REVIEW

Interstitial fluid flow in cancer: implications for disease progression and treatment

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Abstract: As cancer progresses, a dynamic microenvironment develops that creates and responds to cellular and biophysical cues. Increased intratumoral pressure and corresponding increases in interstitial flow from the tumor bulk to the healthy stroma is an observational hallmark of progressing cancers. Until recently, the role of interstitial flow was thought to be mostly passive in the transport and dissemination of cancer cells to metastatic sites. With research spanning the past decade, we have seen that interstitial flow has a promigratory effect on cancer cell invasion in multiple cancer types. This invasion is one mechanism by which cancers can resist therapeutics and recur, but the role of interstitial flow in cancer therapy is limited to the understanding of transport of therapeutics. Here we outline the current understanding of the role of interstitial flow in cancer and the tumor microenvironment through cancer progression and therapy. We also discuss the current role of fluid flow in the treatment of cancer, including drug transport and therapeutic strategies. By stating the current understanding of interstitial flow in cancer progression, we can begin exploring its role in therapeutic failure and treatment resistance.

Keywords: tumor microenvironment, interstitial flow, therapeutics, invasion

Tumor microenvironment

Although cancer arises from transformation of single cells, once a tumor initiates, the surrounding tissue is altered to promote cancer survival and growth. This tumor microenvironment includes all of the cells and tissue components aside from the cancer. These components, including cells and extracellular matrix proteins, and growth factors, can vary based on the tissue stroma in which the cancer forms. That being said, there are parallels amongst the different solid tumors and their associated microenvironments that may impede the efficacy of cancer therapies. These include biophysical and biochemical barriers. Biochemically, cancer and cancer-associated cells secrete a number of cytokines to sustain their growth and development that can interfere with the success of antiproliferative therapeutics, including antimitotics, DNA intercalating agents, and receptor tyrosine kinase inhibitors.[1,2] Biologically, cancer-associated cells, such as fibroblasts and immune cells, can affect therapeutic resistance in ways that are still being elucidated.[1] Biophysical forces such as stromal stiffening,[3] interstitial pressure,[4] and fluid flow[5] have a number of effects on the ability of therapy to both reach the tumor microenvironment and induce the desired response in cancer cells.

Biophysical microenvironment

The biophysical microenvironment of the tumor has been best studied in breast cancer, the second most frequently occurring cancer next to skin cancer. Here we know that

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the tumor microenvironment becomes mechanically stiff due to accumulation and reorganization of extracellular matrix proteins and activation of stromal fibroblasts.[3] These changes in tissue reorganization have broad-reaching effects on cancer cells.[6] Stiffening of tissue has been correlated with a poor prognosis and softening of tissue reduces metastasis and disease progression in mouse models of breast carcinoma.[7] Not only has this effect been seen in breast cancer, but has been used as a diagnostic and surgical tool in many other cancers, including those of the brain, skin, liver, and lung, where the mechanical stiffness of the native tissue can increase up to 100 times that in the healthy state.[8,9]

The tissue in which a cancer develops is a confined space with limited inlets and outlets for cells, fluids, and waste. As the cancer grows, it pushes on the surrounding environment until it eventually invades the tissue. This process results in a buildup of pressure from the inside out and can lead to a vast pressure differential between the tumor and the tissue in which it resides. This increased interstitial pressure has been studied for over 30 years, with documentation in human clinical samples and mouse models of cancer.[10–12] Heldin et al recently identified this high pressure as a crucial factor in therapeutic failure for a number of reasons, and Ariffin et al have nicely reviewed the implications of and strategies to reduce interstitial pressure in cancer.[4,13] The high pressure of the tumor next to the normal pressure of healthy tissue leads to pressure gradients resulting in the development of fluid flow through the tumor stroma.[14] This increased fluid flow was first documented in murine mammary tumors,[15] and has since been documented using fluorescence recovery after photobleaching in neoplastic tissue in rabbit ears and via magnetic resonance imaging (MRI) in multiple models of cancer.[16,17] Table 1 outlines the studies that have, to date, quantified interstitial flow (IF) in tumors, along with the methodology used and the measured flow velocities.

One observation is that there are relatively few experimental measurements of IF in tumors, and even fewer in normal tissues. With the measurements that have been made, the velocities in animal models are an order of magnitude or more lower than the measurements made in human patients with cervical carcinoma. Some of these differences may arise from the particular animal models used, such as xenografts implanted ectopically into nude mice[18,19] or cells injected into a rabbit ear chamber.[16] These tumors and their surrounding microenvironments may be substantially different from spontaneous human tumors, particularly in those cases where the immune system is compromised and cells are implanted in sites other than their tissue of origin. Nonetheless, some of these experimental measurements of interstitial pressure and flow have been corroborated by a variety of mathematical models of the tumor fluid environment. These models predict maximum IF velocities in the range of 0.01–1 µm per second. One common theme shared by these mathematical models and the aforementioned experimental measurements is that peak velocities occur at the edge of the tumor, where the pressure gradient is steepest.[20–23] However, each of these mathematical models is limited by their specific assumptions regarding tumor geometry and hydraulic properties of the tumor and the surrounding stroma, which strongly affect predicted IF velocities. In most cases, the significant heterogeneity of the tumor and its microenvironment are not captured in any meaningful way. Based on these mathematical models, as well as the few experimental measurements of IF that have been made, it is typically assumed that IF velocities range from 0.1 to 10 µm per second; however, it is possible that these results are biased by the use of models of human cancer, rather than naturally occurring human cancers. Thus, while these studies suggest that IF is altered in the tumor microenvironment, they also emphasize the dearth of information available regarding IF in tumors and

Table 1 Measurements of interstitial flow in vivo for different cancer types

Cancer and model	Method	Interstitial flow velocity (µm/sec)	Reference
MTW9 and Walker 256 mammary carcinomas in rats	Implanted micropore diffusion chamber	4–5 times greater interstitial drainage in tumors	Butler et al[15]
Rabbit ear chamber with normal and VX2 carcinoma neoplastic tissue	Fluorescence recovery after photobleaching	Normal: 0.59±0.16 Neoplastic: 0.55±0.16	Chary and Jain[16]
Inducible VEGF 165-expressing C6 tumors in mice	Dynamic contrast-enhanced MRI	No VEGF 165: ~0 VEGF 165: 0.1–0.5	Dafni et al[19]
TS-415 human cervical carcinoma xenografts in mice	Dynamic contrast-enhanced MRI	2–8	Hompland et al[18]
U-25 human melanoma xenografts in mice		1–8	
Locally advanced squamous cell carcinoma of the uterine cervix (human patients)		Nonmetastatic: 5–25 Metastatic: 10–55	

Abbreviations: MRI, magnetic resonance imaging; VEGF, vascular endothelial growth factor.

the effects that this flow can have on cancer. This review focuses on the current data elucidating the role of IF on cancer progression and therapeutic approaches, ending with an outlook of the unstudied aspects of IF in cancer and how it might be contributing to treatment resistance.

Origins of interstitial flow in cancer

Interstitial fluid refers to the fluid that is found in the stroma of tissue. In healthy tissue, this fluid results from an influx of plasma from the blood vasculature as nutrients and oxygen are transported into the stromal space and from cells as they undergo metabolism.[24] The majority of the fluid that leaks from the blood capillaries is reabsorbed by the post-capillary venules, but a fraction of the fluid filters through the interstitium and drains through the lymphatic vasculature downstream, eventually emptying into the venous bloodstream. Under healthy conditions, this system prevents buildup of fluid in the interstitial space. In cancer, the amount of fluid is greatly increased and poorly drained, resulting in an increase of interstitial pressure in the tumor that leads to a steep gradient of pressure between the tumor and the surrounding healthy tissue.[10] This gradient results in higher interstitial fluid flow into the healthy tissue and draining lymphatics.

In cancer, there are a host of irregularities in the vasculature that contribute to the malfunction of this perfectly coordinated system and lead to increased flow rates through the tissue. Angiogenesis and lymphatic co-option are correlated with poor tumor prognosis and progression of cancer, and are the primary contributors to the changes in fluid flow and interstitial pressure in the tumor microenvironment.[25]

Angiogenesis is the generation of new blood vessels from existing vessels and occurs in and around the periphery of the tumor. Tumor cells secrete a number of factors to induce angiogenesis, most notable of which is vascular endothelial growth factor.[26] Vessels that develop in the tumor microenvironment are largely irregular. They are marked by large gaps in the endothelial cell layer, reduced pericyte and smooth muscle coverage, and oddly sized and shaped structures.[27] The increased vessel number together with increased hydraulic conductivity, or the relative ease with which fluid moves across the vessel wall, results in an irregular and increased influx of fluid into the tumor stroma.[17] Modeling approaches for IF have shown that the irregularities in tumor vasculature are tightly linked to changes in interstitial pressure and fluid flux.[28,29]

Unlike angiogenesis, which is seen in a majority of solid tumors, intratumoral lymphangiogenesis, or the growth of new lymphatic vessels, is rarely seen.[30] Tumors rely on lymphatic vessels already present in the co-opted tissue to drain the fluid from the cancer. These vessels enlarge, become more permeable, and have increased drainage capacity as the cancer progresses.[31] In some cancers, there are increases in lymphatic vessels peritumorally or in the draining lymph node that can contribute to altered drainage in the peripheral tissue. However, these new vessels do not effectively balance the increase in fluid from the blood vessels, resulting in an overall pressure increase in the tumor bulk with a steep decrease in pressure at the periphery.

This increased pressure is due not only to the fluid that cannot properly drain out of the interstitium, but also to a number of other physiological changes in the tissue microenvironment. This includes both the increase in cell number and the increase in extracellular matrix deposition in the stroma.

Cell growth

The best known hallmark of cancer is the unchecked growth and proliferation of the transformed cancer cells.[32] As cancer cells expand in a confined environment, they press on the surrounding stroma and extracellular matrix. This leads to an increase in the elastic stress on this tissue, and since the area cannot expand, translates to an increase in the intratumoral pressure.[33] This pressure increase has been documented in numerous cancers and can be 10–100-fold greater than that of the healthy surrounding tissue.[10,12,34,35] This high pressure differential drives fluid flow at the periphery of the tumor mass corresponding to the invasive front. Interstitial fluid flows from the tumor toward the healthy tissue and eventually empties into the tissue lymphatics.[33] Aside from the expansion of the cells, the increase in metabolic activity of cancer cells yields an increase in extracellular secretion and accumulation of biomolecules and electrolytes that enhance the overall mass of the tumor. Stromal cells and infiltrating immune cells respond to the cytokines secreted in the tumor microenvironment, contributing to the cell-associated pressure and expansion of the tissue.[36]

Extracellular matrix

Cancer and stromal cells in the tumor microenvironment alter the extracellular matrix components as they grow and invade the healthy tissue. This includes irregular deposition and degradation of protein components, such as fibronectin and collagen, and glycosaminoglycans such as hyaluronan.[37] Stromal cells in the activated microenvironment alter the architectural and physical properties of the extracellular matrix, crosslinking, and aligning fibrils. These changes

contribute to the characteristic stiffening of the tumor that is well documented as a prognostic and diagnostic indicator in multiple solid cancers.[3] Due to these changes in the extracellular matrix structure, and in spite of the enhanced deposition of extracellular matrix proteins, the hydraulic conductivity increases in the tumor towards healthy tissue.[38] These changes in extracellular matrix contribute to the enhanced invasion and metastasis that correlate with heterogeneous fluid flow pathways through the tissue.[21,39]

Interstitial flow and progression of cancer

Fluid flows from solid cancers have been documented in animal models and clinical settings. The high interstitial pressures result in efflux of fluid from the tumor into the surrounding tissue, resulting in increased swelling of the draining tissues in lymph nodes or ventricular spaces of the central nervous system.[40,41] Understanding the role of IF in the progression of cancer is expanding. Its incorporation and measurement in vitro and in vivo is important to properly developing and implementing new therapeutic strategies.

Effects on cancer cells

The direct effects of IF on cancer cells are the best studied phenomena thus far. Using two-dimensional and three-dimensional tissue culture models with controlled fluid flow and pressure differentials has allowed examination of a number of malignant behaviors. The development of microfluidic devices and cell analysis strategies has been integral to the understanding of the behavior of cells in the microenvironment and has recently been reviewed by Wu and Swartz.[42] Recent experiments examining multiple models of cancer and the response to interstitial or low shear flows are summarized in Table 2.

Invasion

Cancer cell invasion and migration has been the best studied of the phenomena related to cancer progression. The role of flow appears to be primarily promigratory; however, other microenvironmental factors can influence how the cell detects and responds to this stimulus.[43] Breast cancer cells exposed to IF in a three-dimensional collagen microenvironment show increased migration both in three-dimensional tissue culture inserts and in specially designed three-dimensional flow chambers.[44,45] Similarly, glioma cells have been shown to increase migration in the presence of IF in multiple ways. First, glioma cells exposed to continuous flow in the absence of chemokine gradients were shown to have enhanced cell

motility and directional cell migration in hyaluronan matrices.[46] Second, glioma cells embedded in collagen gels showed enhanced chemotaxis after exposure to IF.[47] The enhanced motility seems to occur via several proposed mechanisms. Flow can trigger increased kinesis of cells via activation of surface receptors, such as the chemokine receptor CXCR4. It has also been proposed that small pericellular gradients can form around cells dispersed in a matrix (like what is seen at the leading invasive edge of a solid tumor) to yield a chemokine gradient across the body of the cell. This gradient can induce directional migration through a mechanism termed "autologous chemotaxis".[48] Studies using higher concentrations of cells and exposing them to pressure gradients have shown changes in cell migration, but no directional migration.[49,50] This could indicate that the chemokine-induced changes may only be relevant at the leading edges of tumors, where the protein distribution is more heterogeneous and fluid flows are more marked.

In vivo, IF in tumors has been measured in human and animal cancers. This flow rate can also be estimated based on an observed drainage rate of dye from the tissue into the lymph or cerebrospinal fluid.[51,52] In melanoma, it has been noted that fluid transport in the interstitium and to the draining lymph nodes actually increases prior to lymph node metastasis of cancer cells.[53] In breast cancer, routes of extravascular fluid flux correspond to invasive fronts in mouse models of carcinoma,[51] and brain cancer invasion tightly corresponds to pathways of fluid drainage.[54] Elevated IF has also been correlated with lymph node metastasis in patients with cervical carcinoma, as well as xenograft models of cervical carcinoma and melanoma.[18] These results indicate the possibility of fluid flow as a prognostic indicator of eventual invasion and metastasis.

Proliferation and growth

Aside from invasion, cancer proliferation and growth are major causative factors in the progression of disease. Low shear flows and IFs have been implicated in inducing proliferation in many normal cells including osteoblasts,[55] adipose-derived stem cells,[56] and endothelial cells. In cancer, early evidence suggests that IF has effects on cellular proliferation and differentiation. In multiple cancer cell lines, application of shear stress resulted in G_2/M arrest, indicative of inhibition of differentiation of cancer cells.[57] This is the first evidence of fluid flows affecting the cancer cell cycle. Interstitial pressure changes are responsible for altering the invasive and proliferative phenotype of cancer cells towards malignancy.[58] These data indicate that IF may have an effect on proliferation and growth, which

Table 2 In vitro studies examining the effects of shear of interstitial flow on cancer cells

Cancer model	Description of flow	Experimental setup	Finding	Reference
MCF7 and ZR75-1 breast carcinoma; MDAMB435S melanoma (human)	Interstitial, continuous (0.2 μm/sec)	Tumor cells in collagen-matrigel matrices. Tissue culture inserts and radial flow chambers. Lymphatic endothelial cells incorporated as a downstream monolayer.	Invasion was increased with lymphatics or flow and exponentially increased with both. This could be blocked by blocking CCR7.	Shields et al[44]
MG63 and Saos2 osteosarcoma; SCC25 oral squamous carcinoma; SW1353 chondrosarcoma (human)	Shear flow (12 dyne/cm^2)	Monolayers of cells exposed to shear stress for 12–48 hours.	Shear stress induced G_2/M arrest and static conditions induced G_0/G_1 arrest. Effect is mediated by $\alpha_v\beta_3$ and β_1 integrins.	Chang et al[57]
MDAMB435S human melanoma with human dermal fibroblasts	Interstitial, continuous (0.5 μm/sec)	Cells cocultured in a collagen matrix in tissue culture inserts.	Synergistic increase in cancer cell invasion with addition of flow and fibroblasts. Fibroblasts migrate in a TGF-β-dependent and collagenase-dependent fashion.	Shieh et al[70]
U87 and U251 human glioma; CNS-1 rat glioma	Interstitial, noncontinuous (0.8–3 μm/sec)	Cells cultured in collagen in tissue culture inserts and exposed to flow. Followed by chemoattractant TGF-α exposure.	Glioma cell lines with low invasive potential show decreased chemotaxis after flow exposure. This is modulated by MMP activation and expression.	Qazi et al[47]
OVCAR-3 epithelial ovarian cancer (human)	Shear flow (0.5–1.5 dyne/cm^2)	Microfluidic chamber with seeded cells in two dimensions exposed to pump-driven shear flow.	Cells elongated and developed stress fibers in response to flow.	Avraham-Chakim et al[117]
RT2, C6 rat astrocytoma, U87 human glioma	Interstitial, continuous (0.7 μm/sec)	Tumor cells in three-dimensional hyaluronan-collagen matrices. Hydrostatic pressure and pump driven flow in tissue culture inserts, radial flow chambers, microfluidic devices.	Flow increased glioma invasion. This was mediated by CXCR4 activation and possible autologous chemotaxis.	Munson et al[46]
SN12L, SN12C human renal carcinoma; MDAMB435S	Interstitial, flow (1 μm per second) followed by static invasion period	Collagen matrices in tissue culture inserts. A period of flow was followed by a period of static with chemoattractant TGF-α for 24 or 48 hours.	Flow upregulates MMP-1 and MMP-2; inhibition of adhesion molecules or degradation of the glycocalyx inhibited invasion.	Qazi et al[64]
MDAMB231 human breast carcinoma	Interstitial flow (4.6 μm per second)	Cells in three-dimensional collagen matrices with pressure driven flow across matrix to induce flow.	IF induces reorganization of focal adhesions in an integrin-dependent manner. IF induces paxillin-dependent cell protrusions.	Polacheck et al[43]

Abbreviations: TGF, transforming growth factor; IF, interstitial flow; MMP, matrix metalloproteinase.

are integral to the testing and validation of antitumor therapeutic strategies.

Sensing of interstitial fluid flow

The mechanism by which a cell senses fluctuations in shear stress and IFs in the extracellular matrix has been better examined in endothelial cells than in cancer cells.[59,60] Recently, studies in cancer cells have indicated that the glycocalyx may be responsible for mechanosensing of flows.[61] The glycocalyx consists of a layer of glycoproteins, glycolipids, and proteoglycans that surrounds the cell membrane. This layer is integral to the function of endothelial cells, particularly in the regulation of cell–cell adhesion and immune detection.[62] Degradation of the glycocalyx layer with glycoprotein-specific enzymes resulted in diminished flow-enhanced invasion in three-dimensional models of cancer. The glycocalyx is important for integrin clustering and binding of cells to their surroundings.[63] If integrin binding and adhesion to the matrix is blocked, cells lose their mechanosensing properties leading to less aggressive disease.[7] Reorganization of integrin binding sites due to IFs is a hypothesized mechanism by which cells sense and respond to fluid flow.[64] Lastly, cancer cells have been shown to have a primary cila that can act as a sensor of the mechanical and

chemical microenvironment around each cell.[65] These cilia are vital to tissue development and homeostasis due to their involvement in cell signaling and may have similar signaling pathway effects in cancer.[66] Regardless of the mechanism, cell sensing of IF is an important attribute of all cells, but in cancer may be a possible pathway to enhance malignancy and resistance to treatment.

Activation of the tumor stroma

In addition to the direct effects of IF on cancer cells, fluid flow also has effects on the stromal compartment of tumors. Since the tissue stroma frequently encounters and responds to interstitial fluid flow, the primary stromal cells are sensitive to fluctuations in fluid changes. Many of the responses seen in cancer-induced fluid flows are similar to the responses seen in wound healing or infection.[67]

Activation of fibroblasts

Fibroblasts are sensitive to changes in fluid flows. During progression of cancer, fibroblasts are recruited and transformed to become cancer-associated fibroblasts.[68] These cells are activated, showing positive expression of cytoskeletal protein alpha-smooth muscle actin, development of stress fibers, and secretion of extracellular matrix proteins and cytokines, such as transforming growth factor beta (TGF-β). Fibroblasts in the absence of cancer cells will respond to high IF rates by becoming myofibroblasts and realigning the extracellular matrix.[69] When cancer cells and fibroblasts are cultured together, there is an enhancement of cancer cell invasion and fibroblast activation in response to IF.[70] This response is mediated by TGF-β. Activation of the tumor stroma, which can include fibroblast activation, can contribute to enhanced metastasis and is hypothesized to mediate much of the downstream immune, angiogenic, and biophysical activation of the tumor microenvironment.[71]

Angiogenesis and lymphangiogenesis

Interstitial flow in vitro increases angiogenesis and lymphangiogenesis in three-dimensional matrices.[62,72,73] Shear flows also increase endothelial cell sprouting, adhesion, permeability, and secretion of cytokines.[74,75] In cancer, it is hypothesized that increases in fluid influx from resident blood vessels will trigger and are necessary for angiogenic processes. Lymphatic endothelial cells exposed to shear or transmural flows undergo a host of changes, including increased immune cell adhesion, cancer cell adhesion, cancer cell transport, and secretion of CCL2, a tumor chemoattractant and proinflammatory chemokine.[60,76] In multiple ways,

blood and lymphatic endothelial cells work in conjunction with interstitial fluid flow to promote cancer. In studies of angiogenesis, we know that blood flow to the cancer area increases before angiogenesis begins via vessel co-option.[77] It is possible that other processes, including cytokine secretion and cell adhesion, start long before we see any evidence of new vessel sprouting. In this way, the interaction between the endothelial vessels that perfuse the stroma and the fluid flows that originate from them are cyclically connected to propagate interstitial fluid flows and progression of cancer.

Interstitial flow in the treatment of cancer

Changes in IF are important for the efficacy of cancer therapy in at times contradictory ways. Although we know that IF can have far-reaching, if not yet fully understood, effects on the progression of cancer, it can also be manipulated in the hope of promoting positive outcomes from treatment. Often changes are incorporated to better distribute therapy to the tumor, without a full understanding of how IF affects cancer.

Transport of therapeutics

Arguably, the greatest effect that IF can have on cancer therapy and, specifically the failure of cancer therapy, is its effect on the transport of systemically delivered therapeutics. Although it seems that IF should be advantageous in terms of getting therapeutics through the extracellular matrix and to invading cancer cells via convective forces, this is not the case. A high pressure gradient forms at the periphery of the tumor, yielding increased IF. However, there are substantial modeling data suggesting that the majority of the tumor, or tumor bulk, has a uniformly high pressure.[11,22] This increased pressure neutralizes or reverses the normal pressure differential that exists between blood vessels and healthy tissue, making it more difficult to transport molecules away from the vessel.[4,21,22] This results in a localized distribution of therapy around the entering blood vessels. This effect, combined with the heightened permeability of neoangiogenic blood vessels, has been called the enhanced permeability and retention effect.[29,78] Depending on the type of therapeutic being delivered and the intended target area of the tumor, this can be either advantageous or not.

In small molecule (<1 kDa) therapy, transport of the therapeutic is diffusion-limited. This means that although the molecule can exit the vessel and often diffuse towards areas of increased fluid flow, these flows do not alter the distribution of the therapy.[11] For larger therapies, such as immunotherapies

and nanoparticles, fluid flow can have a substantial effect on the distribution.[11] However, these therapies are subject to heightened retention near blood vessels, and thus rarely enter the area of high IF flows. Therefore, although IF should yield increases in drug distribution, systemically delivered drugs are limited at the tumor entry point. Thus, many adjuvant therapeutic strategies aim to either bypass this delivery route or alter it in order to re-equilibrate the drainage of the tissue to the advantage of drug delivery.[79]

Changes in IF with therapy

Although manipulation of fluid flows in cancers has obvious therapeutic implications, standard of care therapies have also been studied to understand the changes in the biophysical microenvironment that occur in patients. Few studies have looked at the effect of therapy on IF velocity. Strategies to target blood vessels, such as antiangiogenic drugs, have been shown to alter the tumor vasculature and decrease interstitial pressure after therapy.[80] Strategies to increase the temperature of the tissue (such as hyperthermia) appear to reduce interstitial pressure.[81] Treatment with standard therapies generally appears to reduce interstitial pressure and presumably IF due to the decreased pressure gradient. After radiotherapy in human xenografts, interstitial pressure decreased,[82] and standard anti-inflammatory therapies such as dimethylformamide and pentoxifylline showed similarly lowered interstitial pressures.[25] The studies of standard of care therapies and IF or interstitial pressure include patients who are receiving anti-inflammatory agents before any chemotherapy or radiotherapy, so it is difficult to assess the effect of therapeutics on IF.

Manipulation of IF as a therapeutic approach

Changes in IF in the tumor to better resemble that seen in healthy tissue stroma are gaining ground as potential therapeutic strategies. These treatment approaches are mostly experimental, but are seeing benefits in patient cohorts.[83] The ability to alter the transport of drugs into and through the tumor interstitium is the primary goal in these therapies. That being said, the method and target of the therapies vary and can limit their implementation for particular cancers.

Increasing fluid flow (convection-enhanced delivery)

Increasing the convective forces in tissues is a technique developed for the delivery of large macromolecules to the central nervous system.[84] This technique is advantageous for bypassing the blood–brain barrier and yielding a distributed profile of larger drugs. In cancers, a catheter is inserted either intratumorally or intrathecally, and drug is slowly infused over the course of several hours. This method can increase the distribution of drug by an order of magnitude.[85] This technique was developed over 20 years ago, and since then has been used in a number of clinical trials. Common chemotherapeutics, such as paclitaxel and BCNU, as well as immunotherapies against interleukin-4 and interleukin-13 have been delivered to patients and shown mild success in reducing tumor bulk.[86]

Unfortunately, this technique suffers from difficulties in catheter design and inefficient distribution of drug in the interstitium due to leakage.[87] The native fluid flow through the tumor tissue, and the potential impact of introducing new fluid flow into this tissue are not well studied. A better understanding of both the fluid flow resulting from direct infusion and the side effects of fluid flow could yield more efficacious implementation of convection-enhanced delivery for brain cancers.

Other cancers benefit from enhanced convection to distribute therapeutics. Besides direct fluid infusion, induction of convection using either heat or ultrasound can lead to higher distribution of drugs throughout tumors.[79,81,86–89] Hyperthermia-based techniques at low temperatures will induce mild convective fluid flows and lead to redistribution of systemically delivered therapies.[88] These changes may be due to alterations in vessel permeability and distribution rather than actual changes in fluid patterns in the tissue, but this remains to be determined.

Decreasing fluid flow

Although there are no identified direct targets to inhibit or reduce IF, techniques to reduce the pressure differential and subsequent increase in fluid flow between the tumor and the surrounding tissue have recently surfaced. The first evidence of benefit over 25 years ago showed that patients receiving angiotensin-converting enzyme inhibitors had an apparent reduction in incidence of cancer.[90] Use of these vasoconstrictors to increase blood pressure in infiltrating blood vessels is a strategy used to counteract the high pressure of the tumor. Use of angiotensin II receptor antagonists in conjunction with chemotherapy in nanoparticle and free form shows increased efficacy in animal models of cancer.[91,92] The change in blood influx reduces the overall interstitial pressure of the tumor, which reduces the fluid flow rate at the tumor border, altering the distribution of blood-delivered nutrients and oxygen throughout the tumor. Hence, increases in blood pressure in the tumor could have far-reaching effects on both the study

and treatment of cancers, and is thought to have a role in "normalizing" the tumor stroma.[79]

A secondary approach to potentially reduce fluid efflux from the tumor is to inhibit drainage via lymphatic vessels.[93] By inhibiting lymphatic activation, which includes increases in permeability, lymphangiogenesis, and vessel dilation, fluid drainage from the tumor is reduced.[94] As antibodies against vascular endothelial growth factor receptor-3, a primary receptor involved in lymphatic activation, move to the clinic, further information on the role of lymphatics in tumor normalization will be established.

Potential for therapeutics related to IF

The ability to manipulate flow through alterations in the biophysical microenvironment or through fluid influx and efflux in the tumor may offer another avenue for treating cancer. However, molecules related to the response of cancer cells to IF could also be used as potential novel therapeutic targets. When examining autologous chemotaxis, several molecules have been identified as modulating flow-enhanced invasion, including chemokine/chemokine receptor pairs.[44,46] These molecules offer potential targets to minimize the effects of IF on cancer progression. Similar co-expressed chemokine/receptor pairs could be identified in patients to facilitate personalized therapy. Biomechanical sensing is regulated by matrix binding integrins and glycans that form the matrix glycocalyx, and thus offer a potential means to reduce flow responsiveness in cancer cells. Inhibition of β1 or α3 integrins or degradation of glycans reduced invasion of cancer cells.[7,64] Novel therapeutics targeted at integrins or glycans have vast therapeutic potential to mediate flow responsiveness in cancer, and also for modulation of a number of other malignancy-associated behaviors.[95] Other adhesion molecules and sensing molecules including focal adhesion complex proteins such as focal adhesion kinase, and cytoskeletal assembly molecules are potential therapeutics that directly process fluid flow signals to cancer cells.

Recent studies have looked at the tumor secretome, or constituents of the interstitial fluid in ovarian,[96] lung,[97] and breast cancers.[98] Proteomic screens on lung pleural effusions and ovarian ascites fluids have revealed potential biomarkers in cancer-associated fluid with the potential for therapeutic development.[96,97] In addition to proteins, exosomes and microRNAs have been identified as playing a role in invasion and metastasis of cancer cells[99,100] and having interactions with the immune system.[101] The role of these markers and the potential they hold for diagnosis and therapeutic development

are still to be determined. The IF that carries these factors away from the tumors is important to their dispersal and targeting of healthy stromal cells and it is possible that inhibition or manipulation of these extracellular markers may have an impact on the role of IF in tumor progression.

Role of IF in therapeutic resistance: hypotheses

Although the distribution and delivery of therapeutics is an important aspect of cancer chemoresistance, there are numerous other mechanisms involved in the inefficacy of current therapeutic strategies. Contributors to therapeutic resistance have been extensively reviewed, and include cancer stem cells,[102] multidrug resistance,[103] hypoxia,[104] cancer cell invasion,[105] and more recently, stromal cells and the extracellular matrix.[106–108] Aside from cancer cell invasion, there is no evidence that IF affects other mechanisms of therapeutic resistance, although correlations and side effects of other therapies may indicate some role. Noncancerous stem cells, such as adipose-derived and mesenchymal stem cells, respond to interstitial fluid flows by proliferating. Cancer stem cells are found in areas of the tumor stroma near neoangiogenic blood vessels in the perivascular niche, where fluid flows will be high and nutrients enriched in both breast[109] and brain[110] cancers. There may be a mechanism of sensing by these cells to home to these niches and remain dedifferentiated, hinted at in shear flow studies indicating G_2/M arrest.[57]

Expression of multidrug resistance transporters and regulation of extracellularly displayed receptors for anticancer drugs have been found to be regulated by integrin clustering. This has recently been suggested to aid in enhanced chemotherapeutic efficacy in cancers pretreated to alter the extracellular matrix.[107,111] It is possible that IF may induce cancer cell resistance to chemotherapy through this type of extracellular matrix-mediated pathway. Similarly, hypoxia has been shown to have downstream effects that promote therapeutic resistance in multiple cancer types.[112] Hypoxic areas of tumors tend to exist in primarily static conditions for long periods of time. Cells in these microclimates of low oxygen express prosurvival proteins and mutate to avoid cell death. Increasing oxygenation to these areas could rescue these cells and make treatment more viable, but the effect of flow in these regions, which is largely static, would be interesting for both a therapeutic and biological understanding of cancer.

Lastly, the far-reaching effect of flow on the tissue stroma may be the most complex and difficult to understand.

It is undeniable that the tumor microenvironment, including the biophysical and cellular components, is affecting the success of anticancer therapy.[1,106,113] Early studies indicate that the tumor stroma can affect multidrug resistance,[1] be altered to create a better therapeutic response,[107,114,115] and act to initiate and progress cancer via multiple cellular pathways.[113] Biophysical parameters, such as matrix stiffness, are associated with therapeutic resistance in humans and animal models. This indicates a role of biomechanical factors in modulation of cancer cell behavior. We have seen already that multiple cell types, including fibroblasts and immune cells, in the tissue stroma will respond to IF by activating and altering the microenvironment for the benefit of the cancer cell.[116] A more holistic picture and experimental approach to therapeutic testing, diagnosis, treatment regimen including IF will yield a greater clinical outcome.

Conclusion

Biophysical effects on tumor progression and response to therapy are beginning to be elucidated. Increased stromal stiffness, interstitial pressure, and IF are apparent in a number of cancers, and increase with progression. Although there is increasing evidence that IF may be significant in the progression of cancer, it is still relatively limited and poorly understood. There is a need for research on the clinical and prognostic significance of changes in fluid flow and its relevance to progression of cancer and its response to therapy. There is a need for further understanding of the multitude of changes that occur when cancer tissue is exposed to the physiological force of fluid flow. There is also a need to understand how and when a cancer cell detects and responds to interstitial fluid and shear stresses beyond the current small number of studies. Although IF is not the single contributor to cancer metastasis and invasion in humans, it is an important part of the tissue stroma, be it healthy or malignant. In order to understand, develop, and model therapeutic delivery and efficacy against cancer, biophysical forces, including interstitial flow, must be taken into account.

Disclosure

The authors report no conflicts of interest in this work.

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