Received: 30 May 2021 | Revised: 14 July 2021 | Accepted: 15 July 2021

DOI: 10.1002/cm.21680

REVIEW ARTICLE

WILEY

Molecular cancer cell responses to solid compressive stress and interstitial fluid pressure

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Funding information
Cancer Prevention and Research Institute of Texas, Grant/Award Number: RR200043; National Institutes of Health, Grant/Award Number: U01 CA225566

Abstract

Alterations to the mechanical properties of the microenvironment are a hallmark of cancer. Elevated mechanical stresses exist in many solid tumors and elicit responses from cancer cells. Uncontrolled growth in confined environments gives rise to elevated solid compressive stress on cancer cells. Recruitment of leaky blood vessels and an absence of functioning lymphatic vessels causes a rise in the interstitial fluid pressure. Here we review the role of the cancer cell cytoskeleton and the nucleus in mediating both the initial and adaptive cancer cell response to these two types of mechanical stresses. We review how these mechanical stresses alter cancer cell functions such as proliferation, apoptosis, and migration.

KEYWORDS
cancer cell, hydrostatic pressure, interstitial fluid pressure, solid compressive stress

1 | INTRODUCTION

A hallmark of cancer is alterations to the mechanical properties of the tumor and its microenvironment (Nia, Munn, & Jain, 2020; Northey, Przybyla, & Weaver, 2017). Mechanical alterations include changes to the mechanical stiffness of the microenvironment as well as elevated mechanical stresses on tumor cells. Two types of mechanical stresses are particularly important in modulating cancer tissue and cell function in vivo—solid compressive stress and hydrostatic pressure (Davies & Tripathi, 1993; Wang & Li, 2010).

Compressive stresses build up on cancer cells in a growing, solid tumor due to mechanical resistance of the surrounding, confining environment to displacement (Figure 1a) (Jain, Martin, & Stylianopoulos, 2014). Solid compressive stress in tumors ranges from 0.7 to 75 mmHg (0.1–10 kPa) for human tumors and 2 to 60 mmHg (0.25–8 kPa) for murine tumors (Nia et al., 2016, 2020; Stylianopoulos, Munn, & Jain, 2018). Compressive stresses also build up on cancer cells migrating through narrow interstitial spaces of the tissue (Friedl & Alexander, 2011).

Interstitial fluid pressure (IFP) is a marker of malignancy in a number of human cancers (Gutmann et al., 1992; Nathanson & Nelson, 1994). IFP is generated by accumulation of fluid in the growing tumor (Heldin, Rubin, Pietras, & Ostman, 2004). Proliferating tumor cells recruit thin-walled, leaky blood vessels to meet their high oxygen demand. Due to a lack of a functioning lymphatic system, build-up of fluid leaked from tumor capillaries results in elevated pressure in tumors (Figure 1b; Ariffin, Forde, Jahangeer, Soden, & Hinchion, 2014; Stylianopoulos et al., 2018). Increased presence of interstitial fibroblasts can also contribute to increased IFP by contracting the extracellular matrix (Astafurov et al., 2014; Heldin et al., 2004). The IFP in human tumors ranges from ~5 mmHg (~0.6 kPa) in brain tumors to ~40 mmHg (~5.3 kPa) in ovarian and renal cell carcinomas (Jain, 2012) and the range is around 3–15 mmHg (0.4–2 kPa) for murine tumors (Boucher, Baxter, & Jain, 1990; Sen et al., 2011).

The elevated solid stress and high IFP in a tumor can impact drug delivery. For example, accumulated solid compressive stress in tumors can be high enough to constrict blood vessels (Griffon-Etienne, Boucher, Brekken, Suit, & Jain, 1999; Padera et al., 2004; Stylianopoulos et al., 2012). The collapse of blood vessels can cause hypoxia (Chauhan et al., 2013; Stylianopoulos et al., 2012) and reduce the efficacy of therapeutic drug delivery (Jain, 2014; Munn & Jain, 2019). Elevated IFP in the tumor can inhibit convective transport of drugs to the tumor core (Jain et al., 2014; Jain & Baxter, 1988). The steep IFP gradient near the periphery of the tumor causes an outward flow of fluid from the interstitial space to the surrounding normal tissue, which can reduce the time of retention of drugs in the tumor (Jain, 2013).

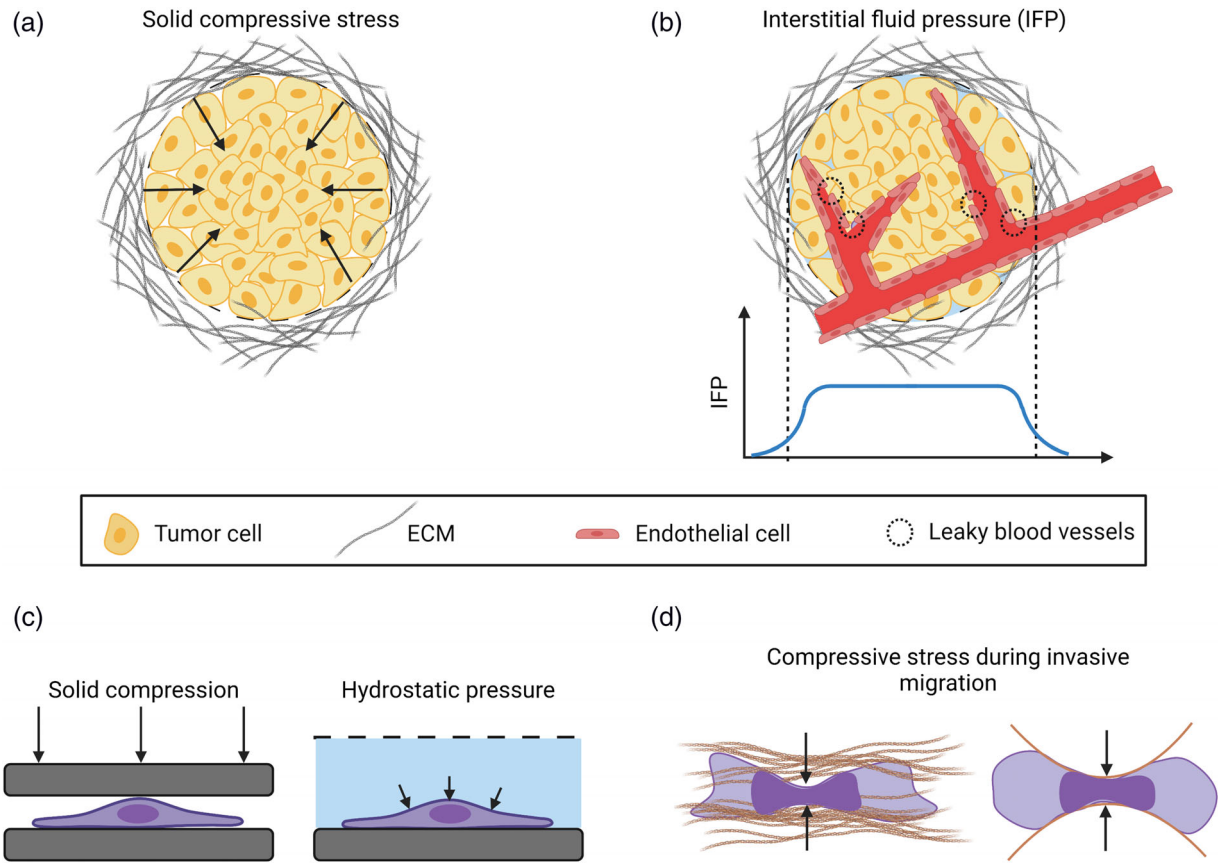

FIGURE 1 Build-up of solid compressive stress or hydrostatic pressure in tumors, and in vitro assays to study them. (a) A solid tumor mass surrounded by dense ECM. Overcrowding of cells in the tumor microenvironment due to abnormal cell proliferation displaces the surrounding ECM and causes a buildup of solid compressive stress (black arrows) (b) Leaky/permeable blood vessels in the solid tumor cause plasma leakage which, combined with a lack of functioning lymphatic vessels, leads to elevated interstitial fluid pressure (IFP) in the bulk of the tumor, with a gradient near the tumor periphery. The IFP distribution in the tumor mass is shown by the blue curve. (c) In vitro approaches to apply solid compressive stress and hydrostatic pressure (black arrows) on cancer cells. (d) Solid compressive stress acts on an invading cancer cell in the confined environment of the extracellular matrix. Right image shows a schematic of a cancer cell migrating through microfabricated confining channels. Compressive stresses are indicated by solid black arrows [Color figure can be viewed at wileyonlinelibrary.com]

Cancer cells in the tumor respond to elevated mechanical stresses, and these responses are important for cancer cell survival (Hope et al., 2021; Moose et al., 2020). Both solid compressive stress and IFP alter cancer cell behaviors such as proliferation, invasion, and apoptosis, and are a factor in cancer progression (Jain et al., 2014; Northcott, Dean, Mouw, & Weaver, 2018; Provenzano & Hingorani, 2013). In this article, we focus on cellular responses to these two types of mechanical stresses.

2 | CANCER CELL RESPONSE TO SOLID COMPRESSIVE STRESS

2.1 | Methods to apply compressive stress

A typical approach to study the impact of solid compressive stress on the cytoskeleton in two-dimensional cultured cells is to confine cells through physical contact of the apical surface of the cell with another solid surface (Figure 1c). The compressing surface can be a soft flat surface like PDMS (He et al., 2018; Le Berre, Zlotek-Zlotkiewicz, Bonazzi, Lautenschlaeger, & Piel, 2014) or agarose (Aureille et al., 2019). Hard surfaces like a glass plate (Caille, Thoumine, Tardy, & Meister, 2002; Peeters, Oomens, Bouten, Bader, & Baaijens, 2005), a cantilever probe of other material, or ~5 μm-sized beads on an atomic force microscope (AFM) have also been used to indent the cell apex (Ofek, Wiltz, & Athanasiou, 2009). Limitations of such methods include the two-dimensional nature of cell culture, which typically involves flat cell and nuclear morphologies that are not typical of in vivo contexts. Yet, such two dimensional (2D) methods allow controlled probing of cells combined with high resolution imaging which has revealed significant information on the cellular response to mechanical stress. More physiologically relevant in vitro methods of compression include the application of compression to cell-containing three dimensional (3D) matrix gels (Boyle et al., 2020), the growth of tumor spheroids in confining gels (Tse et al., 2012), and osmotically driven collapse of the extracellular matrix to compress tumor spheroids (Dolega et al., 2021).

2.2 | Effect of compressive stress on the cytoskeleton

While the methods to apply compressive stress differ in the spatial distribution of force applied (local versus entire cell, direct contact versus compression of cell containing gel) which can elicit differing responses from the cell, the cytoskeleton, and more recently, the nucleus, are consistently implicated in resisting solid compressive stresses as discussed below. The specific cytoskeletal components that are important in the cellular response may be cell-type dependent, and dependent on the magnitude of the applied stress and on the time/frequency of stress application.

The effect of an increase in the compressive stress on a cell can be understood with a simple force balance at a curved cellular interface. The main components of the force balance over a portion of a free (i.e., nonadherent), stationary cell interface, in the absence of extracellular mechanical stresses, are tension in the contractile, curved actomyosin cortex, which is balanced by the difference in the hydrostatic pressure across the cell membrane (Li et al., 2015). In a resting cell, the hydrostatic pressure difference across the membrane is primarily due to an osmotic pressure, which exists because of a difference in the concentration of ions between the cytoplasm and extracellular space. The Law of Laplace applies at the curved interface:

$$P_{\text{int}} - P_{\text{ext}} = T(2\text{H}) \tag{1}$$

where P_{int} is the internal pressure, P_{ext} is the external pressure, 2H is the mean curvature, and T is the cortical tension.

An external compressive stress P_c applied by an AFM probe or by a confining barrier to such an interface will modify the above force equilibrium. Rapid adjustments can occur to the terms in Equation (1), followed potentially by longer time adaptive changes. The modified equation is

$$P_{\text{new}} - P_c = T_{\text{new}}(2\text{H}_{\text{new}}) \tag{2}$$

where P_c is the new external pressure, which is greater than P_{ext}, P_{new} is the new internal pressure, T_{new} is the new tension, and 2H_{new} is the new curvature. Equation (2) can be considered to apply in the following two simple ways (or combinations of these two ways): if, at constant tension $T_{\text{new}} = T$ and curvature $2\text{H} = 2\text{H}_{\text{new}}$, the hydrostatic intracellular pressure P_{int} increases to P_{new}, or if the cortical tension T decreases to T_{new} and/or the curvature 2H reduces to 2H_{new} at constant internal pressure P_{int}.

An instantaneous increase in the internal pressure P_{int} upon application of a compressive stress to cells is plausible given that water is incompressible. Such an increase in pressure is evident from the fact that application of confining compressive stress to rounded Hela-Kyoto cancer cells caused substantial blebbing of the plasma membrane (Lomakin et al., 2020). Increased intracellular pressure promotes bleb formation by causing membrane delamination from the actin cortex or causing local ruptures in the actin cortex (Charras & Paluch, 2008). To reduce blebbing, cells can adapt to the increased pressure by upregulating cortical actomyosin tension (Lomakin et al., 2020). As an example of cellular adaptation resulting in a potential decrease in cortical tension T, compression applied to HT1080 fibrosarcoma cells reduced RhoA activity through the activity of a membrane ion channel TRPV4, which is permeable to calcium ions (He et al., 2018); a reduction in RhoA activity should reduce cortical actomyosin tension.

The extent to which actomyosin networks remodel under confining compression differs between normal and cancer cells in 2D culture. For example, continuous compression (5.8 mmHg or 0.77 kPa) of cultured 67NR breast cancer cells under a weight applied to an overlaid agarose gel, for example, oriented F-actin stress fibers perpendicular to adjacent vacant areas and caused longer filopodia to develop, while such effects were absent in noncancerous MCF10A cells (Tse et al., 2012). Individual actin filaments in vitro have been reported to stiffen and resist confining compression (Greene, Anderson, Zeng, Zappone, & Israelachvili, 2009) although the extent to which this contributes to cellular responses to compression is unclear.

Because solid tumors are crowded environments, proliferating tumor cells must undergo rounding, assemble a mitotic spindle, and perform cytokinesis against confining barriers. These changes in shape can only occur if cells exert outward pushing forces to deform the confining extracellular matrix. Rounding of cultured Hela-Kyoto tumor cells against a cantilever produces a force of ~60 nN, corresponding to a rounding pressure of ~0.14 nN/μm^2 (Stewart et al., 2011). Cells that are unable to push against confining barriers are unable to round up; these cells have an increased likelihood of entering apoptosis(Sorce et al., 2015). The rounding is driven by de-adhesion from the substrate, but may also be driven by an increase in intracellular osmotic pressure. Cell rounding may also involve a cytoskeletal stiffening mechanism in order to round up against the confining barrier and divide. For example, transient induction of oncogenic Ras^{V12} stiffens MCF10A cells during mitotic rounding in an actomyosin-dependent manner, allowing them to undergo mitosis without chromosome segregation errors during cellular confinement by a stiff gel (Matthews et al., 2020).

Subsequent to rounding, spindle assembly causes an elongation of the rounded cell that also exerts an outward pushing force on the confining matrix. An elegant demonstration of the mechanism by which mitotic cancer cells push against the matrix was provided by Nam and Chaudhuri (2018). Nam et al. observed direct deformation of the surrounding, confining alginate matrix caused by single, mitotic MDA-MB-231 breast cancer cells. Laser ablation of microtubules in the mitotic spindle or inhibition of the actomyosin contractile ring that causes cytokinesis, relaxed some of the matrix deformation caused by mitosis. Further, spindles had a buckled appearance in confined mitotic cells. These experiments showed that at least part of the pushing force against confining barriers is due to mitotic spindle assembly and actomyosin contraction that splits the mitotic cell into daughter cells.

Cytoplasmic vimentin intermediate filament networks in cells can undergo extreme deformations (Hu et al., 2019) without damage and

undergo strain hardening (Janmey, Euteneuer, Traub, & Schliwa, 1991). These properties of the vimentin network help protect mouse embryonic fibroblast (MEF) nuclei from rupture during migration through confined environments (Patteson et al., 2019), and help nuclei maintain mechanical homeostasis against local mechanical forces (Neelam et al., 2015). In epithelial cells, keratin networks may stiffen cells (Ma, Yamada, Wirtz, & Coulombe, 2001) and inhibit their migration (Seltmann, Fritsch, Käs, & Magin, 2013). More studies of the mechanics of intermediate filament networks in cancer cells are needed to understand their role in balancing and adapting to compressive stress applied to cells.

2.3 | Effect of compressive stress on the nucleus

Owing to its size and stiffness, the nucleus is substantially compressed during cancer cell migration through narrow interstitial spaces typically present in tissue (Vortmeyer-Krause et al., 2020; Wolf et al., 2013). The resistance of the nuclear lamina to extension and the resistance of the nuclear volume to changes are key parameters that determine the mechanical response of the nucleus to such compressive stresses (Hobson et al., 2020; Lele, Dickinson, & Gundersen, 2018).

Mechanical cell compression can impact several nuclear structures in cancer cells. Compressive stress can cause the nuclear envelope to delaminate from the lamina to form a bleb, which can then rupture (Denais et al., 2016). The mechanical response of the nuclear envelope during this process is complex and not fully understood (Agrawal & Lele, 2019; Q. Zhang et al., 2019). Rupture causes an intermixing of the cytoplasm and the nucleus, exposing DNA to cytoplasmic DNAses such as TREX1, which can in turn cause DNA damage (Nader et al., 2020). Rupture of the nuclear envelope in cultured cancer cells can also occur in the absence of external cell compression, as a result of an increase in nuclear pressure due to compression by apical F-actin structures (Hatch & Hetzer, 2016). Mechanisms to repair envelope ruptures include early recruitment of the Barrier-to-Autointegration factor (BAF) to the site of envelope rupture (Halfmann et al., 2019), and repair through the recruitment of LEM domain proteins (Halfmann et al., 2019), and the ESCRT family of proteins (Raab et al., 2016).

Deformation of the nucleus during confined migration of MDA-MB-231 and BT-549 breast cancer cells or by mechanical compression of static cells can cause DNA damage in the S/G2 phase of the cell cycle without requiring mechanical rupture of the envelope (Shah et al., 2021). The DNA damage is likely due to a stalling of the DNA replication fork. Mechanical compression induces chromatin condensation in fibroblasts, which correlate with changes in transcriptional response (Damodaran et al., 2018). Chromatin dilates when fibrobast nuclei change shape during cell migration from elongated shapes to circular shapes (Katiyar et al., 2019). These shape changes are also accompanied by an unfolding of the nuclear lamina.

Mechanical compression of the nucleus may cause mechanical adaptation of the cell. For example, mechanical confinement of Hela-Kyoto cancer cells stretches the nuclear envelope, and upregulation of actomyosin contractility, which was attributed to signaling by stretch-sensitive nuclear envelope proteins (Lomakin et al., 2020).

2.4 | Effect of solid compressive stress on cancer cell proliferation, apoptosis, and migration

An early study by Jain and coworkers found that the final size of human cancer cell spheroids in agarose gels was lower at higher gel concentrations (Helmlinger, Netti, Lichtenbeld, Melder, & Jain, 1997). This suggested that at high gel concentrations, the tumor spheroid was unable to displace the mechanically resistant gel matrix beyond a certain size. At the cellular level, no measurable effects on cell proliferation rate were found, and a slight decrease in apoptotic rate was observed, which was consistent with the observed increased cell packing density in confined spheroids. In contrast, in a subsequent study, Munn, Jain, and coworkers reported that cell proliferation was suppressed and apoptotic rates were increased in regions of high solid compressive stress in the spheroid (Cheng, Tse, Jain, & Munn, 2009). In both studies, care was taken to establish that the effect was indeed due to solid stress on cells, by ruling out changes in other factors such as gel toxicity, limitations of nutrients, growth factors, or oxygen in gels at higher concentrations, or by showing similar results through direct mechanical compression of cells and spheroids (Cheng et al., 2009). The increased apoptotic rate under mechanical compression could be reduced by over-expression of Bcl-2, a protein, which inhibits multiple caspases in the mitochondrial pathway.

The original approach by Jain and coworkers or variations on it have been used by others to study effects of solid compressive stress on multicellular tumor size. Solid compressive stress caused a decrease in tumor spheroid size formed by H4 and A172 brain cancer cell lines (Kalli & Stylianopoulos, 2018), breast cancer cell line BC52 (Delarue et al., 2014), mouse sarcoma cell lines AB6 and CT26, and the human colon carcinoma cell line HT29 (Delarue et al., 2014; Table 1). The mechanisms for these effects are not fully understood, but it is possible that some cancer cells may be arrested in mitosis. For example, confined HCT116 colorectal cancer cells are arrested in cancer spheroids due to perturbations of bipolar spindle assembly (Desmaison et al., 2013). Overall, mechanical confinement of tumor spheroids causes an increase in compressive solid stress, which inhibits cell proliferation and increases apoptosis (Table 1).

In addition to modulating proliferation and apoptosis, solid compressive stress impacts cancer cell migration. Compressive stress applied to breast cancer cells 67NR, MDA-MB231, and 4T1, promoted the formation of leader cells that promote coordinated migration (Tse et al., 2012). These effects depended on the cell type, as migration was actually impaired in noncancerous MCF10A cells and in noninvasive MCF7 cells (Tse et al., 2012). Migration also increased in glioma (H4) and pancreatic cancer cell lines CFPAC-1, PaCa-2, and BxPC-3 under compression (Kalli, Minia, et al., 2019; Kalli et al., 2018; Kalli, Voutouri, et al., 2019; Table 1). Cancer cell migration under confinement by solid interfaces formed by the tissue microenvironment

TABLE 1 Survey of papers that reported cancer cell responses (proliferation, migration, and apoptosis) to solid compressive stress or hydrostatic pressure

	Type of model		Mechanical stress		Stress parameters		Effect on			
Cancer cell type	2D in vitro culture	3D culture	Solid compr-essive stress	Hydro-static pressure	Magnitude (mmHg)	Duration	Proliferation	Migration	Apoptosis	References
H4 brain tumor		✓	✓		60	21 days	↓			(Kalli, Voutouri, et al., 2019)
A172 brain tumor		✓	✓		26	21 days	↓			(Kalli, Voutouri, et al., 2019)
MDA-MB 231 breast cancer		✓	✓			6 days		↑		(Liu, Lewin Mejia, Chiang, Luker, & Luker, 2018)
HCT116 colorectal cancer		✓	✓				↓		No change	(Desmaison, Frongia, Grenier, Ducommun, & Lobjois, 2013)
HT29 human colon carcinoma		✓	✓		35–75		↓		No change	(Delarue et al., 2014)
CT26 mouse colon adenocarcinoma		✓	✓		35–75		↓			(Delarue et al., 2014)
BC52 human breast cancer		✓	✓		35–75		↓			(Delarue et al., 2014)
AB6 mouse sarcoma		✓	✓		35–75		↓			(Delarue et al., 2014)
LS174T human colon adenocarcinoma		✓	✓		45–120	30 days	No change		↓	(Helmlinger et al., 1997)
4T1, 67NR, MDA-MB 231 breast cancer	✓		✓		5.8	16 hr		↑		(Tse et al., 2012)
MCF 10A, MCF7 breast cancer	✓		✓		5.8	16 h		↓		(Tse et al., 2012)
67NR breast cancer	✓		✓		> 5.8–58	16 hr		↓	↑	(Cheng et al., 2009; Tse et al., 2012)
H4 brain tumor (glioma)	✓		✓		4	16 hr		↑		(Kalli, Voutouri, et al., 2019)
A172 brain tumor	✓		✓		4	16 hr		No change		(Kalli, Voutouri, et al., 2019)
CFPAC-1 (pancreatic cancer)	✓		✓		1–6	6 hr		↑		(Kalli, Papageorgis, Gkretsi, & Stylianopoulos, 2018)
PaCa-2/BxPC-3 (pancreatic cancer)	✓		✓		4	16 hr		↑		(Kalli, Minia, et al., 2019)
hTRET-AM Ameloblastoma epithelial cells	✓			✓	30–90		↓	↑		(Yang et al., 2018)
CL1-5 & A549 lung cancer cells	✓			✓	0–20	8 hr	↑	↑		(Kao et al., 2014; Kao et al., 2017)
SCC-4/SCC-9 oral squamous carcinoma	✓			✓	0–30	24 hr	↑	↑		(T. Yu et al., 2013)
U2OS osteosarcoma	✓			✓	0–50	72 hr	↑		↓	(DiResta et al., 2005; Nathan et al., 2005)

(Continues)

TABLE 1 (Continued)

Cancer cell type	Type of model		Mechanical stress		Stress parameters		Effect on			References
	2D in vitro culture	3D culture	Solid compressive stress	Hydro-static pressure	Magnitude (mmHg)	Duration	Proliferation	Migration	Apoptosis	
SaOS2 osteosarcoma	✓			✓	0–50	72 hr	↓			(DiResta et al., 2005)
HOS osteosarcoma	✓			✓	0–100	72 hr	↓		↑	(DiResta et al., 2005; Nathan et al., 2005)
MCF7 breast cancer	✓			✓	0–100	72 hr	↓			(DiResta et al., 2005)
H1299 lung carcinoma	✓			✓	0–100	72 hr	↓			(DiResta et al., 2005)
A431/A 549 epithelial tumor		In vivo		✓	10/5		↑			(Hofmann et al., 2006)

Note: Model systems used, magnitude of stress and time of stress application are also included.

requires the displacement of these surfaces by the migrating cell. This should place greater energy demands on cancer cells. Consistent with this, MDA-MB-231 breast cancer cells moving through confined spaces have been found to consume more energy for migration (Zanotelli et al., 2019). The energy demands scaled with cell stiffness and with matrix stiffness. Consistent with these findings, migration of different cells through confined environments correlates inversely with stiffness of the cell and nuclear volume (Lautscham et al., 2015) and the levels of nuclear lamins, lamin A/C, and lamin B2 (Vortmeyer-Krause et al., 2020; Wolf et al., 2013).

The extent to which confinement in solid tissue microenvironments triggers shared pathways in invading cancer cells and cancer cells in solid tumors is presently unclear and deserves further investigation.

3 | CANCER CELL RESPONSE TO HYDROSTATIC PRESSURE

3.1 | Methods to apply hydrostatic pressure to cells

To study the response of cancer cells to elevated IFP, in vitro studies have studied the impact of hydrostatic pressure on cancer cells in culture. The application of hydrostatic pressure to cells in vitro is simpler compared to cell confinement (Figure 1c), and is typically achieved by connecting an external liquid reservoir (Haberstroh, Kaefer, Retik, Freeman, & Bizios, 1999; Mandal, Shahidullah, & Delamere, 2010) or syringe pump (Liu et al., 2010; Daisuke Yoshino, Sato, & Sato, 2015) to the cell culture dish (Kao et al., 2017), or alternatively through applying pneumatic compression (S. Liu et al., 2019; Shang et al., 2021; Stover & Nagatomi, 2007; J. Yu et al., 2011). Limitations of pneumatic compression include potential increases in the dissolved concentration of gases and related changes in pH, while connections to closed liquid pump systems can result in longer-time decreases in dissolved gas concentrations. Ruling out such complications is important for reliable interpretation of results.

3.2 | Effect of hydrostatic pressure on the cytoskeleton

Equation (2) can be similarly used to conceptualize the effect of external hydrostatic pressure; P_c can be interpreted as extracellular hydrostatic pressure in the equation. Instantaneous response to a step increase in hydrostatic pressure can be an increase in the intracellular pressure (Kao et al., 2017), while longer-time scale cellular adaptations could include a slower increase in the cell volume through changes to aquaporin 1 expression (Kao et al., 2017), which would result in a reduction in the curvature of the cell periphery and the reaching of a new equilibrium (Equation (2)).

Cancer cell cytoskeletal responses to hydrostatic pressure are relatively under-studied in the literature. At least one study suggested

that cancer cells respond to hydrostatic pressure differently from noncancerous cells. Unlike normal bronchial epithelial cells, CL1-5 and A549 lung cancer cells responded to 20 mmHg pressure applied with a syringe pump, by assembling F-actin containing filopodia (Kao et al., 2017). A large number of studies that applied pressure through an external liquid reservoir or pump, have examined microtubule behavior at pressures of the order of MPa (Gao et al., 2018; Nishiyama, 2017; Nishiyama, Kimura, Nishiyama, & Terazima, 2009; Nishiyama, Shimoda, Hasumi, Kimura, & Terazima, 2010), but these pressures are orders of magnitude larger than those prevalent in cancers in vivo (Stylianopoulos et al., 2018).

3.3 | Effect of hydrostatic pressure on cancer cell migration and proliferation

The elevated pressure in solid tumors in vivo is spatially uniform through the majority of the tumor, and declines rapidly toward the periphery (Figure 1b; Boucher et al., 1990). The decline in the pressure drives outwardly directed fluid flows in the peripheral region (Boucher et al., 1990). Fluid flows can exert shear stresses tangential to the cellular surfaces which can trigger molecular cellular responses that are distinct from responses to hydrostatic pressures which act normal to the cellular surface. There are at least two types of studies in the literature in the context of cancer cellular responses to fluid pressure: those that involved flows under pressure gradients imposed across cells embedded in 3D extracellular matrices (e.g., Polacheck, Charest, & Kamm, 2011; Polacheck, German, Mammoto, Ingber, & Kamm, 2014; Tien, Truslow, & Nelson, 2012), and studies in which a hydrostatic pressure was applied to cells in the absence of any flows (e.g., Kao et al., 2017). Here we focus specifically on papers where the cellular responses were solely due to hydrostatic pressure and not flow.

Application of hydrostatic pressure to cultured cancer cells alters their proliferation in a manner that depends on the magnitude of the pressure and on the cell type (DiResta et al., 2005). Pressures in the range of 100 mmHg suppressed the proliferation of cultured osteosarcoma cancer cell lines SaOS2 and HOS, breast cancer cell line MCF7 and lung cancer cell line H1299 (DiResta et al., 2005). Conversely, lower pressure ranges of 0–50 mmHg caused an increase in proliferation in some of these lines and a decrease in others (DiResta et al., 2005; Hofmann et al., 2006; Kao, Lee, & Kuo, 2014; Yu et al., 2013). Pressure ranges of 0–30 mmHg increased proliferation in oral squamous cell carcinoma cell lines SCC-4 and SCC-9 (T. Yu et al., 2013). Likewise, lung cancer cells CL1-5 proliferated more at elevated pressures in a similar pressure range (Kao et al., 2017).

The proliferation of the hTERT^{+}-AM epithelial cell line, on the other hand, was suppressed in pressure ranges of 30 mm–90 mmHg (Yang et al., 2018). Finally, relieving the tumor IFP in nude mice caused a decrease in the proliferation of epidermal carcinoma A431/A549 cells in the tumor cortex, which may be due to a decrease in IFP-induced stretching of cells (Hofmann et al., 2006). Overall, these contrasting results suggest that hydrostatic pressure is clearly important in terms of its impact on tumor cell proliferation, but whether it is pro- or anti-proliferative depends on the pressure magnitude and on the specific tumor cell types. Hydrostatic pressure may also promote tumor cell proliferation indirectly by modulating the release of pro-proliferative molecules by other cell types (Sottnik, Dai, Zhang, Campbell, & Keller, 2015).

Elevated hydrostatic pressure has been reported to increase cell migration in a range of cancer cell types, over a broad range of pressures (0–90 mmHg, see Table 1). Application of hydrostatic pressure to CL1-5 and A549 lung cancer cells caused an increase in cancer cell migration (Kao et al., 2017). Elevated hydrostatic pressure promoted migration and invasion of ameloblastoma cells by upregulating the expression of matrix metalloproteinases MMP-2 and MMP-9, which are targets of the Wnt signaling pathway (Yang et al., 2018). Pressure upregulated the expression of ~1,800 genes in SCC-4 and SCC-9 oral squamous cell carcinoma cells (Yu et al., 2013) associated with metastasis, the Wnt pathway and cell adhesion pathways, consistent with the observed increase in cell migration.

Different noncancerous tissue cells have been reported to respond to hydrostatic pressure, including human chondrocytes (Correia et al., 2012) and human endothelial cells (Prystopiuk et al., 2018; Shin, Bizios, & Gerritsen, 2003; Yoshino & Sato, 2019). In contrast with these and the above studies, one study reported no effects of hydrostatic pressure in the range of 100 mmHg on the F-actin cytoskeleton, nor on cell functions like proliferation or apoptosis in endothelial cells or neuronal cells (Tworkoski, Glucksberg, & Johnson, 2018). Other studies have similarly raised uncertainty about whether there are any effects of hydrostatic pressure on cell function at all (Astafurov et al., 2014; Osborne et al., 2015). The reasons for the inconsistencies remain unclear.

4 | CONCLUSIONS AND FUTURE OUTLOOK

There is a growing body of evidence that solid compressive stresses and interstitial fluid pressure alter tumor cell behaviors like proliferation and invasion. The molecular mechanisms underlying these responses are not as well-understood. Studies so far suggest that response mechanisms are likely to be distinct depending on the cancer and cancer cell type, the type of mechanical stress, and the magnitude of stress.

Studies of cell responses to mechanical stresses have traditionally involved mechanical sensitization of cells over time scales of hours to a few days. Emerging evidence suggests however that cells may adapt to mechanical stimuli over periods of several days to weeks (reviewed in [Lele, Brock, & Peyton, 2020]). Pathways that mediate adaptation of cancer cells to mechanical stresses, such as Rho signaling, can protect cancer cells from therapy-induced death (Misek et al., 2020; Orgaz et al., 2020). Knowledge of the molecular mechanisms of long term adaptation of tumor cells (weeks to months) is crucial if clinical strategies that target cancer cellular adaptation pathways to mechanical changes in tumors (J. Zhang & Reinhart-King, 2020) are to become a reality.

Further, given that cancer cells in a tumor are genetically highly heterogeneous, it is possible that cancer cell responses to mechanical stresses depend on genetic heterogeneity. Mechanical stresses may act as agents of natural selection, causing evolution of cancer cell populations in the tumor. For example, we have shown that substrate stiffness can exert selection pressure on genetically variable fibroblast populations, resulting in the enrichment of specific genotypes over periods of weeks (Purkayastha et al., 2021). It is tempting to speculate that tumor mechanical stresses cause similar significant cancer cellular evolution, and that the resulting selected sub-populations are resistive to tumor therapies, or prone to higher invasion.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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How to cite this article: Purkayastha, P., Jaiswal, M. K., & Lele, T. P. (2021). Molecular cancer cell responses to solid compressive stress and interstitial fluid pressure. *Cytoskeleton*, 1–11. https://doi.org/10.1002/cm.21680