

Mechanobiological Strategies to Augment Cancer Treatment

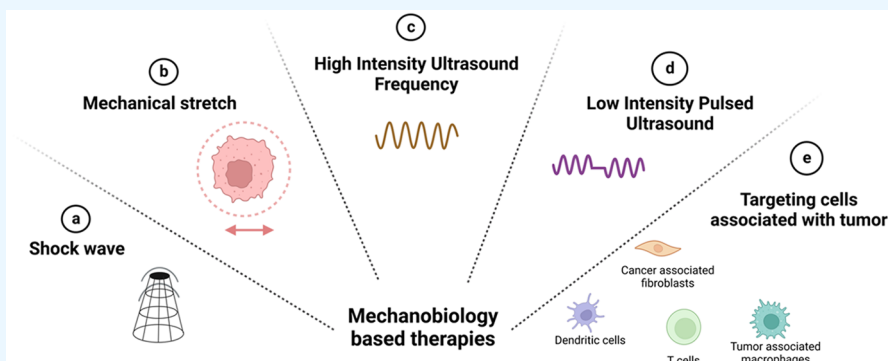
Alka Kumari,[#] S Manasa Veena,[#] Rashmita Luha,[#] and Ajay Tijore*Cite This: *ACS Omega* 2023, 8, 42072–42085

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ABSTRACT: Cancer cells exhibit aberrant extracellular matrix mechanosensing due to the altered expression of mechanosensory cytoskeletal proteins. Such aberrant mechanosensing of the tumor microenvironment (TME) by cancer cells is associated with disease development and progression. In addition, recent studies show that such mechanosensing changes the mechanobiological properties of cells, and in turn cells become susceptible to mechanical perturbations. Due to an increasing understanding of cell biomechanics and cellular machinery, several approaches have emerged to target the mechanobiological properties of cancer cells and cancer-associated cells to inhibit cancer growth and progression. In this Perspective, we summarize the progress in developing mechano-based approaches to target cancer by interfering with the cellular mechanosensing machinery and overall TME.

1. MECHANOBIOLOGY OF THE TUMOR MICROENVIRONMENT

Contemporary cancer research has confirmed that the tumor microenvironment (TME) is a crucial driver of tumor development and progression. On examination, the TME has often displayed unique characteristics, such as a dense extracellular matrix (ECM), increased stiffness, leaky vasculature, and inflammation. These features have been known to be associated with tumor growth.¹ Owing to the dense microenvironment and the fact that the TME harbors other types of cells apart from cancer cells, such as fibroblasts, epithelial cells, immune cells, and stem cells, the cancer cells constantly interact with other cancer cells, cancer-associated cells, and the ECM. This interaction often occurs through mechanical forces generated in the pushing and pulling of the ECM and the surrounding cells. Cancer cells also experience mechanical forces due to the interstitial fluid pressure and vascular flow during invasion and metastasis.

The studies published mainly in the last two decades have demonstrated that these mechanical forces generated in TME contribute to the onset of cancer and its subsequent progression. These forces have been placed in three broad categories: compressive stress, tensile stress, and fluid shear stress (Figure 1).² Compressive stress is the compression

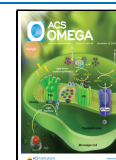
caused in the dense tumor interior due to the indefinite growth of cancer cells at the primary site. Corresponding to this, reports show that a 35–142 mmHg value of mechanical loading on human tumors induces cancer cell proliferation and invasion.^{3–5} Tensile stress is the force generated by the push and pull of the cells on cross-linked ECM fibers. This cross-linked ECM fiber network is developed due to an excessive ECM secretion, mainly by surrounding cancer-associated fibroblasts. Thus, both tensile stress and excessive ECM deposition result in an increase in the tissue stiffness. For instance, the stiffness of breast cancer tissue is in the range of 4–12 kPa, while the stiffness of normal breast tissues is 0.4–2 kPa. Likewise, other tumor tissues, such as the lung, brain, bone, and liver, exhibit higher stiffness than matched normal tissues.⁶ Fluid shear stress is the force exerted by interstitial fluid and blood flow in cancer cells. This mechanical force is a significant cue for cancer progression. Cancer cells migrate

Received: August 29, 2023

Revised: October 17, 2023

Accepted: October 18, 2023

Published: November 3, 2023



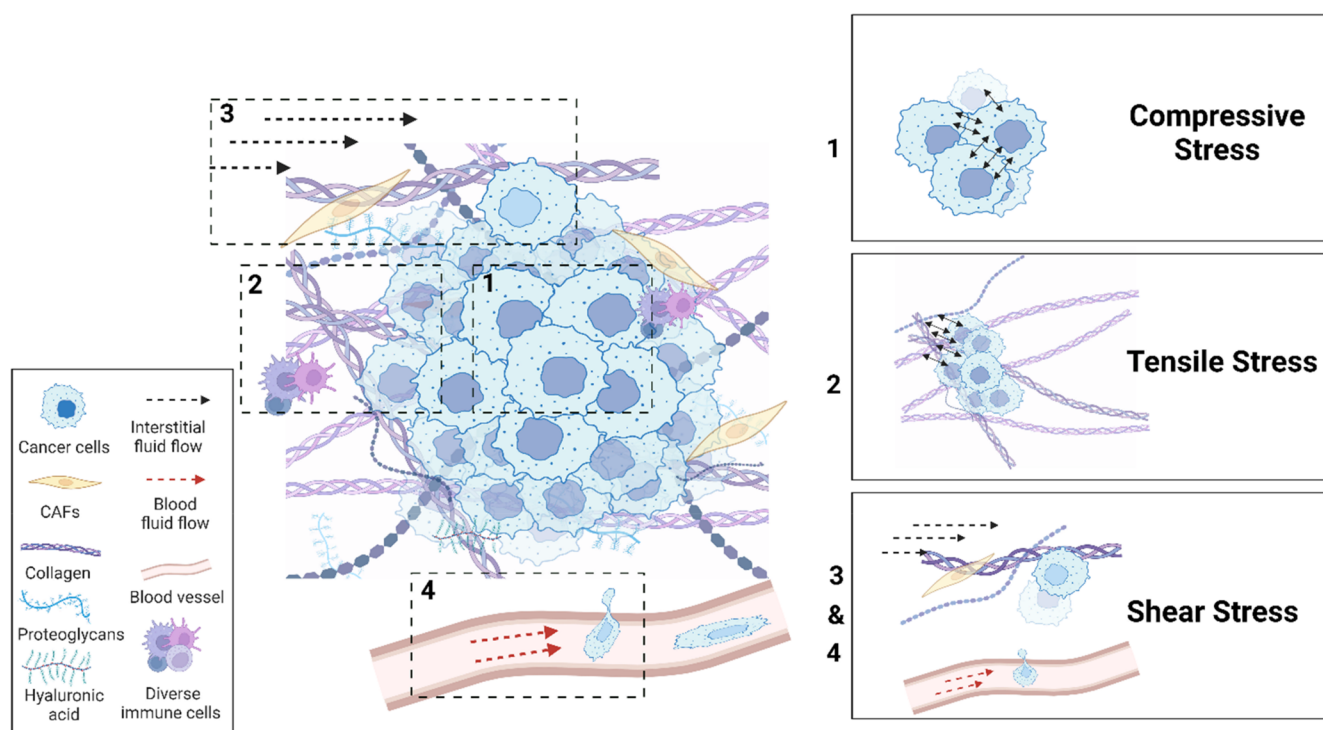


Figure 1. Types of mechanical forces experienced by cancer cells during the beginning stage of cancer and its progression. Compressive forces are generated due to the indefinite growth of cancer cells in the dense tumor interior. The pushing and pulling by the cancer cells on a dense network of cross-linked ECM fibers generates tensile forces. Cancer cells experience fluid-generated shear forces during their migration through interstitial fluid and blood flow.

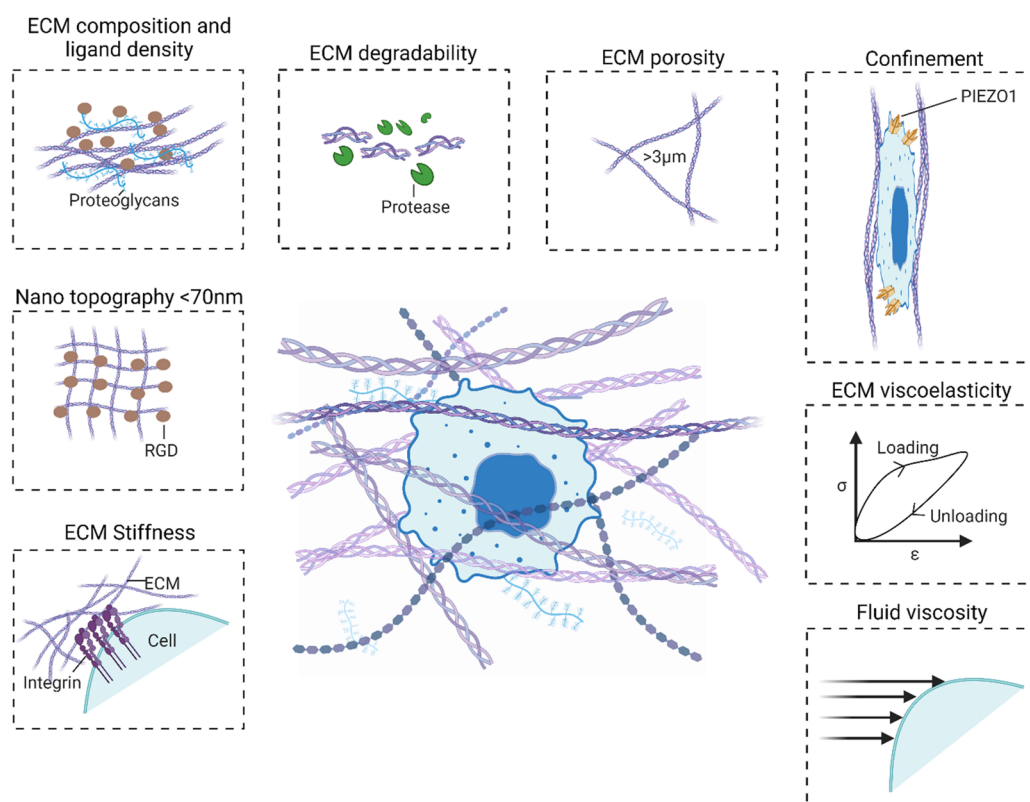


Figure 2. Cancer cell–TME interactions in 3D. Cancer cells are mainly surrounded by a dense network of ECM, cancer-associated cells, and vasculature within the tumor site. Cancer cells sense biomechanical properties of the TME, such as ECM characteristics (stiffness, ligand density, confinement, degradability, porosity, and viscoelasticity), RGD nanospacing, and fluid viscosity, and then activate intracellular signaling pathways, which in turn regulates cancer progression.

through confined tissue interstitial spaces, dense ECM fibers, and blood capillary networks associated with the TME during the invasion process, and the fluid viscosity experienced during migration has been shown to facilitate cancer cell dissemination from the primary site.^{7,8}

2. IMPACT OF THE TUMOR MICROENVIRONMENT ON CANCER CELL BEHAVIOR

In the preceding section, we discussed the major types of mechanical forces and their effects on the ECM. In this section, we focus on how changes in the ECM architecture determine cancer cell behavior (Figure 2).

2.1. ECM Stiffness and Spatial Distribution. Cells sense the ECM stiffness using the transmembrane receptor integrin, which induces downstream cellular changes. Consequently, focal adhesions and membrane protrusions are formed at the cell–substrate interface. This, in turn, leads to the emergence of large lamellipodia and the recruitment of nonmuscle myosin II, which results in directional cancer cell migration on 2D substrates.^{9,10} On the contrary, cancer cell migration through 3D confined collagen channels in the TME suppresses focal adhesion and the formation of lamellipodia.^{11,12} Apart from the ECM stiffness, the spatial molecular arrangement of the ECM also plays a pivotal role in cell adhesion and proliferation. Repetitive binding domains, such as the RGD domain, are present in ECM fibers that provide a binding site to the cell.¹³ Many studies have revealed that this interligand spacing should be less than 70 nm to form stable focal adhesion.¹⁴

TME's stiffness gradients impact tumor progression, metastasis, and drug resistance via durotaxis. Durotaxis refers to cells moving toward an increasing stiffness gradient. There is growing evidence that durotaxis plays a role in tumor cell migration and invasion in the TME. Lu et al. conducted a study demonstrating a higher tendency for tumor cells to invade soft tissues than stiff tissues.¹⁵ The process of durotaxis remains complex, and *in vivo* evidence has yet to be found.¹⁶ Nonetheless, increasing evidence indicates that it may contribute to the migration and invasion of tumor cells within the TME. As such, targeting durotaxis could hold promise as a potential avenue for cancer therapy.

2.2. ECM Density and Degradability. Other characteristics of the ECM that determine the phenotypic fate of cancer cells are the ligand density, degradability, and composition. An ECM with high ligand density and degradability enhances tumor growth, while that with reduced ligand density (adhesivity) and degradability promotes balanced cellular dormancy. These features play a coordinated role with ECM stiffness and induce the invasiveness of cancer cells at the intermediate-stiffness substrate.^{17,18} Adding to this, the size, alignment, spaces, and gaps in the collagen bundle of the ECM determine cancer cell invasion.

2.3. ECM Confinement. Studies have identified that the ECM pore size also controls cancer cell migration. For instance, the HT-1080 fibrosarcoma, a common 3D tumor cell motility model, cannot migrate from less than 3 μm width pores.^{19–21} When the cancer cells move out from the primary site, they migrate through confined spaces in the ECM that are 3–30 μm wide and ~ 600 μm long.²² Physical confinement such as this has been shown to regulate several biomechanical properties of cancer cells. These include the upregulation of mechanosensitive channels (Piezo1), the suppression of focal adhesion, and the disassembly of microtubules. It has also been observed that confined cancer cells are resistant to therapeutics

and display cancer stem cell-like properties.²³ Additionally, physical confinement also affects the migratory behavior of cancer cells. In this regard, it has been noted that confined cancer cell migration depends on microtubule dynamics rather than cell contractility.^{24,25}

2.4. ECM Viscoelasticity and Viscosity. In recent years, the viscoelasticity of the ECM and cancer cells has been found to affect tumor progression. For example, the latest report revealed that ECM viscoelastic properties promote cancer cell proliferation as an added function of ECM stiffness.²⁶ Conversely, cancer cells show more deformability and fewer viscoelastic properties than normal cells, which provide them an edge during the invasion process through a highly dense TME. In fact, different cancer cell states (epithelial and mesenchymal) express different levels of viscoelasticity. Such properties have the potential to become reliable mechanical biomarkers for cancer.

It has been recently observed that the viscosity of the interstitial fluid in the TME influences cancer metastasis. For instance, Konstantopoulos' lab and others have recently shown that the high viscosity of matrix fluid increases the invasion and metastasis of various cancer cell types using 3D microfluidic and *in vivo* assays. Briefly, viscosity enhances the integrin-dependent cell spreading and actin cytoskeleton rearrangement, further enhancing cell migration and mechanosensing properties.^{8,27}

3. MECHANOBIOLOGICAL STRATEGIES TARGETING THE TUMOR MICROENVIRONMENT

3.1. Extracellular Matrix (ECM). As described in the earlier sections, ECM stiffness plays a crucial role in altering the mechanical properties of TME and thus promoting tumor growth, metastasis, drug resistance, and immune system evasion. The expression of ECM proteins is upregulated in tumors, and their cross-linking causes an increase in tissue stiffening. Thus, targeting ECM synthesis, ECM cross-linking, ECM mechanosensors and mechanotransducers, TME cells, and other physical means of ECM degradation has been considered a strategy to alleviate tumor stiffness (Table 1). We have discussed some of these targets and the mechanobiological strategies investigated at various levels.

Different types of cancers exhibit distinct mechanobiological characteristics due to variations in the ECM arrangement, composition, and tumor microenvironment. For example, the stiffness of breast cancer, brain tumor, and pancreatic cancer tissues have been found to be 20,²⁸ 26,²⁹ and 6 kPa,³⁰ respectively. The variations in the mechanotransduction pathways and tumor mechanical properties must be studied to identify cancer-specific targets for mechanobiological-based therapeutic strategies.

3.1.1. Targeting ECM Protein Synthesis and Stiffening. TGF- β is a vital target to curb ECM stiffening due to the versatility of its function. TGF- β signaling activates collagen synthesis (a significant contributor to ECM stiffening), heat shock protein 47 (a collagen chaperone that promotes collagen folding and organization), and lysyl oxidase enzyme LOX (causes collagen-elastin cross-linking). TGF- β contributes to cancer progression by promoting epithelial–mesenchymal transition (EMT) and mesenchymal–epithelial transition (MET).⁸⁰ In addition, TGF- β promotes T-reg cell differentiation to induce immunosuppression in the TME.⁸¹ LH2 and FKBP10 also contribute to ECM cross-linking. TGF- β is pro-apoptotic in the initial stages of cancer, while in the later

Table 1. Existing Mechanobiological-Based Strategies for Cancer Treatment

aim	targeting	targets	targeting agents	stage of clinical trials and cancer type
ECM stiffness reduction	collagen production	TGF- β	fresolimumab (NCT01401062), (NCT02581787)	I/II, metastatic breast cancer, early-stage non-small cell lung cancer ⁴¹
	halofuginone			animal models of pancreatic, lung, melanoma, and breast cancer ^{30–35}
	LY-2109761			<i>in vitro</i> (liver metastasis of colon cancer, pancreatic cancer metastasis), an animal model (breast cancer bone metastasis) ^{36–38}
	AS1409	extra domain B (EDB)		I, malignant melanoma or renal cell carcinoma ³⁹
	pirfenidone	HSP7		<i>in vitro</i> , lung fibrosis ⁴⁰
	ND-L02-s0201 (NCT03241264)			I, fibrosis ⁴¹
	PXS-5505 (NCT04676529)	Pan LOX, LOX, LOXL2		II, myelofibrosis ⁴²
	sintuzumab (NCT01472198), (NCT01479465)			II, pancreatic adenocarcinoma, ⁴³ colorectal adenocarcinoma ⁴⁴
	low power of Pulse-HIFU (20 W/cm ²)	collagen		Animal model ⁴⁵
	cilengitide (NCT00093964)	integrins		III, glioblastoma ⁴⁶
	ATN-161 (NCT00352313)			II, malignant glioma ⁴⁷
	anti- $\alpha v \beta_3$ antibody etaracizumab (MEDI-522),			I/II, metastatic melanoma, renal cell, prostate cancer, lymphoma, small intestine cancer, colorectal cancer ^{48,49}
	anti- $\alpha v \beta_1$ integrin antibody volociximab			II, metastatic pancreatic cancer, ovarian cancer peritoneal neoplasms, melanoma ⁵⁰
	anti- αv antibody intetumumab (NCT00246012), (NCT00537381)			II, melanoma, ⁵¹ prostate cancer ⁵²
	anti- αv antibody Abituzumab (NCT01008475, NCT01360840)			II, colorectal cancer, ⁵³ prostate cancer ⁵⁴
	low-frequency LIPU	Piezo1		Breast cancer cells, malignant melanoma, breast epithelial cell ⁵⁵
	GSK2798745 (NCT02119260)	TRPV4		II, healthy subjects and patients ⁵⁶
	IONS37 (anti-YAP DNA antisense oligonucleotide) (NCT04659096)	YAP/TAZ		I, advanced solid tumors ⁵⁷
	IAG933(NCT04857372)			I, ongoing mesothelioma and other solid tumors ⁵⁸
	verteporfin (NCT04590664, (NCT03067051, NCT03033225)			I, ongoing mesothelioma and other solid tumors ^{59–61}
	high-frequency LIPU	nuclear mechano-transduction		I/II, glioblastoma, prostate cancer, pancreatic cancer ^{59–61}
to utilize mismatching of the mechano-phenotype of cancerous and normal cells				<i>in vitro</i> , breast carcinoma and a malignant melanoma, ⁶² mice cervical cancer (HeLa cell) ⁶³
	shock wave therapy			<i>in vitro</i> , bladder cancer cells and prostate cancer cell, ⁶⁴ human renal epithelial, cancer cell, ⁶⁵ hamster melanomas ⁶⁶
	mechanical stretch therapy			<i>in vitro and in vivo</i> , breast cancer cells, ^{67,68} p53PTEN ^{-/-} mice breast cancer model ⁶⁹
	low-frequency LIPU			<i>in vitro and in vivo</i> , breast cancer cells, chick embryo grafted tumors, ⁶⁸ murine mammary sarcoma and murine mammary sarcoma, ⁷⁰ Hecat, and Cal33, <i>in vivo</i> mice injected with Cal33 HNSCC cell line ⁷¹
depletion of blood flow to the tumor	tumor blood vessels			animal models, hepatocellular carcinoma, ⁷² rabbit ⁷³
	dendritic cells			<i>in vitro</i> , primary effector CD4 ⁺ T-cells obtained from TCR-transgenic OT-II mice, ⁷⁴ primary human CD4 ⁺ T-cells, ⁷⁵ human breast cancer sample ⁷⁶
	TAM			<i>in vitro</i> , murine prostate cancer cells (RM-1) and bone marrow derived DCs from BALB/c mice ⁷⁷
	ultrasound-targeted nanobubble destruction (UTND)			<i>in vitro</i> , mouse macrophage cell line RAW264.7 (M0) and Lewis lung carcinoma (LLC) cell line ⁷⁸
	shock wave therapy			<i>in vitro</i> , CAFs extracted from tumors of prostate cancer patients ⁷⁹

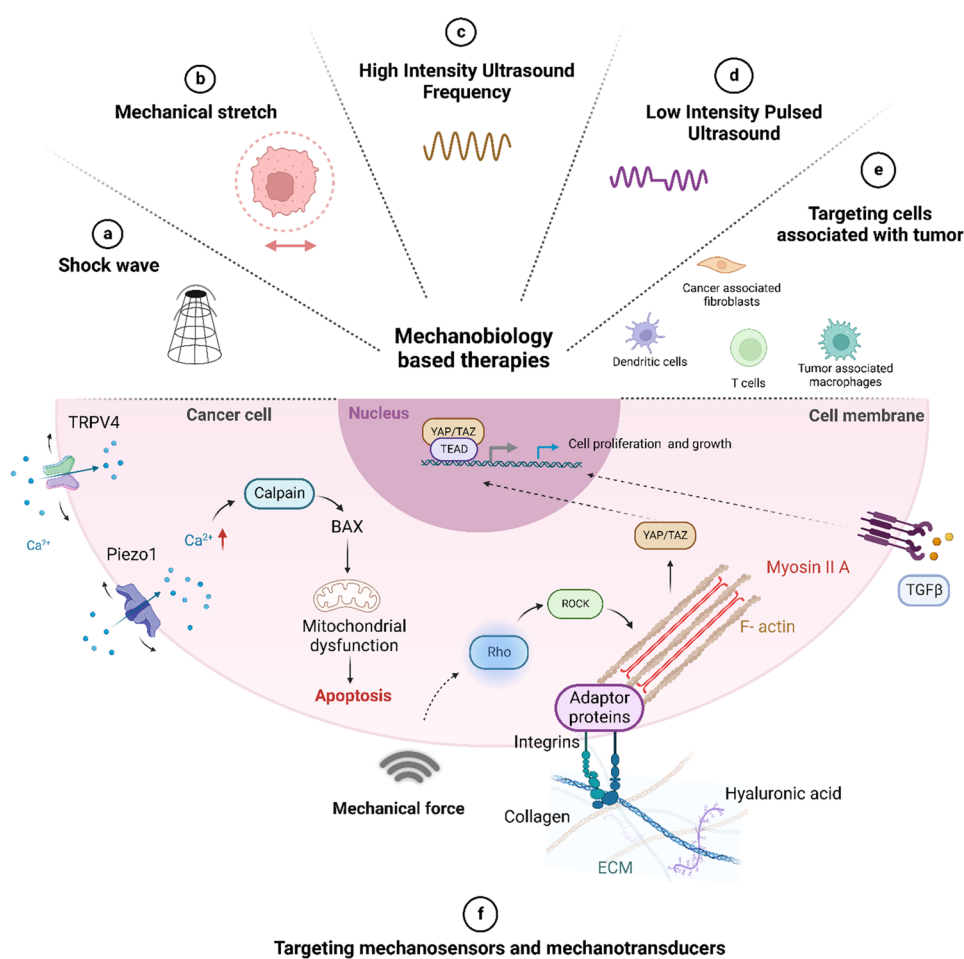


Figure 3. Schematic representation of mechanobiology-based therapies. Targeting cancer cells by mechanical perturbations like (a) shock wave,^{65,79} (b) mechanical stretch,^{151,152,160} (c) high-intensity ultrasound frequency,^{45,75,136,138,139} (d) and low-intensity pulsed ultrasound.^{55,68,70,71} (e) Targeting cells associated with tumor-associated T-cells,^{114,119} dendritic cells,^{130,132,138} tumor-associated macrophages,¹⁴⁹ and cancer-associated fibroblasts.⁷⁹ (f) Targeting mechanosensitive channels, mechanosensors, and mechanotransducers of the cancer cell, including TRPV4,^{55,89} Piezo1,^{55,68} Rho/ROCK,^{10,55} YAP/TAZ,^{56–59} and TGF- β .^{33,35,40,81}

stages it shows an oncogenic function.⁸² TGF- β R is down-regulated in many cancers;^{83,84} thus, the proapoptotic pathways are not activated. Hence, targeting TGF- β and its receptor should be wisely done depending on the cancer stage and considering its diverse roles. A clinical trial (NCT01401062) to study the efficiency and feasibility of a monoclonal antibody fresolimumab (GC1008) to block TGF- β has shown a longer median overall survival at higher doses with an increased level of tumor-specific CD8 T-cells.³¹ Another monoclonal antibody (NCT02581787)⁸⁵ targeting TGF- β in non-small cell lung cancer patients is currently under clinical trials. Halofuginone (anticoccidial drug) inhibits TGF- β signaling and decreases collagen synthesis in cancer animal models.³² LOX inhibitors such as pifrenidone and ND-L02-s0201 injection (a lipid nanoparticle containing siRNA against Hsp47) demonstrated antifibrotic activity in lung and hepatic fibrosis, respectively. PXS-5505, a pan-LOX inhibitor, was found to be safe in clinical trials.⁴²

Along with collagen, fibronectin is also up-regulated in many types of cancer and contributes to increased stiffness. Extra domain A and additional domain B (EDB) of fibronectin were found to be upregulated in tumors.^{86–88} Hence, EDB has been targeted in several strategies. A clinical trial of huBC-1-mIL-12 (a murine monoclonal antibody against the cryptic domain

adjacent to human fibronectin EDB, BC-1 was fused with murine IL12) showed \sim 46% of patients had a stable condition after at least six cycles of treatments.³⁹ Another antibody targeting EDB, L19, was fused with IL-2 (L19-IL-2) and significantly improved the tumor inhibitory efficiency of IL-2 in renal cell carcinoma and melanoma patients showing stable conditions without treatment-related death during its clinical trial.⁸⁹

3.1.2. Physical Disruption of the ECM. Increased production of ECM proteins promotes chemoresistance by inhibiting the entry of drug molecules into the TME. Several strategies have been exploited to disrupt collagen, enhancing drug penetration into the TME. Cancer invasion and migration are promoted by various ECM properties mentioned in the above sections, leading to the degradation of ECM and cell–ECM and cell–cell interactions. Cancer cells secrete matrix metalloproteinases (MMPs) that work in cascades and activate other MMPs in the process, rendering proteolytic activity. Mechanical forces are known to modulate the levels of MMPs that are usually regulated at moderate mechanical loading.⁹⁰ For example, it has been shown that MMP-14, involved in pancreatic cancer metastasis, is mechanically regulated to further induce other MMPs to act in action. Hence, particular

MMPs involved in a cancer type must be known to target them for therapeutic purposes.⁹¹

Apart from MMPs and collagenase enzymes, pulsed high-intensity focused ultrasound (HIFU) is a promising strategy to degrade ECM by ablation. An animal model study with A549 tumor tissues has shown ECM structural remodeling upon treatment with a low power of Pulse-HIFU (20 W/cm²), leading to decreased collagen fibers, increased blood flow, and enhanced penetration of nanoparticles without acute tissue damage.⁴⁵

3.1.3. Targeting Mechanosensory Proteins and Mechano-transducers of ECM Stiffness. In the past, efforts have been made to target mechanosensory proteins such as integrins, mechanosensitive channels (Piezo and TRPV4), and Rho-ROCK pathways (Figure 3). Integrin is an important receptor that mediates tumor progression effects of ECM stiffness. Although targeting integrin seemed to be promising in ECM stiffness-related tumor-promoting pathways, and despite the positive outcomes in preclinical trials with integrin inhibitor, Cilengitide, several other antibodies, such as ATN-161, anti- $\alpha_v\beta_3$ antibody (etaracizumab), anti- $\alpha_3\beta_1$ antibody (volociximab), and anti- α_v antibodies (intetumumab and abituzumab), failed in the clinical trials.^{42,92}

TRPV4, another mechanosensitive ion channel, contributes to tumor progression. Several antagonists have been developed in recent years,^{93,94} of which GSK2798745 is the first TRPV4 blocker in clinical trial studies and was tolerated by patients in the early phases.⁵⁶ YAP and TAZ are transcription coactivators activated by mechanical stress and other types of signals that act as signal transducers to the nucleus. The constitutive activation of YAP and TAZ was responsible for the uncontrolled growth of tumors. Cancer progression is promoted by YAP/TAZ in multiple ways through malignancy, metastasis, and chemoresistance. Although YAP and TAZ are undruggable molecules,⁹⁵ RNA interference methods reduce their expression. ION537, an anti-YAP DNA antisense oligonucleotide, inhibits YAP expression and suppresses the growth of tumor xenografts.⁹⁶ Verteporfin restrains YAP/TAZ binding to TEADs, which reduces YAP/TAZ-induced transcription and activity;⁹⁷ however, its cytotoxic effects may be YAP-independent.^{98,99} Both compounds in phase I clinical trials (NCT04659096 and NCT04665206).

3.2. Cancer Cells. Cancer cells vary in their biomechanical properties compared to normal cells, such as stiffness, contractility, and mechanosensing, where the nucleus contributes to higher degrees of alteration in these properties, as reviewed by Liu et al.¹⁰⁰ Such mismatched mechanical properties of cancer cells help cells during tissue invasion. The ECM confinement experienced during cancer invasion also plays a significant role in the alteration of mechanical properties of the cells, which includes a reorganization of the cytoskeleton component of the cells by up-regulating CXCR2 chemokine receptor and protein kinase 2. Confinement also promotes myosin II activity by vertical compression and F-actin and myosin II localization at the back of the cell cortex. The activity and location of myosin II are influenced by forces experienced by the cell, which is crucial for facilitating the mesenchymal–amoeboid transition.¹⁰¹ Confinement also affects nuclear size, integrity, and cell motility by disrupting nuclear flux homeostasis through an RhoA-dependent pathway. This hindrance leads to volume expansion and blebbing.¹⁰²

Interestingly, similar altered mechanical properties of cancer cells are targeted in several mechanotherapy strategies to kill cancer cells selectively. For example, different modes of ultrasound have been used to target cancer cells (Figure 3). Due to the added advantage of its noninvasiveness and ability to penetrate deep tissues, ultrasound has been widely explored for developing cancer mechanotherapy. High-frequency low-intensity pulsed ultrasound (LIPUS) has been found to selectively ablate melanoma and breast cancer cells by triggering the intracellular explosion of nanobubbles with negligible damage to normal cells.⁶² Low-frequency LIPUS has been found to produce nonthermal mechanical effects,^{103,104} such as stretch, compression, and shear stress,^{105,106} through cavitation and acoustic streaming. *In vitro* studies using low-frequency LIPUS (33 kHz, ISPTA = 7.7 mW/cm²) on breast cancer cells, melanoma cells, and breast epithelial cells exhibited a significant increase in the death rate of cancer cells (52%) compared to the normal cells 18% with the application of ultrasound.⁵⁵ Mouse cervical cancer models have shown an increase in survival rate to 52% from 16% in the control upon the use of therapeutic ultrasound irradiation and folate-conjugated nanobubbles.⁶³ On the contrary, the high-intensity pulsed ultrasound (HIPUS) showed nonselective thermal ablation of cancer and normal cells.

Shock wave therapy has also shown potential by killing 30–50% of bladder and prostate cancer cells and 10% of normal cells.⁶⁴ Other studies found smaller deformation and more damage to cancer cells than normal cell types after the shock wave treatment.⁶⁵ Surprisingly, a similar treatment caused over 90% reduction of the local melanoma tumors in hamsters.⁶⁶

Recent studies found that mechanical stretching caused the selective apoptosis of many cancer cell types under cyclic stretching (5% strain and 0.5 Hz amplitude). The molecular mechanistic studies showed that mechanical stretching activates Piezo1 channels, causing an influx of calcium ions and subsequently activating the calpain-dependent apoptotic pathway.⁶⁸ The authors termed such mechanical-force-induced cancer cell apoptosis as mechanoptosis.

3.3. Immune Cells. Although recent developments in cancer immunotherapy have yielded promising results, many solid tumors have failed to respond effectively due to low immunogenicity, lack of universal tumor-specific antigens, poor infiltration of immune cells, and immunosuppressive effects of the TME.^{107–110} Investigating the underlying mechanical characteristics of cancer cells, the TME, and immune cells that can be used as a treatment strategy is essential given the growing understanding of the significance of mechanical forces in cancer and the immunological response. Thus, conditioning immune cells using mechanical forces is covered in this section.

3.3.1. T-Cells. The ability of T-cells to recognize and destroy cancer cells has been extensively used in cancer immunotherapy. T-cell dysfunction brought on by tumor-induced immunosuppression limits the immunological response in the TME. The mechanobiology of T-cells, which is becoming better understood, strongly suggests that these cells can detect and respond to mechanical stimuli that modify their behavior. As a result, directing mechanical forces to modulate T-cell function may represent a new immunotherapy approach.¹¹¹

T-cell mechanics plays a critical role during cognate peptide presentation by APCs to the T-cell receptor (TCR), immunological synapse formation, and downstream transmission of the mechanical signals.^{112–114} The T-cell generates force through the TCR via cytoskeletal rearrangement,

modulating the T-cell activation. Interestingly, providing external cyclical forces to dysfunctional T-cells (that cannot generate their forces) could reactivate these T-cells.^{112,114} During immunological synapse formation, the mechanosensor channels Piezo1 sense a vertical mechanical force in the form of a cell membrane stretch. The downstream signaling involves calpain activation and actin assembly organization, thereby generating an optimal T-cell activation. It has been shown that Piezo1-deficient T-cells failed to achieve optimal TCR activation.¹¹⁵ These studies provide insight into mechanical forces and a mechanosensor as an immune checkpoint for cancer immunotherapy.

T-cell migration requires mechanosensitive cytoskeletal elements, including actin filaments and microtubules, other T-cell features that are essential for immune response. Microtubule stability controls the transition between the amoeboid and mesenchymal modes of T-cell migration. According to studies, unstable microtubules cause contractility by the Rho-dependent pathway, which improves T-cell migration on an ICAM1-nanotextured surface.¹¹⁶ On the other hand, T-cell migration *ex vivo* in 3D collagen matrices is inhibited by microtubule-stabilizing drugs. Mechanosensitive cytoskeletal components offer a unique platform to optimize T-cells for efficient navigation through the TME.

Hydrogels that resemble the body's ECM are another intriguing material for T-cell-based immunotherapy. Hickey et al. investigated the role of an artificial T-cell stimulating matrix (aTM). The studies showed that adjusting aTM's stiffness can modulate T-cell activation. These studies suggested promising results to enhance the effectiveness of T-cell treatment *in vitro* and extend to *ex vivo* T-cell stimulation that can be injected into mice for effective tumor suppression.¹¹⁷ Recently, nanoparticles coated with engineered T-cell membranes (with specific receptors against targeted cells) have been employed in a murine model to eliminate glioblastoma and glioblastoma stem cells using photothermal therapy. The surface membrane on the NPs helped in penetrating through the blood–brain barrier.¹¹⁸ Other studies have demonstrated the optogenetic activation of bulk immune cells, including T-cells and dendritic cells, using wide-field illumination and calcium actuators.^{119,120}

The spatiotemporal control of T-cells in the tumor region is crucial for effective cancer therapy. Recently, mechanogenetically engineered CAR-T systems have been mechanically sensitized to guide and control CAR-T cell activities spatiotemporally.^{121–123} Thermal induction of CAR-T cells by focused ultrasound showed a significant reduction in the tumor growth rate in mice with prostate cancer.¹²⁴ The nonthermal induction of CAR-T cells using ultrasound waves is through the mechanosensitive Piezo1 channel.^{125,126} The mechanical signal transduces via the calcineurin-mediated nuclear factor of activated T-cells (NFAT) transcription factor, which subsequently transcribes anti-CD19 CAR genes in T-cells.¹²⁷ It has been envisioned that these mechanogenetic CAR-T systems will be the future generation tool for cancer treatment, which wireless devices can control.¹²⁷ Although ultrasound is a promising option in CAR-T therapy, further research and technological advancements are needed for clinical use. Overall, an in-depth understanding of the T-cells' mechanobiology and mechanosensitive machinery can give insight into novel strategies for cancer treatment by overcoming the present limitation in the TME.

3.3.2. Dendritic Cells (DCs). Most research on cancer immunobiology focuses on T-cells' actin and actin-binding proteins. However, cytoskeletal remodeling in APCs (DCs and macrophages) also plays a role in antigen presentation and immunological synapse formation, which are crucial for effective immune response.^{128–131} In bone-marrow-derived macrophages, Jönsson et al. demonstrated that the actin-binding protein *n*-cofilin (F-actin depolymerizing factor) regulates various immune response-related events. In addition to mediating macrophage motility and cell polarity, *n*-cofilin-mediated F-actin remodeling is essential for T-cell antigen presentation.¹³¹

DCs, which are potent antigen-presenting cells, greatly aid primary T-cell activation. On DCs, a structure known as a podosome that is rich in actin senses the substrate rigidity. It has been demonstrated that podosomes apply mechanical stresses and locally break down the ECM at substrate-soft areas (low physical resistance). When the pore size is $>1 \mu\text{m}$, podosomes develop into a protruding structure where pathogen recognition receptors (PRR) occupy the region and mediate active antigen uptake, followed by antigen presentation to T-cells.¹³² An effective immune system depends on the navigation of these cells across the endothelium and ECM cells. A greater infiltration of DCs and other immune cells at the TME may be facilitated by reducing the ECM stiffness.

Activated DCs release effector molecules that kill cancer cells; however, in the TME, immunosuppressive cytokines co-opt dendritic cells to withstand cancer. Therefore, one promising strategy is to promote dendritic cell (DC) activation by utilizing mechanical forces. It has been observed that after HIFU therapy on breast cancer, a significant amount of tumor debris and heat shock proteins accumulate *in situ*. This debris not only induces local infiltration of activated DCs but also triggers more significant lymphocyte proliferation.^{76,133,134} HIFU therapy has been clinically used to treat cancer.¹³⁵ However, HIFU has been linked to tissue necrosis near the tumor due to heat dissipation.¹³⁶ Therefore, research into low-frequency ultrasound is being investigated. In a study focusing on angiogenesis and DC-related immunotherapy, low-frequency ultrasound combined with microbubbles in murine prostate cancer cells reduced VEGF expression and significant DC differentiation, activating T-cell mediated immune response *in vitro*.¹³⁷ Thus, DCs are potent targets for cancer immunotherapy. However, further studies are necessary to comprehensively understand mechanical force sensing, induction, and transmission in DCs.

4. TUMOR-ASSOCIATED MACROPHAGES (TAMS)

Various mechanical signals have been demonstrated to control macrophage polarization and determine their antitumor (M1) or protumor (M2) nature. For instance, reduced actin polymerization brought on by spatial confinement and decreased substrate stiffness causes macrophages to change from the M1 to the M2 phenotype.^{138–140} YAP detects the substrate stiffness and causes the macrophages in the TME to exhibit an antitumor M2 phenotype.^{141,142} Thus, changing the phenotype of M2 macrophages to M1 is beneficial, as these cells are responsible for building an immunosuppressive environment for the cancer cells. Inhibitors of class IIa histone deacetylase (HDAC) in breast cancer patients^{143,144} and both IL-12 and IL-37 in hepatocellular carcinoma patients were able to reverse the phenotype of

TAMs in TME. The FAK/NF- κ B signaling pathway, as demonstrated by Shan et al., facilitates the M1 polarization of macrophages upon mechanical stretch.^{145,146} TAMs are more effectively reprogrammed from the M2 to the M1 (antitumor) phenotype *in vitro* when ultrasound-targeted nanobubble destruction and nanobubbles containing drugs (low molecular weight hyaluronic acid) are combined. The potential cause of the enhanced reeducation effect on UTND may be attributed to oxidative stress.⁷⁸ In addition, studies showed that TAMs also interfere with immune checkpoint inhibitors; as a result, the therapeutic targeting of TAMs may be more advantageous for immunotherapy. Their mechanobiological aspects need further focus in this regard.

4.1. Cancer-Associated Fibroblasts (CAFs). Cancer-associated fibroblasts (CAFs) are heterogeneous fibroblast populations with diverse origins.¹⁴⁷ These are found in the tumor stroma and help in tumor progression and drug resistance. CAFs play a crucial role in cancer development by directly exerting mechanical forces. These forces are transferred by a cadherin-composed adhesion structure that bridges the CAF with the cell membrane of cancer cells.¹⁴⁸ CAFs exert a compressive force on the cancer cells that activate YAP-mediated cancer cell proliferation.¹⁴⁹ CAFs also play a significant role in drug resistance in several cancer types through mechanisms including immunological regulation, cancer metabolism, desmoplasia, and neo-angiogenesis.^{150–154} It has been well documented that the increased ECM stiffness brought on by CAFs presents a considerable barrier to immune cell migration.¹⁵⁵ Therefore, researchers are focusing on preventing CAF-induced ECM-stiffening events.

Zhang et al. demonstrated the feed-forward cycle between a stiff ECM and CAFs. During the stage of cancer, the ECM stiffness is maintained by increasing the activity of the mechanical responsive transcriptional regulator SNAIL1 in the CAFs. Depletion of SNAIL1 resulted in altered CAF function, including tumor fibrosis.¹⁵⁶ Moreover, the mechanics of CAFs in blood vessel growth in the TME have recently been explored.^{157,158} CAF generates mechanical forces that induce large deformations in the ECM, subsequently causing neo-angiogenesis in the TME. Sewell-Loftin et al. showed the involvement of Rho-ROCK and YAP signaling in the CAF-mediated neo-angiogenesis, providing insights into targeting these signaling pathways for anticancer therapeutics.¹⁵⁷ These studies suggest that targeting the mechanosensitive factors and pathways in CAFs could result in better therapeutic outcomes. *In vitro* studies of prostrate CAFs showed that subjecting patient-derived CAFs to extracorporeal shock waves (ESWs), a form of acoustic waves, reduced the gene expression and protein level of mesenchymal markers of CAFs α -smooth muscle actin and type I collagen. Lower levels of these markers consequently reduced the growth of prostate cancer epithelial cells.¹⁵⁹ Thus, targeting CAFs may improve treatment outcomes given the prevalence of CAFs and pro-tumorigenic role.

5. CONCLUSION AND OUTLOOK

It has become increasingly clear that biophysical properties of the TME, including ECM characteristics, play a dominant role in cancer onset and progression. For example, increased ECM stiffness is associated with cancer progression and is widely used for breast cancer detection. Mechanical forces generated in the TME due to cell–cell or cell–ECM interaction, mainly compressive, tensile, and shear forces, also contribute to cancer

progression. Thus, targeting stiffness and biophysical forces associated with the TME by targeting ECM has been considered in several preclinical/clinical studies. In recent years, several mechanical-force-based strategies have emerged that target the mechanosensory machinery of the cancer cells and cancer-associated cells to induce selective killing (mechanoptosis). Although these mechano-based treatments look promising due to their selectivity in killing cancer cells over normal cells, comprehensive knowledge of the molecular mechanism of the killing is not yet fully understood. Further studies in this direction will help to harness the mechanical forces to develop a mechano-based treatment for personalized use.

■ AUTHOR INFORMATION

Corresponding Author

Ajay Tijore – Department of Bioengineering, Indian Institute of Science, Bangalore, Karnataka 560012, India;
orcid.org/0000-0002-0803-2168; Email: ajaytijore@iisc.ac.in

Authors

Alka Kumari – Department of Bioengineering, Indian Institute of Science, Bangalore, Karnataka 560012, India
S Manasa Veena – Department of Bioengineering, Indian Institute of Science, Bangalore, Karnataka 560012, India
Rashmita Luha – Department of Bioengineering, Indian Institute of Science, Bangalore, Karnataka 560012, India

Complete contact information is available at:

<https://pubs.acs.org/10.1021/acsomega.3c06451>

Author Contributions

#A.K., S.M.V., and R.L. contributed equally.

Notes

The authors declare no competing financial interest.

Biographies

Alka Kumari is a graduate student in the Department of Bioengineering at the Indian Institute of Science, Bangalore. She works at the department in Dr. Ajay Tijore's "Mechanobiologics lab". Her research focuses on developing PDMS elastomer-based microfluidic platforms to study cancer cell growth under mechanical force treatment.

S Manasa Veena is a graduate student in the Department of Bioengineering at the Indian Institute of Science, Bangalore. She works at the department in Dr. Ajay Tijore's "Mechanobiologics lab". Her research focuses on developing nanopatterned platforms to study cancer cell growth under mechanical force treatment.

Rashmita Luha is a graduate student in the Department of Bioengineering at the Indian Institute of Science, Bangalore. She works at the department in Dr. Ajay Tijore's "Mechanobiologics lab". Her research studies the effect of ultrasound-mediated mechanical forces on patient-derived oral cancer cell survival.

Ajay Tijore received a Ph.D. in stem cell bioengineering from Nanyang Technological University, Singapore, in 2016. He then joined Michael Sheetz's lab at the Mechanobiology Institute, Singapore, as a postdoctoral research fellow to study in the field of cancer mechanobiology. In November 2021, he joined the Department of Bioengineering, where his lab works on investigating the effect of mechanical forces on cancer cell growth and regulating stem cell fate using custom-built microfluidic devices and micro/nanoscale biomaterials.

ACKNOWLEDGMENTS

We acknowledge the Science Engineering Research Board (SERB), India, for funding. A.T. acknowledges support by Dr. Kiran Mazumdar-Shaw, Chairperson and Managing Director, Biocon Limited, Bangalore, for supporting the R.L. Mazumdar Young Investigator position.

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