

Review Article

Fluid shear stress and tumor metastasis

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Abstract: The tumor microenvironment (TME) is a key factor regulating tumor cell invasion and metastasis. The effects of biochemical factors such as stromal cells, immune cells, and cytokines have been previously investigated. Owing to restrictions by the natural barrier between physical and biochemical disciplines, the role of physical factors in tumorigenesis is unclear. However, with the emergence of interdisciplinary mechanobiology and continuous advancements therein in the past 30 years, studies on the effect of physical properties such as hardness or shear stress on tumorigenesis and tumor progression are constantly renewing our understanding of mechanotransduction mechanisms. Shear stress, induced by liquid flow, is known to actively participate in proliferation, apoptosis, invasion, and metastasis of tumor cells. The present review discusses the progress and achievements in studies on tumor fluid microenvironment in recent years, especially fluid shear stress, on tumor metastasis, and presents directions for future study.

Keywords: Fluid shear stress, metastatic cascade, tumor microenvironment, mechanotransduction, circulating tumor cells

Introduction

Metastasis is a complex dynamic cascade, accounting for approximately 90% of tumor-related mortalities [1]. Previous studies have focused on the effects of biochemical factors, such as stromal cells, immune cells, and cytokines, on tumor metastasis. However, during metastasis, tumor cells also interact with various biochemical and biophysical factors in the tumor microenvironment (TME). Therefore, it is essential to elucidate the dynamic response of tumor cells to different physical and chemical factors in the TME.

In 2015, a landmark study reported that long-term 1-kPa magnetic load in intestinal crypts can upregulate the oncogene c-Myc and lead to carcinogenesis, which indicates that a simple physical process can induce tumorigenesis [2]. Moreover, the biophysical characteristics of tumor cells have been gradually unveiled with developments in biomechanics for approximately 60 years [3]. Biomechanics refers to the study of the deformation and movement of living bodies and validates the laws of mechanics in life. In the 1990s, with the emerging

mechanics tools such as atomic force microscopy and Förster resonance energy transfer (FRET), physicists shifted their focus on biomechanics from the tissue level to the cellular or gene level, and subsequently gradually shifted from biomechanics to mechanobiology. During this shift, a series of mechanosensitive molecules such as Cav-1, BMP, IGF-2, VEGF [4, 5], and nuclear transcription factors YAP/TAZ [6], c-Myc [2], and Atoh8 [7] were identified, which play an important role in tumorigenesis or tumor progression [8, 9]. This aspect triggered a widespread concern among oncologists, especially in the past 4 years, and numerous studies then focused on the mechanobiological mechanism of tumorigenesis and metastasis.

As a classic mechanical feature, matrix hardness has been considered a peculiar mechanical feature in predicting tumor metastasis and prognosis [10, 11]. Wei *et al.* confirmed that matrix hardness can activate the TWIST1-G3BP2 pathway to promote tumor cell invasion and metastasis [12]. Nonetheless, the flow of biological fluids is a vital physical property of the TME; however, owing to continuous changes in the parameters including flow diameter and

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Table 1. Tools for the study of fluid mechanics of cancer

Tool or technique	Application	Refs	
Mechanical measurement	Microfluidic traction force microscopy	Observe the traction and shear deformation of cells under fluid environment; To study mechanotransduction in angiogenesis and the initial growth of tumors;	[23, 24]
	Intracellular tension sensors/FRET	Realize the visualization of intracellular forces; To detect the location and interactions of cellular structures (including integrins and membrane proteins);	[25]
	Confocal microscopy or optical coherence tomography	Investigate the effect of shear stress on cell-cell interactions and mechanotransduction mechanism; To measure biomechanical properties of developing, engineered, and natural tissues and to understand the role of mechanical stimuli such as shear stress;	[22, 26, 27]
	4-dimensional flow magnetic resonance imaging	Analyze the flow and wall shear stress; To monitor the chemoembolization of hepatocellular carcinoma;	[21, 28]
Mechanical simulation	Parallel plate flow chamber	Mimic the fluid environment of cancer cell growth; To investigate the effect of shear stress on cell-cell interactions and mechanotransduction mechanism;	[29, 30]
	Bionic chips or microfluidic platforms	Model and study the cell-cell interactions and mechanotransduction mechanism under shear stress; To detect cancer biomarkers and to isolate characteristic cancer cells;	[31-33]
	Computational fluid dynamics modeling	Simulate drug distribution in a single tumor nodule or tumor-induced angiogenesis, etc.; To observe various cell behaviors on micro-rheology of cancer cells in 3D environments;	[34-36]

fluid velocity *in vivo*, *in vitro* modeling of the tumor fluid microenvironment has been faced with numerous technical challenges. In recent years, with the application of microfluidic technology and mechanical measurement methods in studies on cancer, developments in tumor fluid mechanics accelerated. Increasing evidence now indicates that fluid shear stress (FSS) is an essential factor affecting fluid mechanics, and its role in metastasis has received increasing attention.

FSS is defined as the internal frictional force between moving layers in laminar flow. Additionally, FSS, the product of fluid viscosity and shear rate, is an important parameter of cellular stress in flowing liquid, measured in Newtons per square meter (N/m²) or dynes per square centimeter (dyn/cm²) [13]. FSS is a key regulator of vascular endothelial phenotypes and to induce polarity in endothelial cell [14], cytoskeletal rearrangement [14], and post-translational modifications (e.g., phosphorylation, etc.) and gene expression [15]. Liquid laminar flow is prevalent in biological systems and is usually categorized as blood, lymphoid, and interstitial flow. Tumor cells primarily encounter interstitial shear stress and blood shear stress during metastasis to the target organs. The former plays a role in promoting tumor metastasis, lymphatic drainage, and anti-cancer drug delivery [16]. Current evidence suggests that on tumorigenesis, blood shear stress has dual

effects. It could promote tumor invasion and metastasis, adhesion, and extravasation under certain circumstances while [17] conversely, mechanically eliminating circulating tumor cells (CTCs) [18], and they promote cell cycle arrest in tumor cells [19]. The development of related technology, four types of tumor-related fluid microenvironments and the mechanism of FSS in various stages of the tumor metastasis cascade are summarized herein to provide a reference for subsequent studies on tumor fluid mechanics.

Technological advancements in microfluidics

In the past few decades, the need to explore the biological significance of mechanical force has led to the development of several innovative approaches. Furthermore, the emergence of pN-level mechanical measurement and visualization tools such as biofilm probes, traction force microscopy, and atomic force microscopy have shifted the focus from traditional biomechanics to mechanotransduction at the cellular and subcellular level [20], and the use of microfluidic chips and 4-dimensional flow magnetic resonance imaging to model *in vitro* and *in vivo* mechanical microenvironments has received increasing attention [21, 22]. The following sections focus on the advancements in fluid mechanic tools and their applications in studies on cancer (Table 1). These novel methods have enhanced the general understanding of

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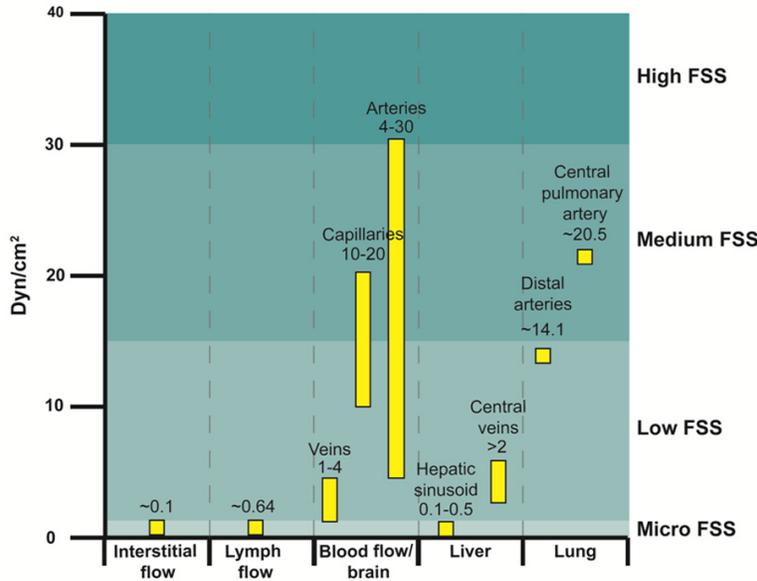


Figure 1. Shear stress levels are variable in tumor metastasis-related fluid microenvironment. Blood shear stress levels are higher than interstitial flow and lymph flow. Additionally, the FSS in hepatic sinusoid and central veins is 0.1-0.5 dyn/cm² and over 2 dyn/cm², respectively. The FSS in the central pulmonary artery and distal arteries are higher, approximately 20.5 ± 4.0 and 14.1 ± 0.7 dyn/cm² in that order.

the correlation between tumor metastasis and fluid shear stress.

Pioneering advancements have been made in fluid mechanics. However, it is important to investigate the biological mechanisms involved in fluid mechanics. At present, the process of integrating mechanical sensing and mechanical simulation, or in other words, during dynamic hydrodynamic sensing, simulating positive and negative feedback regulation mechanisms and adjusting the parameters of the microfluidic model in real time to restore the complexity of fluid mechanics may be the direction for future studies. In addition, the need to establish a reliable *in vivo* model of fluid dynamics is still urgent for the development of mechanical technology.

Tumor metastasis-related fluid microenvironment

Tumor growth and metastasis are influenced by changes in the fluid microenvironment, such as interstitial flow, lymph flow, blood flow, and other organ-specific components.

Interstitial flow

The gradual flow of fluid in tumor tissues is known as interstitial flow. In a physiological

state, most of the fluid that leaks out of capillaries is directed back to the capillaries, and only a fraction of fluid that passes through tumor tissues is recycled by the lymphatic vessels. The aforementioned process completes the exchange of material between the capillaries and the surrounding tissues and prevents the accumulation of fluid in interstitial spaces. In tumor tissues, however, it was reported that owing to the increased flow rate and high vascular permeability [15], interstitial pressure increased and therefore interstitial shear stress approached approximately 0.1 dyn/cm² [13, 37] (**Figure 1**). Under continuous flow of interstitial fluid in an *in vitro* 3D culture, the migration rate of breast cancer cells tended to increase [38]. Munson *et al.*

reported a similar result in glioma cells [39]. Apart from promoting tumor invasion and metastasis, higher interstitial flow rate is also an independent predictor of poor prognosis in cancer patients [40].

Blood flow

When primary tumor cells intravasate into blood vessels, they become CTCs and are then widely disseminated through circulation. Many clinical studies have suggested that CTCs are responsible for most postoperative recurrences and distant metastasis in patients with malignant tumors [41]. Moreover, metastatic colonization of CTCs is much less efficient. Although millions of tumor cells differentiate into CTCs per day, only 0.02% survive to successfully undergo metastasis [42]. Apart from anoikis and killing by natural killer cells, the mechanical damage from FSS is the main cause of death of CTCs. The mean FSS in veins, capillaries, and arteries is 1-4 dyn/cm², 10-20 dyn/cm², and 4-30 dyn/cm² [43], respectively (**Figure 1**). In addition, FSS is significantly higher in close proximity to large vessels, heart turbulence, and blood vessel bifurcations, where the FSS of tumor cells encountering may approach 3000 dyn/cm² [13, 44]. FSS is omnipresent in circulation; hence, CTCs are exposed

to varying levels of FSS. There is growing evidence that blood shear stress can bilaterally regulate tumor cell proliferation [19], induce apoptosis [45], promote CTC adhesion and extravasation [46], etc., thereby serving as a vital factor affecting tumor metastasis.

Lymphatic flow

In general, the lymphatic system participates in blood and body fluid circulation by assisting the re-entry of body fluid into the circulatory system. In addition, it can also transport immune cells and deliver antigens, shouldering imperative immune function. The average FSS of lymphatic vessels is 0.64 ± 0.14 dyn/cm² and the peak is 4-12 dyn/cm² [47] (**Figure 1**), which is far lower than blood shear stress. Recent studies have reported that the FSS generated at sentinel lymph nodes can significantly upregulate ICAM-1 in lymphoid endothelial cells, thereby facilitating lymph node metastasis [48]. Overall, lymphatic shear stress is considered to probably increase lymph node metastasis and affect immune regulation in cancer.

Target organ-specific blood microenvironment

When primary tumor cells infiltrate the circulatory system, they usually stagnate in the vasculature of the target organs minutes after being in rapid blood flow [18]. Clinically, the liver [49], lung [50], and brain [51] are highly metastatic organs; all of these have unique blood microvasculature, and their FSS is enlisted in **Figure 1**.

For instance, the hepatic sinusoid and alveolar walls both have a dual blood supply system. A key process of organ-specific metastasis is that CTCs are captured by the different vasculature. Weiss *et al.* have analyzed the relationship between the metastatic rate of eight target organs and their arterial blood flow in colorectal cancer and esophageal squamous cell carcinoma. They found that the frequency of organ metastasis is positively correlated with blood flow [52]. Recently, researchers have developed a series of organ-specific microfluidic chips, such as liver, lung, brain chips, etc. [53-55]. Using these chips as experimental systems, they confirmed that FSS affects the metabolism and secretion from hepatocytes, the immune response of lung tissue, and the integrity and penetrability of the blood-

brain barrier [53-55]. Clinically, the mechanism underlying organ-specific metastasis and whether fluid mechanics is a contributor warrant further investigation. Hence, it seems worthwhile to investigate the following two aspects using microfluidic chips: first, to set up various tissue/organ-specific microfluidic chips in series and observe tumor cell invasion and metastasis under different fluid dynamics; second, to seed different cell types (tumor cells, endothelial cells, immune cells, etc.), and investigate the interactions between tumor cells and other stromal cells, using the microfluidic chips.

Fluid shear stress plays significant roles in the tumor metastasis cascade

Dynamic response of tumor cells to FSS

FSS induces tumor cell death: The size, action time, and acting form of FSS changes with time for CTCs. In general, CTCs undergo an FSS that can vary from 0.1 dyn/cm² to 1-40 dyn/cm², sometimes approaching 3000 dyn/cm² [13, 44]. To better understand the effects of FSS of various strengths on tumor metastasis, we have defined four grades of FSS, based on the existing literature: micro, low, medium, and high; these correspond to FSS ranges of 0-0.5 dyn/cm², 0.5-15 dyn/cm², 15-30 dyn/cm², and >30 dyn/cm², respectively (**Figure 1**). Lien *et al.* reported that laminar shear stress (LSS) of 0.5-12 dyn/cm² can induce apoptosis of Hep3B, MG63, SCC25, and A549 cells, unlike oscillation shear stress (OSS), which suggests that the different effects of FSS-induced apoptosis might depend on the model of FSS (**Figure 2**) [45]. Under certain circumstances, such as liver fibrosis or high interstitial pressure, blood flow tends to be reversible [15, 56] and easily induces OSS, leading to decreased FSS-induced apoptosis in tumor cells. Accordingly, a high metastatic characteristic in a particular region is likely to attribute to a variant FSS model.

Notably, FSS primarily induces tumor cell apoptosis or autophagy rather than necrosis. The human colorectal carcinoma cell line HCT116 has been reported to undergo almost no cell death within the first 2 min under continuous FSS of 8-60.5 dyn/cm²; however, tumor cell death rate increases to 60% after 20 h [57]. Similarly, Sagar reported that high FSS (60 dyn/cm²) eliminates more circulating tumor cells

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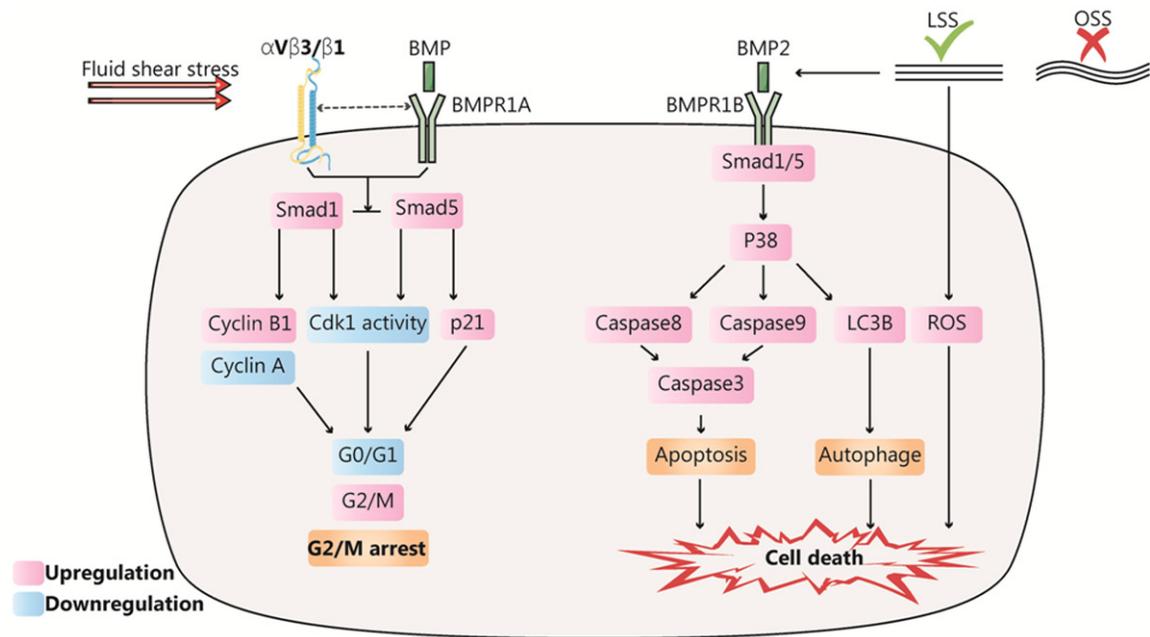


Figure 2. Dynamic response of tumor cells to fluid shear stress (FSS) related to cell survival. FSS targets bone morphogenetic protein (BMP) and integrin, and then accelerates the process of G2/M cell cycle arrest and cell death. On one hand, FSS upregulates BMPR1B, activating the Smad1/5/p38 MAPK signaling pathway, enhancing cleavage caspase-3 or LC3B-I and further promotes apoptosis or autophagy. On the other hand, FSS increases the levels of reactive oxygen species in tumor cells and directly induces tumor cell death. Moreover, FSS activated Smad1/5, causing cell cycle arrest at the G2/M phase and inhibiting cell differentiation.

than low FSS (15 dyn/cm²), and high FSS can eliminate more than 90% of tumor cells within 4 h; apoptosis of tumor cells continued even after termination of FSS in 16-24 h [58]. These results indicate that FSS-specific cell death has a residual effect that is positively correlated with FSS size and action time. In terms of the molecular mechanism, Fu reported that high FSS increased reactive oxygen species (ROS) levels in tumor cells, which could cause oxidative stress and ultimately induce tumor cell death (Figure 2) [38]. Furthermore, FSS also upregulates BMPR1B, thereby activating the Smad1/5/p38 MAPK signaling pathway, enhancing protein expression of cleavage caspase-3 or LC3B-I, to promote apoptosis or autophagy (Figure 2) [45]. In addition, it has been reported that FSS-induced cell death may be attributed to cytoskeleton destruction, thereby preventing cell adhesion and inducing anoikis.

FSS regulates tumor proliferation: In addition to cell death, FSS was reported to influence cell proliferation. Studies have reported that tumor cell proliferation can be obviously reduced by increasing the FSS stimulation time [19].

Further experiments have indicated that FSS can lead to G1/S or G2/M cell cycle arrest in tumor cells. A previous study reported that 61% of colon cancer cells were arrested in the G1 phase with sustained FSS stimulation at 15 dyn/cm², compared to 24% in the FSS-free group [59]. Similarly, Chang reported that low FSS stimulation of 2-20 dyn/cm² activated Smad1/5, causing cell cycle arrest at the G2/M phase and downregulating cell differentiation in the adherent human osteosarcoma cell line (MG63) (Figure 2) [60]. Simultaneously, the study reported that p-Smad1/5 was upregulated with increasing FSS strength [60]. Fan reported that when FSS of different strengths were applied to circulating human colon cancer cells (HCT116) for a constant period, cancer cells in the high FSS group (60.5 dyn/cm²) had higher cell vitality and β -catenin expression than those in the low FSS group [19]. Recent studies have also reported that tumor cells have shear stress resistance, implying that tumor cells can adapt to shear stress stimulation up to 6000 dyn/cm² and show increased survival after repeated exposure to FSS, relative to normal epithelial cells [61]. The findings of these two studies are not

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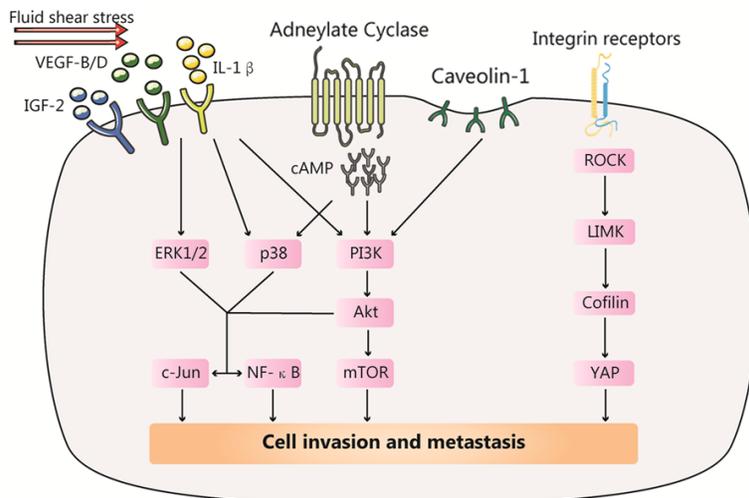


Figure 3. Various factors activated by fluid shear stress (FSS) can induce tumor cells metastasis. FSS stimulation upregulates a series of cytokines or mechanosensitive molecules, such as IGF-2, VEGF and Cav-1, activating PI3K/AKT, c-Jun and NF- κ B pathway and promoting invasion and metastasis; FSS can also combine integrin receptors, activating the ROCK-LIMK-cofilin signaling axis, inducing nuclear translocation of YAP1 and promoting invasion and metastasis.

consistent with those of Chang, and we surmise the reasons may be related to the FSS range and cell state employed in the study. Although FSS has been reported to have various regulatory effects on tumor proliferation, FSS is consistently reported to play a vital role in tumor proliferation.

FSS promotes tumor invasion and metastasis: Increasing evidence indicates that low FSS stimulation upregulates or activates a series of cytokines or mechanosensitive molecules, such as IGF-2, VEGF, ROCK, and Cav-1, triggering downstream molecular pathways and promoting invasion and metastasis of tumor cells (**Figure 3**) [62, 63]. For instance, Wang reported that application of low FSS (2 dyn/cm²) to chondrosarcoma cells promoted the synthesis of cAMP and IL-1 β or activation of IGF-2 and VEGF-B/D, targeting the PI3-K, p38, or other signaling pathways, ultimately enhancing the invasion of chondrosarcoma cells *in vitro* [64, 65]. Lee supported this conclusion by demonstrating that FSS (0.05 dyn/cm²) activated the ROCK-LIMK-cofilin signaling axis, inducing nuclear translocation of YAP1, and regulating transcription of metastasis-related genes in prostate cancer cells [17]. Yang's team also verified that Cav-1 can activate the downstream PI3K Akt/mTOR pathway and promote metastasis of breast cancer cells under low

FSS, using *in vivo* and *in vitro* experiments [66].

In conclusion, FSS has an important effect on proliferation, death, invasion, and metastasis of tumor cells, which is largely dependent on the type, size, and action time of the FSS.

FSS-dependent interaction among tumor cells and other blood components

Platelets: Clinically, thrombocytosis is often observed in metastatic cancer patients, suggesting that platelets may contribute to tumor metastasis [67]. Platelets can down-regulate the NK cell-surface receptor NKG2D through paracrine TGF- β signaling [68]; meanwhile, platelet-derived

VEGF can also repress antigen presentation in mature dendritic cells, thereby inhibiting their immune surveillance function. Besides mediating immunity escape of carcinoma cells, platelets can directly trigger EMT of CTCs [18] or mobilize neutrophils via the secretion of TGF- β /PDGF, and leading to tumor micrometastasis [69]. However, in most situations, platelets will directly bind to CTCs to form cell complexes, thus promoting the evasion of CTCs from the immune, inhibiting CTC apoptosis, and mediating CTC extravasation [70].

At low FSS of 1.84 dyn/cm², thrombin-activated platelets can produce a 5-fold increase of endothelial adherence in cervical cancer cells (HeLa) [71]. At low FSS (5 dyn/cm²), tumor gangliosides can drastically enhance the dynamic adhesion of platelets in the bloodstream, facilitating the capture of platelet-CTC complexes by endothelial cells (**Figure 4**) [72]. However, at an FSS of 50 dyn/cm², the adhesion efficiency was not further enhanced, thereby indicating that low FSS is sufficient to enhance the adhesion of platelet-CTC complexes to endothelial cells [72]. In addition, Egan *et al.* reported that low FSS would similarly reduce LDH levels in tumor cells; LDH can be a quantitative molecular marker of membrane damage induced by FSS, which indicates that platelets can protect cancer cells from FSS-induced mechanical

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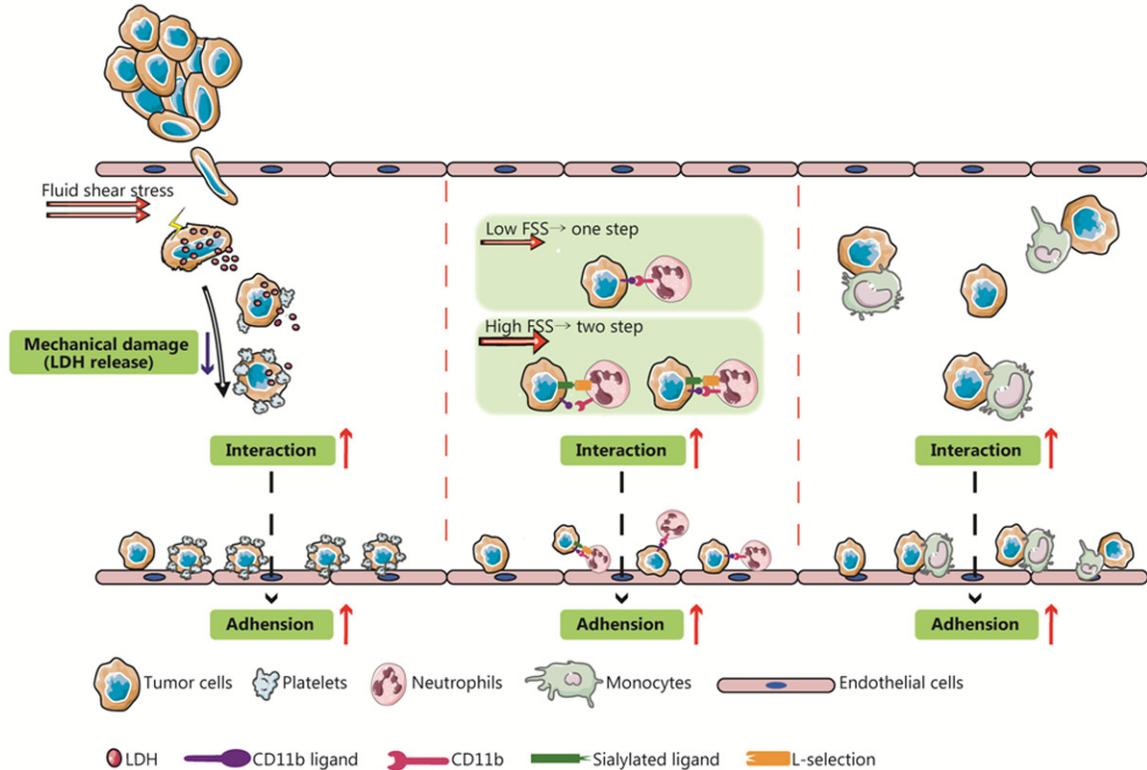


Figure 4. Fluid shear stress (FSS) regulates the interactions among tumor cells and other blood components. Blood shear stress can enhance the interactions between circulating tumor cells (CTCs) and platelets, neutrophils, monocytes, and other blood components, thereby protecting them from mechanical damage and promoting CTC adhesion to endothelial cell. Moreover, different magnitudes of shear stress can regulate the binding of CTCs to neutrophils.

damage (Figure 4) [73]. However, with the increase in FSS, although a similar number of platelets adhered to tumor cells, the protective effect from platelets in FSS-induced tumor cell death decreased [73]. Thus, low FSS can enhance platelet promotion of tumor metastasis; however, high FSS may reverse the effect.

Neutrophils: There is growing evidence that neutrophils may play a dual role in tumor metastasis. On one hand, when neutrophils have immediate contact with tumor cells, they can produce TNF- α , IL-1 β , protease, membrane perforators, and other compounds to eliminate tumor cells [74]; on the other hand, gastrointestinal and other malignant tumors are characterized by neutrophil infiltration [75], and neutrophils can enhance tumorigenic potential [76].

The formation of neutrophil-tumor cell complexes is mediated by the sizes of the FSS; specifically, the number of neutrophils binding to tumor cells decreases with an increase in FSS. The mechanism underlying this phenomenon is that at low FSS, neutrophils can bind directly to

tumor cells through the surface molecule CD11b, while at high FSS, binding is a two-step, sequential process. In detail, neutrophils first bind transiently to tumor cells via L-selectin, followed by conversion of the transient binding into stable adhesion via the synergistic effect of CD11a and CD11b (Figure 4) [77]. In 2008, another study confirmed that the accumulation of neutrophils and melanoma cells into a cellular mass is dependent on FSS size and shear rate, mainly via β 2 integrin and selectin [78].

Monocytes and macrophages: Similar to neutrophils, macrophages are important immune cells, with phagocytosis, antigen presentation, and other immune functions, while various macrophage subtypes play different roles in tumor metastasis. Previously, tumor-associated macrophages (TAMs), which specifically infiltrate tumor tissue, were the focus of studies on cancer associated with macrophages. However, in recent years, *in vivo* studies have reported that circulating monocytes/macrophages are closely linked to the process of extravasation of breast cancer cells [79]. Based on an *in vitro*

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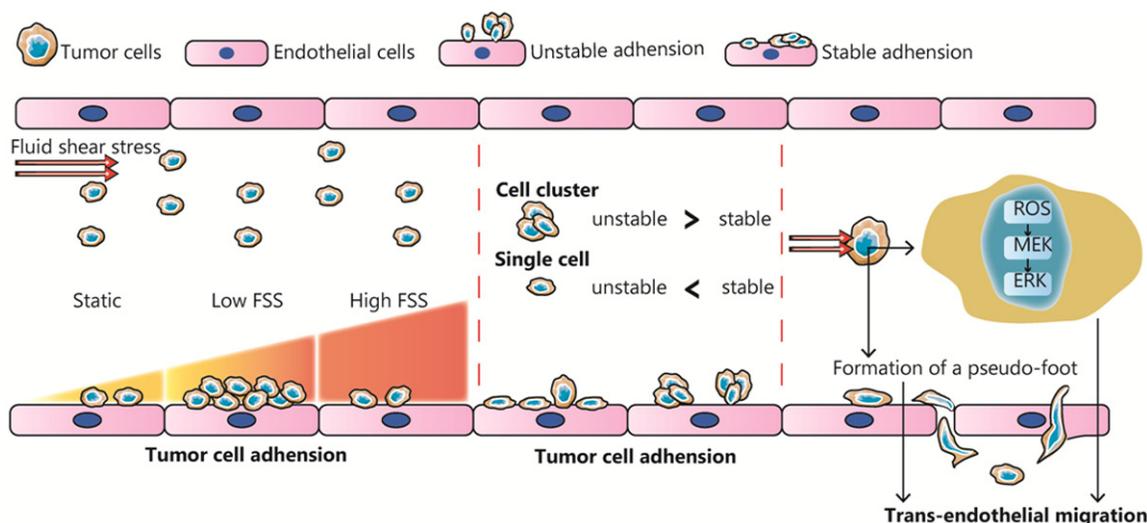


Figure 5. Fluid shear stress (FSS) is an essential regulator of tumor cell adhesion and extravasation. FSS plays a dual role in the adhesion of tumor cells to endothelial cells, and as the FSS increases, the adhesion efficiency first increases and then decreases. There may be different tendencies in the manner of adhesion of single tumor cells and tumor cell clusters in blood. FSS stimulation can promote intracellular generation of reactive oxygen species and pseudopodia formation, thereby triggering trans-endothelial migration.

model loaded with a dynamic FSS, Evani *et al.* reported that breast cancer cells would not adhere to endothelial cells directly under low FSS (0.5-2 dyn/cm²), but instead formed a tumor cell/monocyte complex before binding to endothelial cells (**Figure 4**) [80]. Briefly, FSS determines the binding of tumor cells/monocyte complexes and endothelial cells.

These results suggest that FSS can mediate the interaction between CTCs and various blood components; moreover, the formation of tumor cell complexes contributes to CTC survival, adhesion, extravasation, etc. In the future, targeting the tumor cell complex-endothelium axis should be investigated as a promising therapy.

FSS is an essential regulator of tumor extravasation

Extravasation is a preliminary step in tumor metastasis. Similar to leukocyte exudation, tumor cell extravasation primarily involves three steps: adhesion, trans-endothelial migration (TEM), and crossing the vascular basement membrane (**Figure 5**) [81]. Previous studies indicate that FSS not only plays an important role in the neutrophil recruitment cascade (capture-scrolling-activating-adhesion), but also is closely related to the extravasation of CTCs [82].

FSS regulates tumor cell adhesion: The adhesion of CTCs to vasculature endothelial cells is a prerequisite for tumor extravasation, wherein selectin, cadherin, and integrin are key proteins. Essentially, cell adhesion involves binding between a specific receptor and its ligand, which can be subdivided into two stages: initial rolling adhesion and stable adhesion. Different adhesion molecules display diverse molecular dynamics of reaction rate or affinity; therefore FSS is likely to affect tumor cell adhesion via regulating adhesion/dissociation efficiency or expression levels of adhesion molecules.

Low FSS has been shown to affect the stable adhesion of tumor cells; furthermore, it has been reported that as the FSS increases, the adhesion efficiency first increases and then decreases. Fennewald reported that the quantity of cancer cells (HNSCCs) adhered to the matrix gel was significantly higher in the group with FSS (0-0.05 dyn/cm²). Similarly, another study reported that low FSS induced a 2-fold increase in the number of breast cancer cells adhered to endothelial cells, compared with the group without FSS [83]. However, Fennewald also reported that tumor cell adherence was gradually reduced to zero as the FSS increased (in the range of 0.05 to 1 dyn/cm²) [84]. Papadimitriou and Richter successively reported the same conclusion that FSS, within a particular range, can inhibit tumor cell adhesion

[85, 86]. It is noteworthy that although FSS is a two-way regulation of tumor adhesion, the response of different tumor cells to varying degrees of FSS may be positive or negative [87], which may be associated with the type or expression level of adhesion molecules at the tumor cell surface. Other than stable adhesion, FSS can negatively regulate rolling adhesion within a certain range (**Figure 5**). Aigner reported that the ratio of rolling adhesion in three cancer cell types (KS, HL-60, and SkW3) significantly declined with FSS stimulation (0.25-2.75 dyn/cm²) [88].

Since the effect of FSS varies with time and site of application, it is worth investigating how the dynamic FSS play roles in tumor cell adhesion. Through *in silico* modeling, Yan observed that the adhesion rate of tumor cells to curving vessels was 1.5-fold times greater than that to straight vessels, and nearly 45% of tumor cells preferentially adhered to medial curving vessels. Based on the hydrodynamic theory, the authors concluded that a positive shear stress gradient enhanced tumor cell adhesion while a negative gradient weakened it [89]. Thus, a constantly increasing FSS (within 50 dyn/cm²) is likely to promote tumor cell adhesion, and vice versa.

FSS has differing effects on cell adhesion in single cells and clustered cells, the two major forms of CTCs. Yano *et al.* compared adhesion rate between N17 single cells and NL17 cell clusters at low FSS. They found that the binding frequency increased in NL17 cell clusters, which was mostly short-term attachment, with less stable adhesion, but single cells are more likely to develop stable adhesion at the same FSS [90]. However, subsequent *in vivo* experiments reported that NL17 cell clusters result in greater tumor metastases than did N17 single cells (**Figure 5**) [90], which may attributed to the stronger killing effect of FSS on single tumor cells.

FSS regulates TEM of tumor cells: TEM refers to the process whereby tumor cells stably adhere to endothelial cells and then penetrate the endothelial tissue. There are two primary TEM pathways in tumors: 1) paracellular TEM, wherein tumor cells reduce the endothelial cell junction and cause endothelial cell retraction and separation via secretion of VEGF, TGF- β , and other cytokines, promoting TEM [91]; and

2) transcellular TEM, wherein tumor cells pass directly through vascular endothelial cells [46, 92].

The potential for TEM increases as the retention time of tumor cells in endothelial cells increases, and that retention time largely depends on FSS [93]. As mentioned earlier, a stronger FSS can not only increase the adhesion between tumor cells and endothelial cells, but also reduce the duration and prevent TEM. However, using a zebrafish model, Ma reported that ROS levels in tumor cells are upregulated with FSS (10-15 dyn/cm²) stimulation, activating the MEK/ERK signaling pathway and promoting TEM (**Figure 5**) [94]. Another study reported that when the shear rate is greater than 400 s⁻¹, that is, physiological shear conditions, FSS could induce the formation of a pseudo-foot in tumor cells, which is also conducive to TEM [95].

Future research directions for fluid mechanics in tumor metastasis

Establishment of guidelines for tumor research using fluid mechanics

Fluid mechanics of tumors, as an emerging interdisciplinary research field, faces many challenges. Unlike solid mechanics and structural mechanics, fluid mechanics involves the investigation of components that continually change their form and are in a constant state of motion. Therefore, it entails more complicated theoretical analysis and numerical calculations. However, in practical research, there are no uniform standards to determine FSS mode or microfluidic devices, leading to poor reproducibility and weak clinical translation of most findings. Hydrodynamics has a significant impact on vascular remodeling and tumor metastasis. Therefore, a multidisciplinary team of professionals including physicists, biologists, and clinicians should be constituted to promote interdisciplinary integration and establish guidelines for research in this field. The background and history of mechanobiology development, its scope, theoretical basis, research and experimental methodologies, existing achievements, scientific problems remain to be solved, and the future developmental directions urgently need to be comprehensively and systematically summarized.

Elucidation of the mechanism of mechanotransduction

Mechanotransduction, which constitutes the core of biomechanics, can be divided into five stages—stimulus, sensing, signaling, gene expression, cellular response, and cellular function [96]. Among these, molecular mechanosensing is the most widely studied process. The activity of biomolecules is closely related to their physical form or conformation, which is the basis for the mechanical sensitivity of mechanosensing molecules. For example, stress can induce a conformational change in p130-Cas adhesion spot, exposing the phosphorylation site of Src protein and allowing it to phosphorylate and activate the downstream p38/MAPK signaling pathway [97]. Numerous studies have reported various mechanosensitive cell-surface molecules such as integrins, adherent proteins, and calcium channels [98], which can dynamically detect changes in mechanical forces and activate downstream signaling pathways, which regulate gene transcription and translation to effect phenotypic and functional changes. However, how these mechanosensitive molecules detect changes in the magnitude and direction of mechanical forces and subsequently mediate the activation of downstream pathways is unclear. Studies using intracellular tension sensors and other new technologies are needed to address these questions.

Furthermore, hydrodynamics influences cell fate through multiple routes, such as epigenetic modifications (DNA methylation) [99], modulation of mRNA and protein levels, and alteration of chromatin structure [100]. Fernandez-Sanchez *et al.* reported that long-term 1-kPa magnetic load can activate the Wnt pathway in intestinal crypt cells, thereby upregulating the oncogene *c-Myc* and leading to carcinogenesis. These effects are not reversed by the withdrawal of the magnetic load, suggesting that mechanical forces can induce stable genetic effects and lead to tumorigenesis [2]. Therefore, the core molecules or pathways that mediate the biological effects of mechanical forces could be unique targets for antitumor therapy.

Strengthening of clinical applications

The tumor microenvironment is a network of biological, chemical, physical, and other signals

that interact with each other. Thus, tumorigenesis and tumor progression cannot be explained without considering both mechanics and biology.

The use of microfluidic chips and computational fluid dynamics modeling tools can enhance our understanding of the biological mechanisms of tumor metastasis. Microfluidic technology, a popular tool, can simulate a wide array of complicated tumor microenvironments, allowing the study of various chemical and mechanical effects and facilitating the comprehensive study of the TME.

In addition, FSS not only directly promotes migration of T lymphocytes [101] and helps screen antigen-specific T cells [102], but also it induces M1 polarization of macrophages [103] and activates the immunoregulatory function of mesenchymal stem cells [104]. Therefore, detailed studies on molecular mechanisms through which FSS stimulates tumor cells, immune cell differentiation, and induces antigen presentation are needed. Furthermore, numerous studies have reported that PD-L1, CD47, and other immunosuppressive molecules expressed on the surface of CTCs are significantly increased compared with that on primary tumor cells [105-107]. Aside from the primary tumor, one of the biggest changes for CTCs in the tumor microenvironment is FSS. We speculate that FSS likely induces evasion from the immune system through upregulation of immunosuppressive molecules in CTCs. In the future, more basic studies are needed to determine whether and how FSS affects tumor immunity.

In recent years, researchers have developed numerous novel mechanosensing carriers such as liposomes and microaggregates for clinical application, especially for treating cardiovascular disease [108]. These materials can sense changes in shear force and respond by releasing their contents, diffusion, cohesion, or inducing polymerization to achieve mechanics-targeted drug delivery. Future studies on hydrodynamics-based antitumor strategies would focus on further clarifying how mechanistic and biological factors together impact tumor growth, designing fluid mechanics-based targeted drugs, and changing the tumor hydrodynamic microenvironment.

Outlook

Fluid dynamics is an important dynamic variable in physiological phenomena. Like Heraclitus famously said that no man ever steps in the same river twice. Despite infinite variations in mechanobiology, the goal of understanding the universal law and the vital signal transduction mechanisms can be attained with persistent effort. To determine the essence and verify the laws, a specific spatiotemporal event or a specific biological aspect for investigation (such as FSS) should be focused on to begin with, investigated progressively, and then the accumulated evidence should be interpreted and explained sequentially, thereby revealing the role of fluid mechanics in tumor development.

The tumor fluid microenvironment, especially the FSS, plays an indispensable role in tumor progression and metastasis. Rapid advancements in research tools and computer algorithms will certainly improve the general understanding of the mechanism underlying the induction of tumorigenesis and tumor progression by mechanotransduction, and fluid mechanics will provide novel strategies and new targets for antitumor therapy.

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Disclosure of conflict of interest

None.

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