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Current Opinion in Biomedical Engineering

Biophysical regulation of cancer stem/initiating cells: Implications for disease mechanisms and translation

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Abstract

Cancer stem/initiating cells (CSCs) are a subset of tumor cells proposed to play privileged roles in seeding tumors and driving metastasis. CSCs have emerged as an increasingly important target of interest in cancer biology and therapy. Recent work has suggested that CSC maintenance and metastatic potential may be modulated by physical inputs within the tissue microenvironment, including interstitial pressure and extracellular matrix stiffness. Here we review recent progress in our understanding of CSC regulation by biophysical signals within the tumor microenvironment. While the mechanistic basis of this signaling remains incompletely understood, we discuss emerging evidence that mechanical inputs can epigenetically regulate CSC behavior and that some CSCs can evade mechanotransductive signals to more efficiently infiltrate tissue. We also describe efforts to leverage these findings to engineer culture platforms for the characterization of CSC mechanics for discovery and screening.

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Current Opinion in Biomedical Engineering 2017, **1**:87–95

This review comes from a themed issue on **Future of BME**

Edited by **George Truskey**

http://dx.doi.org/10.1016/j.cobme.2017.02.006

Keywords

Cancer stem cell, Tumour, CSC, Biophysical signal.

Introduction

Despite tremendous progress in research and treatment over the past half-century, cancer remains one of the leading causes of mortality worldwide [1,2]. Conventional cancer treatment, which includes tumor resection, chemotherapy, and radiation therapy, is designed to remove or kill rapidly dividing cancer cells, and although this paradigm has had important clinical impact, it is typically based on nonspecifically targeting dividing cells, making no fine distinctions between tumor cells and normal cells, or between subpopulations of cells within the tumor. In particular, these approaches do not specifically target tumor cells most likely to seed metastasis, the process by which cancer cells escape from the primary tumor and seed a new tumor [3] and the main cause of mortality in most cancers [3–5]. In 1855, Rudolf Virchow suggested that stem cells may be involved in the etiology of cancer, a concept that was revisited by Lapidot and colleagues over a century later [6,7]. Over the past decade, the evidence for stem-like cells within tumors has been dramatically expanded and reinforced that not all cancer cells are equally able to maintain the growth of a secondary tumor, and that a specialized subpopulation of cancer stem/initiating cells (CSCs) critically drive tumor formation and metastasis [8–11].

The American Association for Cancer Research defines a CSC as "a cell within a tumor that possesses the capacity to self-renew and to cause the heterogeneous lineages of cancer cells that comprise the tumor" [12]. Although much controversy surrounds the origin and prevalence of CSCs, these cells have been identified in several solid tumors including breast, colon, brain, pancreas, liver, and prostate [13–20]. The emergence of CSCs has ushered in a completely new organizing principle for tumor initiation and progression in which tumors are composed of a hierarchy of cells (e.g., stem cell, progenitor cell, and mature cell), with some but not all members of the hierarchy capable of recapitulating all components of a tumor when injected into immunocompromised mice.

CSCs have been shown to possess many characteristics that are essential to tumor initiation, invasion, and recurrence. Most notably, many CSCs are highly resistant to radiation and chemotherapy, suggesting that these cells drive recurrence [10,21]. CSCs employ a stunning arsenal of strategies for evading chemotherapy, including a slow division rate, high expression of efflux pumps, and enhanced capacity for DNA repair [21]. There is also evidence that certain CSCs can invade tissue more rapidly than other tumor cells, consistent with a special role in invasion and metastasis [22]. For this reason, significant energy has been devoted to identifying CSCs, elucidating how they contribute to tumor progression and metastasis, and developing therapies that preferentially target these cells.

Initial efforts to develop CSC-specific therapies have focused on targeting signaling pathways within CSCs that contribute to self-renewal and survival, with the goal of promoting a more differentiated phenotype susceptible to conventional cancer drugs [23,24]. However, targeting specificity remains a major challenge, given that many of these pathways (e.g. Notch, Hedgehog, Wnt) are also active within non-tumor cells. Moreover, many of the relevant targets are intracellular proteins, creating significant challenges in drug delivery. An important opportunity to circumvent both challenges has come in the discovery that CSCs display enhanced expression of specific cell adhesion receptors, such as integrins and CD44, which cells use to attach to and transduce mechanical signals from the extracellular matrix (ECM) [25–29]. This is important, because dramatic changes to the physical environment are experienced within the tumor microenvironment, including increased mechanical stress due to interstitial pressure and increases in the density and stiffness of the ECM [26,30]. Furthermore, it is now well established that these physical changes can activate mechanotransductive signaling systems within the cell, which may collude with canonical mitogenic signaling systems to drive tumor progression [26,31]. While the lion's share of past studies has focused on "bulk" tumor cells, several recent studies have indicated that these mechanobiological cues have special implications for CSC generation, maintenance, and metastasis [27,28]. A deeper investigation into the biophysical regulation of the CSCs represent a promising approach to unravel new CSC specific targets for pharmacological intervention.

In addition to the extrinsic biophysical signals that may regulate CSC behavior, recent reports have also shown that CSCs themselves exhibit changes in their mechanical properties when compared to the bulk tumor population [32–34]. Several reports have indicated an increase in mechanical deformability as an important identifier of CSC populations [32,33]. The identification of such biophysical "markers" has generated much excitement as it can allow for the application of advanced engineering platforms for diagnostic use, drug screening, and mechanistic discovery for the CSC population [32,33,35,36].

In this review, we will focus on how CSCs regulate and are regulated by the biophysical cues within the tumor microenvironment and how these reciprocal interactions contribute to tumorigenesis and metastasis. Further, we will describe the current state of bioengineering platforms designed to characterize CSC mechanical properties for the development of diagnostic and drug screening tools that can help accelerate the development of new therapies that target CSCs.

Regulation of CSC function by the tumor microenvironment

The tumor microenvironment is remarkably complex and dynamic, providing a host of signaling inputs to the resident tumor cell population [30,37–39]. These cues include changes in oxygen tension, juxtacrine and paracrine signals from tumor and stromal cells, and biophysical signals such as matrix stiffness and applied loads [30,40]. The impact of these alterations is tremendous, and while much attention has been directed towards the role of hypoxia and the influence of soluble factors, this review will focus on the biophysical changes and their role in tumorigenicity and metastasis.

Over the past two decades, it has become clear that the behavior of a wide range of cell types is sensitive to mechanical inputs encoded within the tissue environment [41–45]. Mechanotransductive pathways contribute to many aspects of normal physiology, ranging from developmental morphogenesis to adult tissue repair and regeneration [46–48]. Moreover, the disruption of physiologic mechanical inputs leads to the initiation and development of a variety of diseases [31,49]. In the tumor microenvironment specifically, two mechanical changes documented in many solid tumors include: (1) an increase in compressive, tensile, and shear stress, often due to increased interstitial pressure, and (2) an increase in matrix stiffness and ECM density [26,30]. As the tumor expands, tumor cells begin to proliferate and grow in a confined volume, elevating radial and circumferential stresses within the tissue. Several studies have noted the presence of these stresses *in vitro* and *in silico* revealing applied stresses of $\sim$0.5 kPa in colon and breast tumors [50,51]. Increased shear stresses are also observed in many cancers where increased interstitial fluid pressure leads to increased fluid flow by generating pressure gradients in the tumor microenvironment [30]. In addition, breast and pancreatic tumors exhibit an aggressive desmoplastic response where tumor cells initiate ECM remodeling by depositing increased fibronectin, tenascin, collagen, and proteoglycans while also overexpressing matrix metalloproteinases, thereby dramatically stiffening the microenvironment [26,52,53]. Within this new mechanical environment, pro-tumorigenic mechanotransductive inputs actively regulate tumor growth and spread [25,54].

CSCs and confinement

The stress experienced within the tumor microenvironment is transmitted to tumor cells, and the resulting activation of mechanotransductive signaling has been implicated in the regulation of both CSCs and bulk tumor cells. In breast cancer cells, it has been observed that increased compressive stress can induce rearrangement of actomyosin stress fibers and microtubules and enhance cell migration, reminiscent of an epithelial-

mesenchymal transition [56]. Strikingly, this responsiveness to confinement was not observed in normal mammary epithelial cells but exclusively in aggressive carcinoma cells, suggesting an alteration in mechanosensing that primes tumor cells for malignant behavior. Observations such as these have spurred interest in dissecting molecular mechanisms of mechanotransduction that may be unique to or amplified within tumor cells or CSCs. In a melanoma model, confinement sensing has been shown to be cooperatively mediated by Piezo1/PKA and myosin II [56,57]. In another example, the activation of myosin II driven contractility via α4 integrin/paxillin-dependent reduction of Rac1 activity promoted more efficient migration under confinement [56]. Collectively, these studies indicate that the applied mechanical stresses can influence cancer cell differentiation into CSCs as well as regulate actin dynamics to promote efficient motility [55,58].

It has been noted by many that the migration of tumor cells within confined environments is likely to require enhanced compliance or deformability. Recent evidence has shown that CSCs specifically display heightened deformability relative to either non-tumor cells or bulk tumor cells. In one study, breast CSCs displayed increased deformability, reduced nuclear stiffness, and higher invasiveness through confined spaces when compared to non-stem cancer cells [22]. Further, Harada et al. demonstrated that nuclear stiffness is regulated by lamin-A and lamin-B expression, which is often altered in cancer cells and stem cells [59]. These studies suggest that deformability and perhaps other mechanical properties enhance the ability of CSCs to invade tissue and potentially metastasize. Exciting recent work has further proposed that the high shear stresses experienced by the nucleus during this confined migration contributes to disruption of the nuclear envelope, chromatin, and nuclear DNA, thereby potentially accelerating genomic instability [59,60].

CSCs and the mechanical microenvironment

Cells sense mechanical inputs from the ECM through adhesion receptors, which trigger mechanotransductive signaling events and transmit these inputs to the cellular cytoskeleton. Engagement and clustering of integrins incudes formation of focal adhesions and related structures, which can in turn modulate signaling of a variety of mechanotransductive and canonical mitogenic pathways including through MAPK, PI3K, and the Rho GTPases [25,30,61]. Interestingly, aberrant cell surface adhesion protein expression is found in many CSC populations, including overexpression of the hyaluronan/osteopontin receptor CD44, and integrin subunits α3 and α6 [3,8,62–66]. While there is evidence that enhanced expression of these markers is predictive of tumorigenicity, it remains unclear to what extent alterations in expression of these receptors are primary drivers of tumor progression.

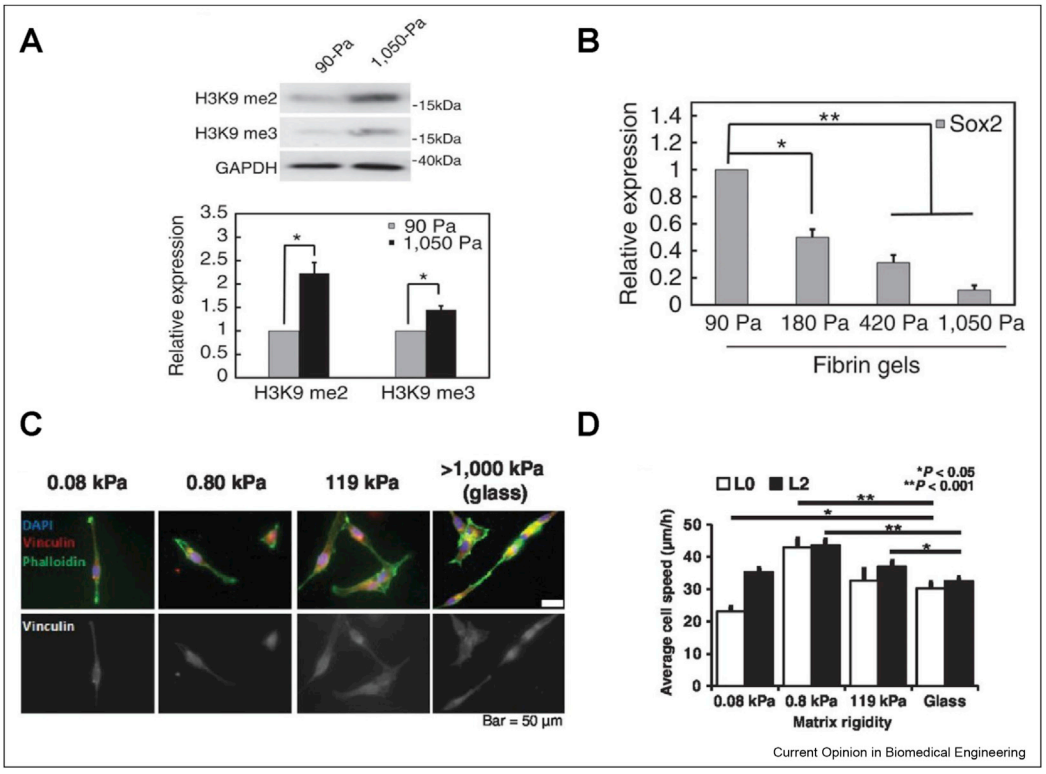
Importantly, integrin signaling is strongly dependent upon the mechanical properties of the ECM, such that stiff ECM environments promote integrin clustering, adhesion assembly, and actomyosin contractility [67,68]. This has motivated interest in the role of ECM stiffness in regulating CSC behavior, which appears to be dependent on tumor type and stage (Fig. 1). For example, tissue hypoxia and matrix stiffening synergistically cooperate to promote the generation of the breast CSC pool through the activation of integrin-linked kinase (ILK) and CD44. Further, suppression of ILK leads to a loss of the CSC phenotype even in the presence of a stiff and hypoxic microenvironment [69]. Conversely, Tan and colleagues revealed that matrix softness plays an important role in CSC maintenance through epigenetic regulation of H3K9 and Sox2 gene expression in melanoma CSCs (Fig. 1A,B) [70]. The authors found that stiff environments decrease Sox2 expression and self-renewal and promote differentiation of CSCs. Our own laboratory has shown that glioblastoma (GBM) CSCs display dysregulated mechanosensing with GBM CSCs exhibiting little change in proliferation, migration, or spreading as a function of ECM stiffness (Fig. 1C,D) [28]. Mechanosensitivity was restored through the constitutive activation of RhoA, which decreased TIC invasive capacity both *in vitro* and an *in vivo* orthotopic xenograft model. Importantly, xenografting with mechanosensitive CSCs extended survival times and decreased tumor size relative to control CSC-based xenografts. These findings suggest that GBM CSCs may evade limitations on migration imposed by soft ECMs to aggressively and efficiently migrate through normal brain tissue, which is highly compliant. These studies highlight the important influence matrix stiffness has on CSC maintenance and behavior and reveal the need for further investigations into these mechanotransductive mechanisms.

Overexpression of integrins and CD44 in CSCs

In an effort to understand the mechanotransductive mechanisms that control CSC maintenance and spread, investigators have studied the consequence of overexpressed cell surface adhesion proteins in the CSC population [14,62,63,71]. As mentioned previously, overexpression of integrin α3 and α6 along with CD44 are present in many types of CSCs [62]. In GBM CSCs, integrin α3 plays a crucial role in the migration and invasion of CSCs. Invasion assays indicate that integrin α3 promotes migration of CSCs in an Erk1/2-dependent manner, and the depletion of integrin α3 via shRNA decreases migratory capacity [63]. Moreover integrin α6 is enriched in glioblastoma CSCs and contributes to CSC tumorigenesis, with α6 knockdown reducing tumorsphere-forming capacity and promoting apoptosis [65].

CD44 has also been under active study as an adhesive regulator of CSC behavior [14,66,71]. CD44 mediates

Fig. 1



CSC mechanotransduction depends on tumor type. The melanoma CSC pool is sustained in soft physical environments; stiff environments increase H3K9 methylation (A) and reduce expression of the stem cell marker Sox2 (B) to promote differentiation and non-stem cancer cells. (C) Glioblastoma CSCs exhibit a loss of mechanosensing with no change in cell spread area and only modest changes in migration speed with matrix stiffness (D). Reproduced with permission from Tan et al. (A,B) [70] from Wong et al. (C,D) [28].

adhesion to a variety of matrix elements, including hyaluronic acid (HA) and osteopontin [72,73]. CD44-HA binding can activate pro-migratory pathways induced by engagement of the CD44 cytoplasmic tail with the actin cytoskeleton through mediators such as ankyrin and the ERM domain-containing proteins [74,75]. Additionally, several studies have linked CD44 to the regulation of Rho GTPases, which modulate cytoskeletal organization and migration [76]. Enrichment of CD44 has been associated with increased invasive capacity and tumorigenesis. For example, lung cancer cells highly expressing CD44 have been reported to form robust spheroid bodies, display enhanced migratory capacity, and exhibit enhanced tumorigenicity *in vivo* [77]. CD44-positive prostate cancer cells were reported to aggressively invade Matrigel and efficiently form tumors when injected into immunocompromised mice [78]. By contrast, CD44-negative cells were non-invasive in Matrigel and formed tumors in only in 40% of cases. Through q-PCR analysis of CD44-positive cells, the authors determined that signaling through the ERM protein Ezrin, which is downstream of CD44 binding, may be associated with invasive behavior of these cells. Collectively, the investigations of cell-matrix adhesion protein function and signaling in CSCs reveals the important role of these proteins in CSC persistence and spread. Additional work is needed to clarify the mechanotransductive components of these interactions and validate their utility as pharmacological targets.

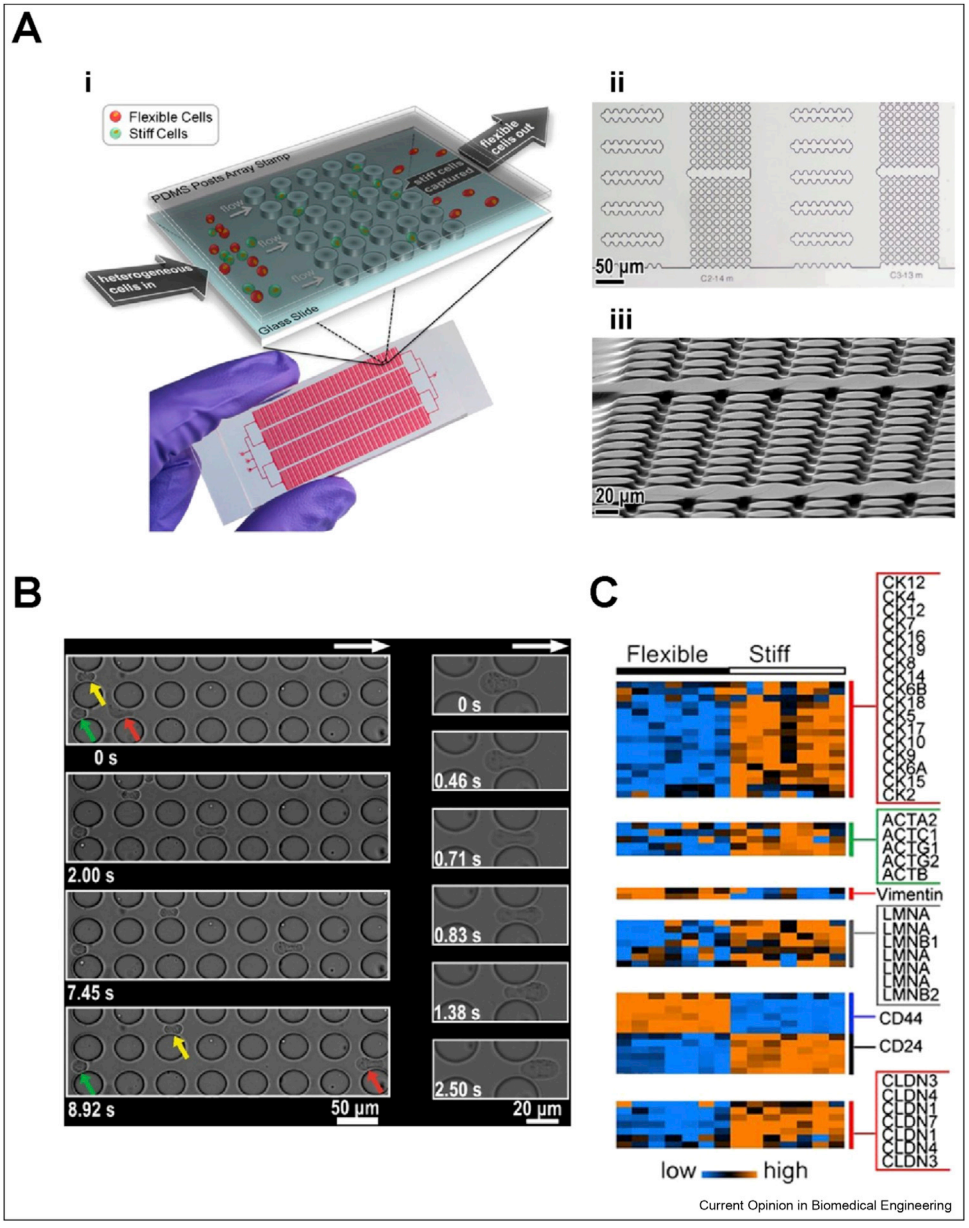
In vitro tools for pharmacological discovery

It has long been hypothesized that deformation of cancer cells can be used as a potential biomarker or predictor of disease status. In breast, prostate, and bladder cancer models, it is established that cancer cells are consistently softer than non-cancerous cells suggesting that cell stiffness is inversely proportional to tumorigenesis and metastatic potential [32,79–81]. This idea is now being extended to CSCs, which are often more deformable than non-CSC cancer cells. For example, ovarian CSCs were found to exhibit 46%, 61%, and 72% increased deformability when compared to late stage, intermediate, and early stage ovarian cancer cells, respectively [81].

These changes in deformability have generated interest in the use of specialized engineering platforms to measure CSC mechanical properties. This in turn has created an opportunity to innovate upon traditional methods for

measuring cellular mechanics, such as atomic force microscopy, micropipette aspiration, and optical tweezers, which are labor-intensive and low throughput [36,82]. Despite the precision of these techniques, their clinical value is limited by their relative inability to rapidly measure cell mechanical properties within large populations. Recently, microfluidic devices have helped usher in a new age of higher-throughput modalities efficient in mechanical characterization of cells [82,83]. One such microfluidic platform can probe single cell deformability at a rapid rate of approximately 2000 cells/second using stretching extensional flow [36]. In this system, cells are fed through a syringe at a set flow rate into a cross-slot chamber that allows for inertial focusing, hydrodynamic stretching, and automated image analysis. This device revealed that undifferentiated cell populations were much more deformable than their differentiated counterparts. Another microfluidic sorting device can separate cells based upon their deformability by combining hydrodynamic force with micrometer-scale flow barriers

Fig. 2



Cell sorting via cell deformability in a microfluidic device. (A) Schematic of microfluidic device, including micropost design for cell separation (i). Optical microscope (ii) and SEM (iii) images show flow channels of microfluidic device. (B) Green, yellow, and red arrows indicate three cells of different deformability flowing through the device with red being the most deformable followed by yellow and green. The most deformable cell is able to traverse a post in under 3 s, while less deformable cells require extended times. (C) Microarray analysis of separated flexible of stiff cells reveal significant changes in mRNA levels of cytokeratins, actins, vimentin, lamins, CD44 and CD24, and claudins. Reproduced with permission from Zhang et al. [32].

(Fig. 2A-B) [32]. Using this system, the authors were able to identify populations of highly metastatic and quiescent cells, which could then be further characterized. Analogous separation tools have been applied to harvest circulating tumor cells and subject them to downstream RNA analysis [84]. Such approaches are being coupled with single-cell sequencing technologies to gain insight into molecular mechanism (Fig. 2C) [85]. These technologies are also actively being deployed in screening format to identify new lead compounds that influence tumor cell deformability [86,87].

While cell deformability has proven to be an enormously valuable metric for describing heterogeneities within a given population, it is important to note that deformability ultimately represents a "snapshot" measurement. In reality, cellular mechanics are highly dynamic and subject to active modulation through cytoskeletal reorganization and contraction. The nature of this parameter is likely to introduce significant variation across measurement approaches and conditions, and so an ongoing challenge in the development of these platforms will be the adoption of more universal and generalizable metrics. Towards this end, we recently introduced an analytical framework that enables extraction of cellular viscoelastic moduli from cross-slot deformability measurements [83]. Important efforts have been underway to create high-throughput approaches to measure other properties, such as active cell contraction [88] and differential adhesion strength [89]. Finally, a new generation of devices is emerging that incorporates stromal elements with increasing sophistication. For example, tumor cell vascular extravasation has been simulated using microfluidic channels seeded with endothelial cells and subjected to fluid flow [90]. Such integrated approaches will provide exciting new avenues for the study of CSCs.

Conclusions

The concept that many solid tumors are initiated and propagated by CSCs represents a major paradigm shift in cancer biology and has motivated the creation of strategies to identify and therapeutically target CSCs. The finding that biophysical signals from the tumor microenvironment can influence CSC behavior raises the exciting possibility that mechanotransductive signaling may be targeted alongside canonical CSC mitogenic pathways. While efforts to do so are in their infancy, much work remains to identify and validate these targets as well as assess generality across multiple tumor types. These efforts will surely be aided by engineered platforms that permit high-throughput analysis of CSC mechanics and invasion in tandem with molecular-scale analyses. As these investments pay off, they should yield new diagnostic and screening tools that should contribute to novel ways of diagnosing, staging, and treating cancer.

Acknowledgements

The authors gratefully acknowledge the support of the NIH (1R21CA174573, 1R01NS074831, 1R21EB016359), the W. M. Keck Foundation, and the California Institute for Regenerative Medicine (RT3-07800).

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- •• of outstanding interest

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