the effect on actin remodeling.	[21]
	CSCs up-regulate RhoC, which can regulate actin organization.	RhoC regulates actin organization and CSC stemness in ovarian, breast, and head and neck squamous cell carcinoma.	[79]
	CSCs have enhanced expression of ACTN4, an actin-binding protein.	ACTN4 knockdown suppresses sphere formation, the expressions of stemness markers, proliferation, and tumor formation ability of cervical CSCs.	[40]
The resistance to chemotherapy and immunotherapy	CTCs (1.6 kPa) that survive under fluid shear flow show reduced stiffness than control cells (2.4 kPa).	Inhibiting actomyosin activity or reducing cellular stiffness enhances the survival of breast CTCs and CSCs in fluid shear flow.	[18]
	Breast CSCs are much softer (0.5 kPa) than control cancer cells (1 kPa).	Soft CSCs endocytose more tumor cell-derived microparticles that carry chemotherapy drugs, which overcome drug resistance of soft CSCs.	[23]
	Melanoma CSCs (0.2 kPa) are much softer than non-CSCs (0.6 kPa).	Soft CSCs show enhanced resistance to T-cell-induced cytotoxicity.	[74]

cytoskeleton-related proteins, indicating the potential cross-talk between cell cytoskeleton/mechanics and CSC stemness.

Among these three classic pathways, Wnt/β-catenin signaling is not only important in promoting self-renewal and tumor progression but also related to cell cytoskeleton and mechanics. As a well-known tumor suppressor in Wnt/β-catenin signaling, adenomatous polyposis coli (APC) is mostly located in the actin-rich area in mammary cells and assumes a dual role-cytoskeleton hub and tumor suppressor [81,82]. On the one hand, APC interacts with actin filaments and microtubules directly through the basic domain and indirectly through cytoskeletal proteins, including IQGAP1, APR-stimulated guanine nucleotide exchange factor, and Rac1-specific guanine nucleotide exchange factor [82,83]. On the other hand, APC binds to β-catenin along with the tumor suppressors Axin and Ser/Thr kinase GSK3 in the cytoplasmic disruption complex [84], which leads to the cytoplasmic retention and degradation of β-catenin and prevents the transcription of the downstream genes, including the stemness-related genes. However, it remains unclear whether the interaction between APC and cytoskeleton influences the binding between APC and β-catenin. Furthermore, loss of CRAD inhibits F-actin polymerization and leads to β-catenin release and Wnt signaling hyperactivation in CSCs [85]. Sorted soft cancer cells up-regulate the Wnt signaling protein BCL9L, which is critical in regulating their self-renewal and tumorigenic function [72]. However, the influence of BCL9L on tumor cell mechanics remains unclear.

Besides, microtubule and intermediate filament are also able to influence the Wnt pathway. For instance, Nestin, an intermediate filament, regulates the Wnt signaling pathway [86]. In non-small-cell lung cancer, breast cancer, and pancreatic cancer, down-regulation of Nestin up-regulates the expressions of GSK3β and APC, which lead to the suppression of the Wnt pathway [87]. Furthermore, microtubule stability can influence the Wnt activity through CDK11-regulated APC [88].

Apart from the Wnt pathway, cell cytoskeleton is involved in the activation of Notch signaling that plays a critical role in regulating CSC stemness [42,89]. For example, actin cytoskeleton is necessary for the activation of Notch signaling in maintaining glioma CSCs properties [42,89]. Fascin, an actin-binding protein, is required to activate Notch-mediated self-renewal pathway [90]. Inhibition of F-actin drives tumor metastasis through Notch-mediated

Essays in Biochemistry (2022) **66** 359–369
https://doi.org/10.1042/EBC20220014

Table 2 Cell cytoskeleton is associated with CSC-related pathways

CSC pathway	Cytoskeleton	Association between cell cytoskeleton and Wnt/Notch/Hedgehog pathways	References
Wnt	Actin	The association of APC with the plasma membrane depends on actin cytoskeleton in breast CSCs.	[81]
	Actin	APC organizes cytoskeletal networks and regulates cell migration.	[84]
	Actin	Wnt/Ca2+ signaling regulates actin cytoskeleton and cell motility in prostate cancer.	[94]
	Actin	Loss of CRAD inhibits F-actin polymerization and leads to β-catenin release and Wnt signaling hyperactivation in colorectal cancer.	[85]
	Microtubule	Microtubule depolymerization redistributes APC from microtubule plus ends to the plasma membrane in breast cancer.	[95]
	Nestin (type VI intermediate filament)	Nestin positively regulates the Wnt/β-catenin pathway and the proliferation, survival, and invasiveness of breast CSCs.	[87]
Notch	Actin	Inhibition of F-actin activates Notch and further promotes EMT-driven metastasis in lung and liver cancer.	[91]
	Arp2/3 complex	Actin cytoskeleton regulator Arp2/3 complex is required for DLL1/Notch1 signaling to maintain glioma CSC phenotype.	[92]
Hedgehog	Actin	MTA1, the downstream of Hedgehog pathway, participates in the regulation of actin cytoskeleton reorganization that affects the metastasis of nasopharyngeal carcinoma.	[93]

EMT [91]. Furthermore, the actin polymerization regulator Arp2/3 complex is required for DLL1-induced activation of Notch1 signaling to maintain the stem cell phenotype of glioma CSCs [92]. Compared with Wnt and Notch signaling, the cross-talk among Hedgehog signaling, CSC stemness, and cell mechanics remains relatively less investigated. MTA1, the downstream of Hedgehog pathway, participates in the regulation of actin cytoskeleton reorganization that affects the metastasis of nasopharyngeal carcinoma [93]. The interaction between cell cytoskeleton and Wnt/Notch/Hedgehog signaling is summarized in Table 2. These findings suggest that cell cytoskeleton and mechanics are related to stemness-related signaling, implicating the possible role of cell mechanics in CSCs.

The potential of cell mechanics for CSC targeting

Malignant CSCs can evade not only chemotherapy and radiotherapy but also the newly developed immunotherapy [74]. Therefore, CSCs hold the essential traits to drive tumor progression, drug resistance, and cancer recurrence, and consequently become the major target in cancer therapeutics. To specifically target CSCs, multiple functional surface markers have been identified, including CD133, CD44, CD90, and aldehyde dehydrogenase [3,7]. However, these CSC markers are mainly surface proteins, which are dynamically evolving during tumor progression [96]. Besides, several studies report that there is no difference in tumorigenic potential between tumor cells with high and low expressions of these CSC markers [96], casting doubt on the reliability of these proteins as functional CSC markers.

CSCs hold unique mechanical characteristics compared with non-CSCs and normal cells across multiple cancer types, which potentiate low cell stiffness as a mechanical marker for CSC targeting. Recent studies report the prospective attempts to combat cancer by targeting CSC mechanics. For example, our recent study shows that soft breast CSCs endocytose more graphene quantum dots (GQDs) than stiff bulk tumor cells. Softening tumor cells increase nanoparticle uptake, while stiffening tumor cells and CSCs reduce cellular uptake. Anticancer drug-loaded GQDs eliminate soft CSCs and suppress tumor growth and xenograft tumorigenicity without obvious side effects on animal growth. The present study demonstrates the potential of a new mechanotargeting strategy for CSC eradication and the prevention of chemoresistance [22]. Another study shows a triangular correlation between cell stiffness, phagocytic capacity, and tumor cell malignancy. Sorted tumor cells with high phagocytic capacity are softer and more CSC-like and exhibit high tumorigenicity in the mouse model [97]. Furthermore, tumor cell-derived microparticles are more likely to be taken up by soft breast and lung CSCs than relatively stiff tumor cells [23], suggesting that the low cell stiffness of CSCs could be exploited to reverse drug resistance. Cell softness prevents the pore formation induced by cytotoxic T lymphocytes (CTL) and thus empowers their evasion from the CTL-mediated cell killing [74]. Stiffening soft and stem-like cancer cells enable effective CTL-induced killing. These findings indicate the feasibility of eradicating CSCs by targeting low cell stiffness. Indeed, several types of cells in a living body, such as immune cells and blood cells, could have as similar mechanical properties as tumor cells or CSCs. Therefore, cell stiffness-based cancer therapy may target these cells, which may compromise the specificity. To overcome this potential off-target effect, the

drugs that stiffen and kill CSCs need to be specifically delivered into malignant tumor cells by targeted delivery, such as the delivery vehicles coated with both targeting drugs and specific tumor cell surface markers, direct local delivery of targeting drugs into the tumor, and the release of targeting drugs only at the stiff tumor sites.

Furthermore, altering the molecules related to cellular stiffness affects CSC functions. For example, inhibition of Rho kinase that can reduce cell stiffness promotes the self-renewal of colon CSCs [98]. A drug 4-hydroxyacetophenone mediates the myosin II cortical distribution and increases cell stiffness through enhancing actomyosin tension, which eventually inhibits tumor cell migration, invasion, and the formation of metastatic tumors [99]. Besides, constitutive activation of myosin II contractility resensitizes glioma CSCs to matrix rigidity and suppresses the invasion and metastasis in glioma tumors [100]. Increasing cell stiffness and F-actin assembly in liver CSCs by salinomycin decreases cell motility [78]. CTCs that survive fluid shear flow exhibit low cell stiffness and high chemoresistance and self-renewal [18,57]. Activating actomyosin sensitizes these cells to shear flow by down-regulating the antiapoptosis genes B-cell lymphoma 2 and superoxide dismutase 2, which may potentially suppress tumor metastasis [18].

Concluding remarks and future perspectives

CSCs are one driving force for tumor malignancy and have become a major challenge for cancer therapy. Numerous evidence has shown the low cellular stiffness of CSCs in many types of cancer and demonstrated the close relationship between low cell stiffness and malignancy, suggesting that cell mechanics may be a generalized marker of CSCs. The reciprocal interaction between tumor cell mechanics and CSC stemness provides the foundation for specific CSC elimination by targeting cell mechanics.

To further push the boundary of this field, several important questions need to be addressed. First, a clear definition of bona fide CSCs is still lacking. Current CSC identification predominantly relies on the functional characterization, such as the self-renewal *in vitro* and the ability to generate tumors *in vivo*, which may cause potential inconsistency or even controversy of research findings among different studies. Second, almost all the measurements of cell mechanics are conducted *in vitro*, which could profoundly influence the cytoskeleton and its mechanical properties. Thus, it is necessary to develop new tools for the *in vivo* stiffness measurement at single-cell level. The combination of the clear definition of CSCs and the *in vivo* high-resolution stiffness measurement will enable the dynamic tracing of CSC mechanics at different stages of tumor metastasis. Third, the causal role of cell mechanics in CSC functions and tumor progression remains elusive. It is pivotal to address whether and how the cytoskeleton and cellular mechanics regulate CSC self-renewal, which will support the idea that low cell stiffness can be truly harnessed for specific CSC targeting and cancer therapy. Further elucidating the influence of cell mechanics on mutation-driven tumor initiation and progression will provide new insight into cancer from the perspective of mechanobiology.

Summary

- The dynamic alteration of cell stiffness accompanies tumor progression.
- Tumor cell malignancy is inversely correlated with cellular stiffness.
- Low cell stiffness is a mechanical marker of CSCs.
- Cytoskeleton-mediated cell mechanics reciprocally interact with CSC-related signaling, which potentiate tumor cell mechanics for CSC targeting.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Funding

The present work was supported by National Natural Science Foundation of China [Project no. 11972316], Shenzhen Science and Technology Innovation Commission [Project nos. JCYJ20200109142001798 and SGDX2020110309520303], General Research Fund of Hong Kong Research Grant Council [PolyU 15214320], Health and Medical Research Fund [HMRF18191421], Germany/Hong Kong Joint Research Scheme [G-PolyU503/20], the Research Institute for Smart Ageing in Hong Kong Polytechnic University [1-CD75], and the Hong Kong Polytechnic University [1-ZE2M].

Essays in Biochemistry (2022) **66** 359–369
https://doi.org/10.1042/EBC20220014

Author Contribution

Y.T. conceived the review. X.C., Y.T., K.T., C.Z., Y.X., X.L., and K.L. wrote and commented the manuscript.

Abbreviations

ACTN4, α-Actinin-4; **AFM**, atomic force microscopy; **ALDH**, aldehyde dehydrogenase; **APC**, adenomatosis polyposis coli; CRAD , capping protein-inhibiting regulator of actin dynamics; CRMP2, collapsin response mediator protein; **CSC**, cancer stem cell; **CTC**, circulating tumor cell; **CTL**, cytotoxic T lymphocyte; ECM, extracellular matrix; **EMT**, epithelial–mesenchymal transition; **ERK**, extracellular-regulated protein kinase; **FAK**, focal adhesion kinase; **GQD**, Graphene quantum dot; **IQGAP1**, IQ motif-containing GTPase-activating protein 1; MAPK, mitogen-activated protein kinase; **MET**, mesenchymal–epithelial transition; **MLCK**, myosin light-chain kinase; **MMP**, matrix metalloproteinase; MSC, mesenchymal stem cell; **MTA1**, metastasis associated gene 1; **RhoA**, Ras homolog family member A; **RhoC**, Ras homolog family member C; **ROCK**, Rho-associated protein kinase; **Wnt/STOP**, Wnt-dependent stabilization of protein.

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