The Fluid Mechanics of Cancer and Its Therapy

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Abstract

Fluid mechanics is involved in the growth, progression, metastasis, and therapy of cancer. Blood vessels transport oxygen and nutrients to cancerous tissues, provide a route for metastasizing cancer cells to distant organs, and deliver drugs to tumors. The irregular and leaky tumor vasculature is responsible for increased interstitial pressure in the tumor microenvironment, whereas multiscale flow-structure interaction processes control tumor growth, metastasis, and nanoparticle-mediated drug delivery. We outline these flow-mediated processes, along with related experimental and computational methods for the diagnosis, predictive modeling, and therapy of cancer.
1. INTRODUCTION

Cancer is a complex phenomenon that can be characterized by a small set of hallmarks that point to a cascade of events from the molecular to the organismal level (Hanahan & Weinberg 2000, 2011). Cancer cells have been found to employ over 11,000 genetic mutations to elicit tumorigenesis (Stoler et al. 1999). This bewildering number of mutations, along with the corresponding wealth of molecular mechanisms, suggests that we should explore the existence of overarching principles and governing physical mechanisms that can be associated with these genetic mutations. As advocated by Folkman et al. (2000), considerable insight and therapeutic benefit may be gained by additionally using a supragenomic, constraint driven approach to cancer that circumvents the problem of genomic instability. Here, the focus is not on the gene alterations within tumor cells, but on physiological constraints imposed on the overall tumor system. Identifying where cancer is constrained is fundamental, because it is at the constraints that variability is reduced.

In the past decade cancer research has progressively adopted this view, integrating physics and mechanics with genomic investigations of cancer and its therapy (Michor et al. 2011, Suresh 2007a). In this review, we outline the role of fluid mechanics as an essential component in the growth, progression, metastasis, and therapeutic techniques for cancer. In writing this article, we recognized that there is a relatively small number of pioneering studies related to cancer from the fluid mechanics community (Popel & Gross 1979, Qutub et al. 2009, Yan et al. 1991). The lack of first principles and the multitude of genetic and molecular cascades may have been hindering factors. However, after a century of rapid advances in theory, numerical methods, hardware, and software, the fluid mechanics community has developed a powerful arsenal of multiscale imaging, analysis, and simulation tools that are highly suitable for the investigation of transport processes in cancer. Here we highlight some of the flow-related processes in cancer, hoping to facilitate the development of a common ground for fluid mechanics and cancer biology researchers.

The interweaving of fluid mechanics in studies of cancer has a long history. Around 160 AD, Claudius Galen, who was possibly motivated by the fluid mechanics-centric scientific world of his time, proposed that black bile was one of the cardinal fluids of living organisms (Mukherjee 2010). For centuries, the field of medicine accepted the existence of four humors until Vasellius’s anatomical studies confirmed that only three were circulating fluids in living organisms (blood, yellow bile/lymphatics, and phlegm). Black bile as a metaphor for disease transported through the cells and organs of a living organism, however, remains relevant. The circulation of abnormal white blood cells, discovered and named leukemia by Rudolf Vichrow in 1847, is one of the most well-studied and today curable forms of cancer.

Blood flow is the essential process of life, and unsurprisingly, it has an important role in many forms of cancer. Blood flow provides oxygen and nutrients to tumors, whereas flow patterns in blood and lymphatic vessels determine the routes of metastasizing cancer cells. Folkman et al. (1971) pioneered research on tumor angiogenesis, the co-option of blood vessels by tumors, which is considered a fundamental aspect of tumor progression and therapy (Carmeliet 2005). The blood vessels in the vicinity of cancer tumors exhibit irregular form and function characterized by abnormal endothelial cell (EC) stratification, altered basement membranes, large gaps between ECs of the vasculature, blood vessel tortuosity, and blood flow irregularities. The large gaps allow blood constituents to extravasate in the tumor microenvironment, thus increasing the interstitial pressure, which in turn affects chemical signaling in the tumor microenvironment. Migrating
tumor cells may intravasate into the vasculature, thus facilitating the hematogenous process of tumor metastasis.

Studies of transport phenomena in blood by Poiseuille in 1846 led to the experimental formulation of one of the most fundamental laws of fluid mechanics, whereas the seminal studies of Krogh (1922) formulated the diffusive transport of oxygen around blood capillaries (reviewed in Egginton 2011). Over the past three decades, Jain and coworkers have made groundbreaking contributions, pioneering the use of fluid mechanics concepts as a complement to genomic and molecular signaling studies for cancer research (Chauhan et al. 2011; Goel et al. 2011; Jain 1988, 1990; Jain et al. 2007). Fluid mechanics of the cancer microenvironment is also prominent in the work of Maeda et al. (2001). With the introduction of the enhanced permeability and retention (EPR) effect, Maeda and colleagues argued for the important role of fluids extravasating from the tumor vasculature and resulting in increased interstitial pressure, a dominant factor in the tumor microenvironment and a guiding principle for cancer therapy.

In this review we highlight aspects of tumor inception, growth, metastasis, and therapy that have direct relevance to flow-related processes in cancer. The article is structured as follows: Section 2 briefly summarizes the genetic and molecular aspects of tumor growth. The tumor vasculature is discussed in Section 3, distinguishing between different modalities of angiogenesis, blood vessel structure, and flows in tumor-induced vascular networks. Section 4 focuses on the process of hematogenous metastasis. We note that important fluid mechanics processes are encountered in lymphatic metastasis, and we refer the reader to a recent review on this subject (Swartz & Lund 2012). Therapeutic techniques that rely largely on the fluid mechanics of vascular transport and extravasation for drug delivery to tumors are discussed in Section 5, whereas related experimental techniques on microfluidics are described in Section 6. Section 7 discusses modeling aspects of cancer fluid mechanics, and we close with a brief summary and outlook.

2. TUMOR GROWTH

A cell transits from its physiological state to a cancerous one by accumulating a set of mutations in its genome. These mutations are linked to genes that have the potential to cause cancer (oncogenes) and genes that play a role in actively preventing a cell from becoming cancerous (tumor suppressor genes) (Nordling 1953). Gene expressions are fundamental to cancer, but today it is recognized that cancer can be described as a complex system, whose components (known as the hallmarks of cancer) have been brilliantly described in the authoritative articles of Hanahan & Weinberg (2000, 2011), and they are briefly summarized below.

Cell proliferation is regulated by signaling pathways that are dependent on the release of growth factors from the tissue environment. Cancer cells acquire the capability of chronic, increased proliferation through accessibility and sensitivity to levels of growth factors, mutations in genes downstream of the growth factor signaling pathway, and alterations in the negative feedback mechanism that normally regulates cell proliferation. In combination with increased rates of proliferation, tumor cells develop mutations to evade mechanisms that negatively regulate cell proliferation. Along with gatekeeper genes that control cell-cycle progression, cell-cell adhesion has been identified as a mechanism that suppresses proliferation. Mutations in genes promoting contact inhibition can disrupt cell-adhesion-mediated regulation of excessive proliferation and neoplasia. The resistance to apoptosis (preprogrammed cell death) and necrosis (premature cell death) marks another stage on the path to cancer. It has been observed recently that necrosis causes the dying cells to release proinflammatory signals into the extracellular environment, stimulating immune response and invasion of inflammatory cells (Galluzzi & Kroemer 2008, Grivennikov et al. 2010). Controversially, the invasion of immune cells has been shown to promote proliferation and

Enhanced permeability and retention (EPR) effect: the extravasation of blood constituents from leaky tumor-induced vasculature and their retention in the tumor microenvironment, increasing interstitial pressure

Hallmarks of cancer: traits and acquired capabilities characterizing cancer, including self-sufficiency in growth signals, tissue invasion and metastasis, limitless replicative potential, and sustained angiogenesis

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facilitate migration of the tumor cells. Furthermore, mutations regulating the limiting mitogenic potential of tumor cells lead to unrestricted proliferation capabilities.

The mutations described above are associated with thousands of genes across different cell lines, and their combination enables uncontrolled tumor growth. As the tumor cells accumulate genetic mutations, the rate of mutations increases as the genomic maintenance machinery breaks down (Negrini et al. 2010, Salk et al. 2010). This leads to the evolution of a tumor consisting of a heterogeneous mass of distinct cell types intertwined with the extracellular matrix (ECM) (Egeblad et al. 2010).

Sustained metabolic activity inside the tumor requires oxygen and nutrients, which may be provided by diffusion through the surrounding perfused tissue. In this so-called avascular stage, tumor volumes usually do not exceed 1 mm³. The diameter of avascular tumors is determined by the diffusion limit of oxygen contained in the nearest blood vessels, which is of the order of 100–200 μm. At this stage, cells in the tumor’s interior that may be deprived of oxygen and nutrients undergo necrosis and start forming a necrotic core region. One of the responses of tumor cells to hypoxia is the secretion of proangiogenic factors into the extracellular space. These factors can stimulate existing blood vessels in the vicinity of the tumor to grow new blood vessels (see Figure 1) to provide the tumor cell with nutrients and oxygen, a process termed the angiogenic switch (Hanahan & Folkman 1996). Not only does the establishment of tumor-associated blood and lymphatic vessels, abnormal in form and function, promote tumor growth beyond the limits set by diffusion, the vasculature may also enable the systematic spread of the disease as carcinogenic cells may detach from the primary tumor and intravasate the vasculature. These circulating tumor cells (CTCs) can be trapped in capillaries, extravasate, find appropriate conditions for growth, and generate a new tumor, leading to cancer metastasis. Left untreated, these processes may continue until the death of the organism.

3. TUMOR VASCULATURE

Tumor angiogenesis refers to the process of blood vessel growth based on cues and actions induced by the tumor and its microenvironment (see Figure 1). Blood vessels are mediators of oxygen and
Tumor-induced angiogenesis: tumor-induced growth of abnormal vasculature from pre-existing blood vessels

VEGF: vascular endothelial growth factor

IA: intussusceptive angiogenesis

MMP: matrix metalloproteinase

Angiogenesis is one of the hallmarks of cancer (Hanahan & Weinberg 2000), an indication of a malignant development in primary and metastatic tumors, and, as such, a therapeutic target (Ferrara & Kerbel 2005, Folkman 2006). The increased vasculature of tumors has been observed since the beginning of the past century as tumors often bleed excessively when operated. The first ideas for tumor-induced angiogenesis were reported by Ide et al. (1939) and were followed by Algire & Chalkley (1945), who demonstrated that the origin of the vasculature was pre-existing blood vessels. Folkman et al. (1971) pioneered the notion that tumor growth is dependent on angiogenesis and that antiangiogenesis could be justified as a clinical tumor therapy. In a series of studies, Hanahan & Folkman (1996) established that in order for tumors to grow beyond 1–2 mm in diameter, it is important that they recruit their own vasculature. Vascular endothelial growth factors (VEGFs) (Dvorak 1986, Leung et al. 1989) are among the key components in this process. VEGFs diffuse through the ECM and bind to the receptors located on the ECs that line the blood vessel walls. In response to VEGF stimulation, ECs offset a cascade of events that leads to the formation of new blood vessels via sprouting or intussusception. In this review we focus on sprouting and intussusceptive angiogenesis (IA) as they entail flow-related processes, but a number of additional mechanisms may also lead to the formation of blood vessels (Carmeliet 2003, Goel et al. 2011, Ribatti & Djonov 2012) including the following: postnatal angiogenesis (the formation of new blood vessels from recruited bone marrow–derived endothelial progenitor cells), vasculogenic mimicry (the participation of cancer cells in vessel formation via transdifferentiation), and mosaic vessel formation (the incorporation of cancer cells into the vascular walls).

3.1. Fluid Mechanics and Modalities of Angiogenesis

Tumor phenotypic variations are reflected in a tumor’s angiogenesis. Certain tumors are hypovascularized (e.g., pancreatic ductal adenocarcinoma), whereas many others are highly angiogenic and densely vascularized (e.g., renal and pancreatic neuroendocrine carcinomas). Flow conditions in the interstitium and inside the existing vessels, in combination with the VEGF level and gradient orientation, have been found to guide the specific response (Carmeliet 2003, Liu et al. 2011, Song & Munn 2011).

In sprouting angiogenesis, new blood vessels sprout from the existing vasculature and grow to form a new network, characterized by intermittent and low-shear-stress conditions inside the vessel and a positive VEGF gradient (Song & Munn 2011). Figure 2 illustrates the steps involved in sprouting angiogenesis. VEGF signaling at the vascular wall initiates a signaling cascade that identifies single ECs as tip cells. Tip cells release proteases that break down the basement membrane, a component of the ECM stabilizing the vascular wall, and lead to detachment of the pericytes, which normally hinder microvessel expansion by more than 30%. EC exposure to the perivascular environment induces a migrative response of the tip cells. Migrating tip cells emit matrix metalloproteinases (MMPs) that degrade the collagen fibers of the surrounding ECM, facilitating the migration process. Proliferating ECs located in regions behind the tip cells lead to a propagation of the sprout as the tip cells move along VEGF gradients toward regions of higher concentration, a directed motion referred to as chemotaxis. Once capillary sprouts have reached a certain distance from the parent vessel, repeated branching of the tips can be observed. Branching is complemented by the fusion of sprouts and the formation of loops, a process known as anastomosis. The formation of lumen within the strands of ECs establishes a network that allows for blood circulation (Iruela-Arispe & Davis 2009, Reddy et al. 2007, Rutkowski & Swartz 2007). The initiation of blood flow leads to active vessel remodeling, maturation, and differentiation into venules and arterioles. During physiologic angiogenesis, the supply of oxygen and nutrients by the blood
Tumor-induced sprouting angiogenesis. Oxygen-deprived tumor cells release vascular endothelial growth factor (VEGF). Upon release, VEGF diffuses through the extracellular matrix (ECM) and initiates sprouting angiogenesis. (a) Tip cell selection and degradation of the basal lamina. (b) Migration, proliferation, and lumen formation, leading to sprout extension. (c) Branching and loop formation, establishing a connected vascular network.

vessels decreases the VEGF levels, completing the process of angiogenesis. In healthy organisms, angiogenesis maintains homeostasis and organismal integrity in situations such as wound healing and inflammation (Adams & Alitalo 2007, Carmeliet 2003, Chung et al. 2010, Goel et al. 2011, Potente et al. 2011). In contrast, tumor-induced angiogenesis is characterized by the persistence of the angiogenesis cascade that is continuously fueled by tumor secretions, further preventing the vessels from undergoing maturation.

IA (also known as intussusive microvascular growth or splitting angiogenesis) is broadly defined as the process of transcapillary pillar formation inside existing vessels that results in the formation of new vessels (Styp-Rekowska et al. 2011). IA involves three distinct steps: microvascular growth, arborization, and branching remodeling (Djonov et al. 2003). Whereas the progression stages in intussusive vessel splitting have been identified (see Figure 3), the molecular mechanisms of this process are not clearly understood. No particular molecule has been linked to this process as opposed to, for example, VEGF for sprouting angiogenesis.
Intussusceptive angiogenesis. The insertion of tissue pillars into vascular vessels leads to the splitting of existing capillaries. (a) Protrusion of opposing capillary walls into the vessel lumen. (b) Establishment of endothelial cell contact and the formation of adherens junctions. (c) Invasion of pericytes and fibroblasts that release extracellular matrix proteins and stabilize the newly established vascular branches.

Fluid mechanics phenomena have been demonstrated to play a fundamental role in IA. Experiments by Djonov et al. (2002) in the chick embryo demonstrated that an increase in blood flow and pressure in the artery had an immediate effect on its branching morphology. As reported by Djonov et al. (2002), IA occurs when opposite EC layers that protrude into the vessel lumen eventually connect, forming a pillar inside the vasculature. The locations of pillar formation are associated with regions of high velocity and low shear in the pre-deformed vasculature. Recent work by Hlushchuk et al. (2008) further elucidated the detailed progression of this process, using electron and confocal microscopy to examine malignant tumor tissue in mice. Vessels with high flow rates widen, and although such high flows are not associated with sprouting angiogenesis, they are associated with intussusception beyond a certain flow-rate threshold (Djonov et al. 2003). Vessel branching leads to a stress reduction in individual ECs while maintaining the overall flow rate. In contrast to sprouting angiogenesis, intussusception is energetically and metabolically more effective (Djonov et al. 2002, Makanya et al. 2009), as no massive proliferation and membrane degradation are involved in the process. Furthermore, sprouting angiogenesis proceeds on much larger timescales as entirely new vessels are grown compared with splitting existing ones (Burri et al. 2004).

One possible scenario for the initiation of IA involves the imbalance of forces experienced by the ECs due to blood flow, cell-cell connections, and the ECM. High velocity in the lumen interior reduces the interior pressure and as such creates a force imbalance that may be compensated by the shear stresses that can be supported by the vessel walls, according to the Young-Laplace equation (Davies 2005). If the structure of the EC layer cannot accommodate the increased pressure difference, the curvature increases locally, and the EC layer protrudes into the vessel. In turn, the narrowing of the passageway for the blood cells may decrease the pressure further, thus accelerating this process. The fusion of the two protruding ECs and the establishment of cell-cell adhesion sheets provide the initial stability for the pillar. Further pillar maturation occurring by the migration of fibroblasts and pericytes into the pillar and the subsequent deposition of ECM proteins increase the pillar’s stability (Paku et al. 2011).
3.2. Structure and Flow in the Tumor Vasculature

The vasculature inside tumors differs drastically from the networks observed in normal tissue in both morphology and structure. Tumor vasculature shows increased vascular density and branching patterns, distorted and enlarged vessels, and highly convoluted, often blind-ended segments (Goel et al. 2011, Narang & Varia 2011). Figure 4 depicts a schematic of the vessel structure and transport processes inside the tumor vessels.

In addition, the structure of the vessel walls in tumors is disrupted. Increased levels of growth factors lead to abnormal levels of EC proliferation. Vascular wall ECs often lack the coverage of perivascular cells, such as pericytes and smooth muscle cells, and tight adherens junctions that stabilize the vessel. The presence of large intercellular spaces renders the vessels leaky, allowing for enhanced macromolecule transport between the lumen and the extracellular space; offers ways for tumor cells to enter the vasculature; and leads to an increase in the interstitial pressure. Elevated levels of growth factor and vasodilation could also be related to the leakiness of the tumor vasculature (Narang & Varia 2011). Moreover, the extravasation of macromolecules due to tumor vascular leakiness plays a dominant role in passive tumor targeting of macromolecular agents (Maeda 2010). These alterations in the vasculature have a stringent effect on the observed flow patterns inside the tumor.

Flow patterns inside vascular networks have been found to control maturation, differentiation, and remodeling. It has not been fully resolved if the regulating function is attributed to mechanical cues, the oxygen delivery of the circulation, or signaling molecules. However, recent findings indicate that mechanical force transduction is necessary and sufficient to induce vessel remodeling in the mammalian yolk sack (Lucitti et al. 2007). Kim & Sarelius (2003) demonstrated that the blood flow shear rate and viscosity influence vascular function independently. The vascular shear rate has further been found to influence vascular lumen formation as well as EC proliferation and migration (Sun et al. 2007, Yamane et al. 2010), whereas pulsatile flow has been shown to stimulate angiogenesis in an in vitro environment (Cullen et al. 2002).

In the tumor-associated vasculature, the highly tortuous vessels increase the resistance to blood flow. Blood flow inside these networks is highly unstable and prone to alterations, even changes in direction (Nasu et al. 1999). In contrast to normal vascular networks, blood flow velocities do not correlate with the vessel diameter (Leunig et al. 1992). The leakage of blood plasma leads to an increase in the interstitial pressure, causing vessel occlusion and acute hypoxia, which leads to the
Persisting release of VEGF. In response, angiogenesis continues, the network structure constantly changes, and maturation is prevented, which in turn promotes vascular leakage. Consequently, flow-induced EC regulation and intercell communication in tumors are impaired. This promotes the formation of shunts (Pries et al. 2010), further deregulating a homogeneous flow distribution inside the tumor.

The transduction of fluid shear stresses on the EC layer is mediated by the glycocalyx layer (Kang et al. 2011). Alterations to cellular glycosylation have been recently observed in malignant cancer progression, contributing to tumor cell invasion, metastasis, and angiogenesis (Borsig 2011). The glycocalyx is a fibrous, brush-like structure of long-chained macromolecules and proteins that covers the luminal endothelium in a thin layer (with an estimated thickness between 60 and 570 nm) and is connected to the actin cortical cytoskeleton of the EC (Tarbell et al. 2005, Weinbaum et al. 2003). The presence of anionic oligosaccharides, such as hyaluronic acid and heparan sulfate, provides the glycocalyx layer with a characteristic negative charge. Stimulation of the glycocalyx layer has been linked to the production of nitric oxide (NO) (Tarbell et al. 2005), a molecule instrumental in the regulation of the mechanical response of ECs (Balligand et al. 2009). Besides mechanotransduction, the glycocalyx layer acts as a transport barrier and as a hydrodynamic interface for the interaction of the ECs with red blood cells (RBCs) and white blood cells. The glycocalyx can retain plasma proteins, and its thickness can be reduced by enzymatic and shear-induced shedding, producing a dynamic equilibrium between the composition of the glycocalyx layer and flowing blood (Reitsma et al. 2007).

In addition to the glycocalyx, the pathway of the fluid and solutes across the vessel wall consists of clefts between neighboring ECs. Detailed structural studies of venular microvessels in rat mesentery performed by Adamson et al. (2004) indicate that clefts are narrow spaces, typically 14–21 nm in width in normal vessels. When present, tight junctions occlude the space between the ECs, forming lines that run approximately parallel to the luminal surfaces of the endothelium. The tight junctions act as barriers for fluid and solutes, which are expected to bypass the tight junctions through the discontinuities or gaps between them. The average gap length is estimated to be 315 nm, and the average spacing between the gaps is 3,590 nm. Experimental studies by Schulze & Firth (1992) and Adamson et al. (2004) indicate that there is a periodic array of cleft-spanning structures that may fill the entire cleft. The radius of cleft-spanning molecules is estimated to be 1.25 nm, and spacing between them is approximately 15 nm. Experimental measurements describing the structure of clefts, fiber matrices in the glycocalyx, and cleft-spanning structures are summarized by Sugihara-Seki et al. (2008).

### 3.3. Flows in Tumor Microvascular Networks

The dynamics of blood flow in a capillary network is much more than just the sum of viscous flows through interconnected narrow pipes. The presence of blood cells affects flow resistance in individual vessels, which varies with several factors, including the vessel diameter, hematocrit, and flow velocity. The distribution of blood flow and RBC flux in a microvascular network depends on the network architecture, the flow resistance in each segment, and the partition of RBCs in diverging bifurcations. Microvascular network architecture and hemodynamics exhibit a high degree of heterogeneity (Pries et al. 1996).

We note that the usually employed estimates of mean parameters for flows in microvascular networks do not provide a sufficient basis for the quantitative analysis of network functions. Estimates of derived quantities based solely on the mean values of the underlying parameters may be erroneous (Pries et al. 1995, Vicaut 1986). For example, blood flow through a vessel is nonlinearly related to vessel diameter, so an estimate based on mean diameter may be incorrect.
Furthermore, the hematocrit profile inside individual vessels varies along the axial direction with the developing flow, whereas the RBC partitions between the daughter branches are in general a function of network architecture. Similarly, solute clearance in a vascular segment or bed is nonlinearly dependent on its permeability–surface area product and the plasma flow (Renkin 1985), and hence its estimate requires consideration of the distribution of these variables (Pries et al. 1996). The tendency of RBCs to follow the higher-flow pathway at each bifurcation results in a strong correlation between the hematocrit and flow velocity. As a result, the transit time through the microvascular network calculated from average values of flow velocity and vessel segment length measured in single vessels can significantly overpredict the true mean value determined by an analysis of the network. This correlation also causes a reduction of up to 40% of the average capillary discharge hematocrit \((H_D)\) compared to the hematocrit of blood flowing through the feed arteriole (the network Fähræus effect) (Levin et al. 1986, Pries et al. 1995).

In tumor-induced vasculature, the composition of the blood plasma may be altered because of an increased presence of fibrinogen and serum globulin, leading to higher viscosities. High viscosity in tumor vessels may also be attributed to the leakage of the vasculature, which may not allow the thin cell-free layer at the vessel wall to be sustained, leading to disorganized flow of the blood in the tumor-induced vessels and high apparent viscosity. The discrepancy in viscosities between healthy and pathological blood flow may impact the routes taken by the blood, with pathological blood preferring larger channels of communication such as arteriovenous anastomoses, as discussed by Fähræus in Frey-Wyssling (1952).

The large diversity of factors that affect the flow in tumor-induced vasculature is reflected by the scatter of data regarding flow rates observed by confocal and multiphoton microscopy for in vivo tumor vasculature (Kamoun et al. 2010). Accurate estimates of flow parameters will require detailed analysis, taking into account the geometry of the network, the particular nature of blood and properties of plasma, and leakage in the tumor microvasculature.

### 3.4. The Tumor Microenvironment

The tumor microenvironment comprises the surrounding tissue cells and the ECM, including a plethora of matrix-bound and soluble growth factors. Fibroblasts, the most abundant tissue cell in the tumor microenvironment, secrete the ECM molecules that compose the tumor stroma. Typically, these structures are altered by cancer cells and their interaction with the microenvironment, leading to an increase in the observed stress, a stiffened ECM, and elevated fluid pressure and interstitial flow (see Figure 5). These mechanical changes to the tumor environment have been shown to play an important role in tumor growth, angiogenesis, tumor invasion, and metastasis (Shieh 2011, Stylianopoulos et al. 2012).

Solid radial stresses in the tissue surrounding the tumor and increased stress inside the tumor are direct consequences of the highly proliferative tumor cells. These forces are further magnified by a stiffening of the ECM and tissue environment. It has been shown that an increase of mechanical stress on tumor cells can cause mesenchymal transition (Gjorevski & Nelson 2010, Gomez et al. 2010, Nelson et al. 2008), modulate cell-cell and cell-matrix adhesion, and trigger changes in gene expression related to invasion and metastasis (Demou 2010). Next to the tumor cells, the cells in the surrounding tissue are exposed to an elevated stress level. Fibroblasts react to this stress and release growth factors, chemokines, and proteases that can promote tumor growth, angiogenesis, and invasion (De Wever et al. 2004, Orimo et al. 2005, Singer et al. 2002). Elevated collagen levels in the ECM produced by the fibroblasts, in addition to contractions that rearrange the ECM fibers, lead to ECM stiffening, further assisting cancer cell migration. These contractile forces have also been shown to facilitate neovascularization of the tumor (KilarSKI et al. 2009).
Figure 5
Mechanical properties of the tumor microenvironment: radial solid stress exerted by the growing tumor (gray arrows), enhanced extracellular matrix (ECM) stiffness (gray fibers), elevated levels of interstitial pressure (blue arrows), and increased interstitial flow (red, purple, and yellow arrows).

Elevated collagen concentrations in the ECM and enhanced tissue stiffness are known risk factors in breast cancer (Provenzano et al. 2009). In vitro experiments have elucidated the importance of substrate elasticity in cell locomotion, differentiation, and proliferation (Engler et al. 2006, Klein et al. 2009). These findings suggest that the alterations in matrix stiffness via active ECM remodeling have an important effect on the microenvironment, leading to increased proliferation of cancer cells and migration of cancer cells, fibroblasts, and immune cells (Hadjipanayi et al. 2009, Levental et al. 2009, Lo et al. 2000, Ulrich et al. 2009). A mechanism of directed migration toward regions of higher matrix stiffness, termed durotaxis, has recently been described by Hadjipanayi et al. (2009) and Lo et al. (2000) and could have an important effect in cancer invasion into the stroma tissue.

High levels of interstitial pressure inside tumors are a consequence of the leaky vasculature inside and surrounding the tumor and have been for years a limiting factor for the successful delivery of therapeutic agents. The increase in pressure has been reported to stimulate proliferation (DiResta et al. 2005, Hofmann et al. 2007, Nathan et al. 2005) and regulate the secretion of angiogenic factors (Nathan et al. 2008). Next to blood vessels, tumors often recruit their own lymphatic system, which drains excessive fluid from the interstitial tissue (Swartz & Lund 2012). In combination with the elevated pressure, the draining lymph nodes establish an interstitial flow.
EMT: epithelial-mesenchymal transition

from the tumor toward the lymphatic vessels. The shear induced by this flow has been linked to the activation of fibroblast differentiation, ECM stiffening, and enhanced lymph angiogenesis. More importantly, the altered flow conditions inside the tumor microenvironment change the gradients of diffusing cytokines and growth factors. For tumor cells secreting chemokines, the flow establishes a small but detectable gradient across the cell, sufficient to trigger a chemotactic response (Shieh 2011), guiding cell migration from high-pressure regions inside the tumor toward the draining lymphatic vessels.

Although the specific features of the tumor microenvironment depend on the tumor cells and the tumor’s original location, it is evident that the microenvironment will experience alterations of its mechanical properties that in turn feed back on the tumor and its vasculature, thus having significant consequences for disease progression and its therapy.

4. FLOW-MEDIATED TUMOR METASTASIS

Metastasis, the process through which tumor cells migrate away from their original location and form distant colonies, is the reason for most cancer-related deaths. Less than 10% of patients who succumb to cancer die because of the presence of the primary tumor alone (Gupta & Massague 2006, Steeg 2006, Valastyan & Weinberg 2011). In many cases, surgery and therapy can provide cures from well-confined tumors. The treatment of cancer detected after it has metastasized is far less successful.

Metastasis is a complex, multistep process (Fidler 2003, Valastyan & Weinberg 2011) in which carcinogenic cells detach from the primary tumor, invade the surrounding tissue, and find their way to nearby blood and lymphatic vessels (Swartz & Lund 2012). The cells can then intravasate into the vessels, enter the circulatory system of the organism, and get arrested in distant sites of the circulation. They may further extravasate into the tissue, proliferate, and sustain their growth by inducing the development of new blood vessels, leading to the formation of a secondary tumor. The process of metastasis hinges on success at any of the substeps, any of which can be rate limiting (Fidler 2003). Importantly, less than 0.01% of thousands of tumor cells that enter the circulatory system survive to produce metastasis (Chambers et al. 2002, Joyce & Pollard 2009). All the substeps involve mechanical interactions between the tumor cells and the different microenvironments they encounter during metastasis (Wirtz et al. 2011). The detachment of carcinogenic cells from the primary tumor is characterized by the process of epithelial-mesenchymal transition (EMT) (Friedl & Wolf 2003, Polyak & Weinberg 2009), leading to significant alterations of the adhesive and mechanical properties of the tumor cells, making them more motile. The detached tumor cells traverse the microenvironment by employing a series of physicochemical processes (reviewed in Joyce & Pollard 2009) to reach the blood vessels. There is limited information on the mechanisms of the intravasation of tumor cells into the blood vessels. However, the damaged endothelium in the tumor microvasculature may provide migrating tumor cells with easy access to the circulatory system.

After entering the circulatory system, the route followed by CTCs depends on their physicochemical interactions with the blood constituents and the vasculature walls, as well as the broader vascular flow patterns. Tumor cells are usually larger than RBCs and white blood cells. Furthermore, CTCs are known to coagulate and to co-opt platelets (Joyce & Pollard 2009). The resulting cell aggregates are among the largest structures circulating inside the human vasculature. Experiments indicate that CTC-platelet interaction is causal for metastatic spread to multiple target organs. Platelets may protect CTCs from immune cells and reduce the shear stresses normally experienced by single circulating cancer cells (Gupta & Massague 2006). Furthermore, recent findings indicate that platelets in direct contact with CTCs release transforming growth factor β, which promotes the EMT-like transition of CTCs (Labelle et al. 2011). This increases the motility...
of the CTCs and in turn the probability of their survival in the circulation until their eventual arrest and extravasation at a secondary site. In turn, platelet-CTC aggregates disrupt the blood flow, induce larger forces on the ECs, and result in larger gradients of pressure and shear along the vascular walls. The segregation of cells is consistent with phase separations of fluid mixtures containing different sizes of particles (Sokolowski & Herrmann 1992).

Reported in vivo and in vitro measurements of CTC flow velocities range from 3 to 12 mm $s^{-1}$ (Sarimollaoglu et al. 2011). This is 10% below the corresponding mean blood flow velocity, with slower velocities expected for cells rolling on the surface of the vasculature. The interaction of the CTCs with the flow constituents induces shear stresses as well as rotational and translational motions on them. Shear stresses inside the vasculature range from $0.01 \text{ N m}^{-2}$ to $4 \text{ N m}^{-2}$ (Resnick et al. 2003), and they may damage the CTCs. Furthermore, shear stresses may result in the upregulation of certain receptors and ligands (Struckhoff et al. 2010) that enhance the potentials of the migrating CTCs to attach to the ECs of the vasculature. There is limited information on this interaction, but one may find similarities with the interactions of leukocytes and RBCs. During inflammation, the aggregation of RBCs in the center of the vessels tends to push leukocytes toward the endothelium, leading to the process of marginalization. A similar fate can be expected for tumor cells, but it appears that, unlike white blood cells, which recirculate until they find a suitable adhesion site, CTCs have a more limited repertoire. Instead, CTCs appear to be transported by the blood flow from their primary location of intravasation, through the vasculature, to locations where they get trapped in capillaries. The arrest of cancer cells by capillaries depends on factors such as their relative size, the blood pressure and shear stresses in the capillary, and the deformability of the cells (Chambers et al. 2002, Wirtz et al. 2011). As the vasculature carries blood from most organs in the body through the heart and then to the lungs for oxygenation, the lung capillaries are among the prime candidates for trapping CTCs. The lungs and liver are efficient at arresting the flow of cancer cells (Chambers et al. 2002) as their capillaries are small (typically 3–8 $\mu m$ in diameter) and are designed to allow the passage of single RBCs, whereas many cancer cells are much larger (20 $\mu m$ or more in diameter) (Chambers et al. 2002). However, not all tumor cells get trapped in lung capillaries, and some continue circulating until they get trapped in other organs that are remote from the location of their primary tumors. For example, prostate tumor cells have been found to metastasize to bones, and colorectal tumor cells to the liver, whereas two-way metastases have been observed between breast and liver cancer cells (Hanahan & Weinberg 2011). Breast cancer tumors are also known to metastasize to the lungs, but circulating breast cancer cells have been detected in humans up to 22 years after successful treatment of the primary tumor (Meng et al. 2004).

The relationship between the organs of the primary tumor and the metastatic location has concerned scientists since the discovery of cancer metastasis. Paget (1889) put forward the concept of “seed and soil,” implying that metastasizing cancer cells (seed) target specific organs that offer a favorable microenvironment (soil) for them to grow. However, an alternative and persuasive view, initially posed by Ewig in 1920 and today supported by intravital imaging, is of a dominant role of circulatory patterns in directing cancer metastasis to secondary organs. This view suggests that the predominant sites of metastases simply reflect the first pass of the cells in the circulation and their entrapment in local capillaries (Joyce & Pollard 2009). These two approaches are not mutually exclusive, and both contribute to the propensity of tumor cells to metastasize. Tumor cells may attain the capability to extravasate from the capillaries at secondary sites and then respond to the specific organ microenvironment to proliferate and elicit a new vasculature (Fidler 2003). Whereas passive entrapment may have a role in metastatic seeding, active adhesion and invasion are essential for the subsequent establishment and persistent growth (Joyce & Pollard 2009, Miles et al. 2008). CTCs may proliferate inside blood vessels, which in turn may eventually rupture as the metastatic tumor grows bigger (Al-Mehdi et al. 2000, Joyce & Pollard 2009). Up to 50% of
all cancer patients and 90% of those diagnosed with metastasis have coagulation abnormalities (Dvorak 1987, Steeg 2006). This relationship can be further quantified through novel imaging techniques and assays that focus on the investigation of the fluid phase of solid tumors (see Kuhn & Bethel 2012, and references therein).

CTCs may also first extravasate and then proliferate (Joyce & Pollard 2009). Folkman and colleagues (Holmgren et al. 1995) suggested that some metastases fail to grow because of the lack of vascularization, a phenomenon termed angiogenic dormancy (Joyce & Pollard 2009). Metastatic tumors can start growing decades after the primary tumor was removed, and both fluid mechanical and biological factors are likely to play a role in the seeding of new colonies (Chambers et al. 2002). It has been found that drugs can modify the mechanical properties of CTCs in vitro (Wuang et al. 2011), and cell deformability can be used as a diagnostic for metastatic and nonmetastatic cells (Lincoln et al. 2004).

5. THERAPY
The understanding that cancer is a systemic disease guides present-day therapeutic efforts (Chauhan et al. 2011). In addition to radiotherapy, chemotherapy, and surgery, a number of techniques (such as antiangiogenic treatment and targeted drug delivery) take aim at the hallmarks of cancer. Fluid mechanics processes, viewed as constraining principles for the genetic instability of cancer, may also serve as routes for its therapy. Some therapeutic techniques engage the tumor vasculature and its microenvironment for drug delivery. Inadequate delivery results in the regrowth of tumors and possibly the development of resistant cells. It is important to note that only 1 in 1,000 to 100,000 molecules reaches its intended destination.

The barriers of molecular delivery to tumors reflect their physiology and their microenvironment (see Chauhan et al. 2011, Swartz & Lund 2012, and references therein). These barriers include the irregular vasculature, the abnormalities of the vessel walls, and the geometric and chemical complexity of the interstitium around tumors (Jain 1990, Kuszyk et al. 2001, Swartz & Lund 2012). Diffusive processes largely characterize the transport of drugs in the tumor microenvironment as pressure gradients near tumors are minimal, except at the tumor margins. Furthermore, nonfunctional lymphatics result in elevated pressure near the vasculature, thus hindering convective transport. The geometric shape and arrangement of the blood vessels and other microenvironment components are critical in determining the infiltration of drugs to the tumor area (Baish et al. 2011, Stroh et al. 2005). Tumors exert mechanical stresses on the surrounding blood and lymphatic vessels (Padera et al. 2004, Young 1959, Stylianopoulos et al. 2012), further hindering hematogenous drug transport.

How do we overcome these barriers? A first approach is to eliminate them by restoring homeostasis in the microenvironment, degrading the ECM, increasing perfusion, and normalizing the vasculature by reducing vascular permeability or repairing the vessel network itself. One must note that drug delivery almost attempts to trace the route of metastasizing tumor cells in the opposite direction. This implies the need for a balance as, for example, the normalization of the tumor vasculature also hinders tumor cell intravasation (Mazzone et al. 2009), whereas degradation of the interstitial matrix also facilitates tumor metastasis. This indicates that the time and location of therapeutic approaches are critical and must be carefully orchestrated with the progression of the tumor.

5.1. Antiangiogenic Therapies
The ECs of newly sprouting and neovascular blood vessels are a natural target for drug therapy, in particular because they are considered a relatively stable cell population when compared with
Figure 6
Response of directed anticancer therapy as characterized by optical frequency domain imaging. (a) Optical frequency domain images of untreated (left) and treated (right) tumors with VEGFR-2-blocking monoclonal antibody DC101. Red and yellow are blood vessels, and blue represents the lymphatic vessels. (b) Quantification of the vessel morphology in response to antiangiogenic treatment. Asterisks indicate a statistically significant difference ($P < 0.05$). Figure reproduced from Vakoc et al. (2009).

genetically unstable, proliferating, and mutating cancer cells. Antiangiogenic therapies include blockers of growth factors, inhibitors of EC proliferation and migration, disruption of tumor vessels, and thrombosis-inducing treatment (Ferrara & Kerbel 2005) (see Figure 6).

Adverse effects are hypertension, wound-healing complications, thrombosis, and hemorrhage. It has also been shown that tumors can develop an evasive resistance to such therapies by genetic mutations that lead to tumors that can survive in a highly hypoxic environment (Yu et al. 2002). The action of antiangiogenic drugs remains a subject of active controversy, in particular because of evidence that antiangiogenic therapies (e.g., bevacizumab) do not have long-term therapeutic effects, despite the fact that they lead to reduction in blood flow and reduce microvessel counts. Numerous mechanisms for resistance to antiangiogenic therapy have been proposed (Bergers & Hanahan 2008), as any disruption of the angiogenesis cascade may trigger a number of adverse phenomena. Antiangiogenic therapies may impact the tumor microenvironment, reducing its defense mechanisms and facilitating tumor invasion. It has been shown that antiangiogenic monotherapy may depend on the stage of the disease and on the particular organs (Ebos & Kerbel 2011), and its advances may be offset by increased metastatic aggressiveness and metastatic potential. Other factors that may be linked to transport phenomena influencing metastasis and invasion after therapy (not necessarily anti-VEGF) include altered adhesion through activation and secretion of enzymes such MMPs, instigation of EMT, induction of stroma autophagy, pericyte dysfunction, and vascular mimicry of cancer stem cells (see Ebos & Kerbel 2011, and references therein). Angiogenic factors may be organ specific, regulating the process of angiogenesis accordingly, so that blocking angiogenesis may not be sufficient to block tumor progression. Moreover, targeting of VEGF/VEGFR pathways may not be appropriate as a minimum threshold of VEGFA is necessary for normal blood vessels and the nervous system (Carmeliet 2003). Furthermore, this therapy may damage only a subset of the tumor vessels and leave unaffected those that have acquired pericytes and smooth muscle cells (Nagy et al. 2009).

Antiangiogenic therapy, however, has led to discernable results when combined with chemotherapy (Ebos & Kerbel 2011). There can be different reasons for such an effect, all
accounting for the systemic nature of the disease. For example, blood vessel normalization (Chauhan et al. 2011) can enhance drug delivery during chemotherapy. Moreover, chemotherapy may damage the neovasculature in tumors and in turn render them more sensitive to anti-VEGF therapies. Finally, chemotherapy drugs can mobilize bone marrow–derived endothelial progenitor cells, which can migrate and colonize drug-treated tumors, leading to a neopopulation. Certain antiangiogenic drugs may have the potential to blunt this reactive host response and thus promote the effects of chemotherapy.

A possible relation between the EPR effect and anti-VEGF therapy is that with reduced growth and permeability factors, the vascular fenestrations are reduced. This leads to the hypothesis that drugs with lower molecular weight may become more effective when extravasating from tumor vasculature. The reason that drugs with lower molecular weight are not useful without VEGF therapy may have to do with the microenvironment and the ability of large particles to extravasate more easily than smaller particles in larger fenestrations.

5.2. The Enhanced Permeability and Retention Effect

The elevation of fluid pressure in blood vessels is controlled by angiotensin, which acts to decrease the diameter of the vessels. Experiments showed that elevating blood pressure by infusing angiotensin increased tumor blood flow by two to six times the normal rate, whereas blood flow in other tissues and organs remained constant, demonstrating that tumors do not possess the homeostatic properties of normal organs (Maeda 2001, Suzuki et al. 1987). As reviewed by Maeda (2010), increasing tumor blood flow through dosages of angiotensin resulted in improved macromolecular drug delivery. Furthermore, delivery to normal organs, such as the kidney and bone marrow, was reduced, as the homeostasis resulted in tighter endothelial gaps, thus permitting less transvascular transfer of macromolecules. Along with angiotensin, NO can be a potent mediator for enhanced drug delivery, as it enhances extravasation. The application of nitroglycerin leads to the generation of NO near tumors and enhances drug delivery. In inflammation, the lymphatic system quickly proceeds to remove such molecules from tissue—this is not the case near tumors (Jain 1987). Because of the defective clearance of such molecules near tumors, they are retained in the area for longer times (see Figure 7). This phenomenon has been called EPR (reviewed in Maeda 2010).

![Figure 7](https://example.com/figure7.png)

**Figure 7**
Illustration of the enhanced permeability and retention (EPR) effect. (a) Time course of Evans blue/albumin complex. (b) Intratumor accumulation of various tagged proteins of different molecular weights in solid tumor–bearing mice. Figure reproduced from Maeda (2001).
The EPR concept implies longer retention times for macromolecules above 50 kDa in the tumor microenvironment. Macromolecules with weights of 70 and 150 kDa may leak from vessels at the periphery of the tumor and may have difficulty penetrating far into it. Smaller molecules reach most organs by diffusion and could enter tumors as well as other tissues (Dvorak et al. 1988). The EPR mechanism is the main reason for the FDA approval of the first nanomedicine drugs in the mid-1990s (Torchilin 2011); however, it has been shown to be practical only for tumors that exceed 100 mm³ in volume. As such, it may also not be applicable for targeting small, prevascularized tumors or unvascularized metastases.

5.3. Flow-Mediated Delivery of Therapeutic Nanoparticles

The irregular tumor vasculature may facilitate drug delivery to tumors by nanoparticles, taking advantage of vessel leakiness and the EPR effect. The effective delivery of drugs carried in nanoparticles hinges on their efficient transport through the vasculature, their extravasation, and their eventual delivery to the collagen-rich tumor microenvironment via the leaky vasculature (Brigger et al. 2002, Peer et al. 2007) (see Figure 8). Nanoparticles must be able to exploit mass-transport differentials such as margination in tumor-associated vasculature, adhesion in ECs, shape, and deformability, as well as surface charge and affinity for ECs (Ferrari 2010). Several works reported that elongated and flexible molecules experience less steric hindrance in the tumor microenvironment in comparison with more spherical and rigid molecules (Stroh et al. 2005, and references therein), which may be confined only near the vasculature. As the structure of the tumor microenvironment changes during the progression of cancer and its treatment, effective therapies may be provided by multistage vectors with nanoparticles of different types attacking the various biological barriers in sequence (Ferrari 2010). Nanoparticles have the potential to target specific tumors, reach subcellular compartments, and interact with malignant cells in circulation. Methods that rely on hematogenous transport for drug delivery may not be suitable in the case of metastasized lesions that have not induced any vasculature.

Nanoparticle targeting is materialized via the surface conjugation of high-affinity ligands, antibodies, aptamers, and other biological moieties that can aid in subsequent drug release at the

Figure 8

The enhanced permeability and retention (EPR) effect. Nanoparticles are loaded with therapeutic agents and equipped with tumor-specific surface proteins. Injected into the circulatory systems, they exit the vasculature through large gaps in the endothelium at the tumor site and diffuse through the extracellular matrix. Upon attachment to target receptors on the tumor cell membrane, the nanoparticles are taken up by the tumor cell and release therapeutic agents inside the cells.
cancer site and that have been investigated for selective delivery. However, nanoparticles with such moieties may experience increased difficulty in overcoming certain barriers. The surface chemistry and the zeta potential of the delivered nanoparticles should be close to neutral or anionic as the luminal surface of the ECs is covered by the glycocalyx, which is slightly negatively charged (cationic drugs will be absorbed on the vessel surface and then dissolve). However, the only therapeutic effect presently available is associated with the EPR rather than detailed molecular recognition for nanoparticle transport. Despite 15 years of research, only a couple of therapeutic formulations have been developed to deliver nanoparticles to tumors (Davis et al. 2008, Perrault et al. 2009). Perrault et al. (2009) found that the size and surface chemistry of nanoparticles are critical for their selective transport to tumor locations. It is important to gear the size of the particles to the size of fenestrations in tumor vessels and to avoid sizes that are small and that may lead to ejection through the urine. Larger particles, however, may accumulate in organs with larger pores such as the kidney and the liver.

6. MICROFLUIDICS AND CANCER

Blood vessels are the main pathways for disseminating tumor cells during metastasis. CTC detection is a challenging task, whereas the evaluation of their clinical significance is still an area of active research. CTC isolation, detection, and molecular characterization can offer a better understanding of tumor biology through the genetic analysis of tumors. CTCs can be thought of as liquid biopsy samples (Kuhn & Bethel 2012). Efficient methods for the isolation and characterization of CTCs can also contribute to better understanding of the metastatic process. The development of such methods capable of capturing a sufficient number of CTCs is difficult because even for patients in advance stages of the disease, CTCs are typically outnumbered by other blood cells by a factor of a billion. For an overview of various methods for CTC isolation, detection, and molecular characterization, we refer readers to recent review papers (Alunni-Fabbroni & Sandri 2010, Hou et al. 2011, Lianidou & Markou 2011, Pantel & Alix-Panabieres 2010, Yu et al. 2011). Here we discuss applications of microfluidic devices for the isolation and enrichment of CTCs. Microfluidics is a relatively new, but promising addition to the CTC isolation approaches (see Figure 9).

CTCs can be distinguished from other cells in the blood sample using various types of biomarkers. The expression of specific cell surface antigens such as EpCAM (epithelial cell adhesion molecule) and CD45 (also known as protein tyrosine phosphatase, receptor type C) by certain types of cells is one of the most commonly used types of biomarker (Lianidou & Markou 2011). CTC

![Figure 9](https://example.com/figure9.jpg)

**Figure 9**

Scanning electron microscopy image of captured NCI-H1650 lung cancer cells (yellow) inside a microfluidic device. The micropillars (blue) have a diameter of ~100 μm. Figure adapted from Nagrath et al. (2007).
enrichment and isolation can be based on adhesion to surfaces coated with antibodies against one of the cell surface antigens. Microfluidic devices are typically designed to minimize CTC exposure to high shear stresses and also to maximize the probability of CTC contact with antibody-coated surfaces. The majority of such devices use positive selection through adhesion of CTCs and are often based on EpCAM expression on the CTC surface. In some cases, however, the EpCAM expression is downregulated because of EMT, in which epithelial tumor cells change their phenotype, reducing cell adhesion and increasing cell motility. This is one of the main limitations of the EpCAM-based approaches. Negative selection, which is based on the removal of leukocytes, can be used instead. However, a very low number of CTCs may also limit its effectiveness.

Physical biomarkers such as cell density, size, and deformability are also utilized. CTCs are often larger than normal cells found in blood, however, owing to the large variation in size for different types of tumors, with cancer cells in some cases smaller than leukocytes (Yu et al. 2011). In several studies, CTCs were shown to have increased deformability compared to normal cells. The alteration of mechanical properties is thought to result from changes in the cytoskeletal structure to a more disordered and softer state (Hou et al. 2011, Li et al. 2008, Suresh 2007b, Wirtz et al. 2011). Microfluidic devices designed for CTC isolation using physical biomarkers are often based on simple physical principles and have a rather simple design. They offer the advantage that they do not require specific antibodies for cell selection. Size is the most commonly used CTC characteristic exploited in devices that filter these cells using different size pores and gaps, arrays of pillars, and various obstacles. The clogging of such devices may limit their use in clinical applications for large volumes of blood samples. The exposure of CTCs to high shear stresses during the filtering process may also be significant. Several filterless microfluidic devices based on size and inertial focusing were developed recently, some of which are reviewed in Hou et al. (2011).

Another application of microfluidic-based devices is in investigations of cancer cell migration. Microfluidic devices can provide a well-controlled microenvironment for the precise placement of cancer cells with delivery of known quantities of the factors and stimuli (reviewed in Huang et al. 2011).

Microfluidic devices have also been used to emulate metastatic conditions. The parallel-flow-chamber technique has been used to study cell dynamics under various shear-flow conditions (Long et al. 2011). Similar to leukocyte adhesion, flow-enhanced CTC adhesion includes several aspects (Zhu et al. 2008): Flow augments the initial tethering of flowing cells to a stationary surface, and adhesion is initiated by glycoprotein-selectin interactions, which facilitate cell rolling on the endothelium (Geng et al. 2012), followed by firm adhesion and eventual extravasation. CTC rolling is highly regulated by fluid mechanical forces, in which physiological shear forces enhance cellular adhesion (Geng et al. 2012). Mechanisms for this intriguing phenomenon may include transport-dependent acceleration of bond formation and force-dependent deceleration of bond dissociation. The former includes three distinct transport modes: sliding of the cell bottom on the surface, Brownian motion of the cell, and rotational diffusion of the interacting adhesion molecules. The latter involves a recently demonstrated counterintuitive behavior called catch bonds, in which force prolongs rather than shortens the lifetimes of receptor-ligand bonds.

7. MODELING ASPECTS OF CANCER FLUID MECHANICS

Blood flow in the tumor-induced vascular network is a central regulator during the vascular phase of tumor development (McDougall et al. 2002). In certain cases of chemotherapy, drugs can be delivered to the tumor through the same network. The analysis of blood flow and transport processes in the growing networks requires accurate modeling of blood flow in microvessels,
solute transport, and angiogenesis. In this section we briefly review some modeling approaches developed to address these issues.

7.1. Blood Flow in Microvessels

Models of vascular transport have been largely based on the Poiseuille equation with the flow rate governed by the pressure gradient and an empirical resistance term that depends on the vessel geometry and the flow viscosity. The Poiseuille model presents a drastic oversimplification of the flow inside blood vessels and capillaries, something that was in fact well appreciated by Poiseuille himself. By understanding the structure of blood inside the blood vessels as a composition of cells within an aqueous solution of ions and molecules, one realizes that the Poiseuille equation loses its validity, in particular for vessels with diameters that are smaller than 10 times the size of the RBCs, such as capillaries. The accurate modeling of blood flow in microvessels should include detailed models of blood cells as well as the glycocalyx layer attached to the endothelial surface, and the dimensional irregularities of vessel diameters (Pries et al. 1996).

RBCs, the most abundant and probably among the simplest cells present in blood vessels, have attracted a lot of attention in recent years. Various modeling approaches have been used for them (Boal et al. 1992; Discher et al. 1998; Eggleton & Popel 1998; Fedosov et al. 2010; Freund & Orescianin 2011; Hansen et al. 1997; Li et al. 2005, 2007; Liu & Liu 2006; Noguchi & Gompper 2005; Pozrikidis 2005; Skotheim & Secomb 2007; Wu & Aidun 2010). These include approaches in which the cells and surrounding fluid are modeled using continuum-based methods. The treatment of the coupling of solid components and fluid flow in these models poses a number of challenging problems. Several approaches have been developed that allow one to reduce computational complexity using methods such as the immersed boundary method (Lai & Peskin 2000). Discrete models of RBCs are often based on the representation of cells using particles connected with links (Dupin et al. 2008, Leble et al. 2011, Noguchi & Gompper 2005, Pan et al. 2011, Pivkin & Karniadakis 2008, Reasor et al. 2012). When coupled with mesoscopic methods for flow discretization such as the lattice Boltzmann method, multiparticle collisional dynamics, and dissipative particle dynamics, these models provide efficient tools for simulations of cells in the flow (Dupin et al. 2008).

Despite their relatively small numbers, leukocytes may have a significant effect on blood flow (Sugihara-Seki & Fu 2005). In simulations, Fedosov et al. (2012) recently analyzed the mechanism of leukocyte margination toward the vessel wall. Leukocyte rolling was studied extensively using modeling approaches. Adhesive dynamics, developed by Hammer & Apte (1992), is among the most popular models for simulations of adhesive interactions between cells and solid surfaces under shear flow. The kinetics of single-bond failure in the model is described by the Bell (1978) model. The method was extended to simulations of cell adhesion to surfaces in a dense multicellular environment. The extended method is called multiparticle adhesive dynamics and combines a stochastic Monte Carlo simulation of single receptor-ligand bonds and calculations of concentrated suspension flow at low Reynolds number (King & Hammer 2001a, 2001b). Numerical studies have indicated that the effect of leukocyte adhesion to the vessel walls on flow is strongly dependent on the number of adherent leukocytes and the vessel diameter (Chapman & Cokelet 1998, Pappu et al. 2008, Sugihara-Seki & Skalak 1997). Leukocytes were also found to affect resistance through interaction with RBCs, which flow behind slowly moving leukocytes in capillaries (Helmke et al. 1997, 1998). Owing to many similarities in the process of leukocyte and CTC adhesion, models developed for leukocytes can be applied to CTCs during the metastatic process.

There have been several analyses for the flow of a uniform Newtonian fluid in vessels with geometries different from circular tubes (Schmid-Schonbein & Murakami 1985, Sugihara-Seki

Damiano et al. (1996) analyzed flow through the glycocalyx, modeled as a porous medium, using the Brinkman equation. Simulations showed that the presence of a deformable glycocalyx layer reduces the transient fluid shear stresses and deformations experienced by RBCs traversing a nonuniform capillary (Secomb et al. 2002).

7.2. Transport of Solutes

The extravasation of nutrients and drug molecules is associated with fluid movement across the vasculature wall. The cleft between the ECs is widely believed to be the principal pathway for fluid and hydrophilic solute transport through the microvessel wall (Sugihara-Seki & Fu 2005). The fundamental principles governing this flow were laid down by Starling (1896), who proposed a model in which the capillary walls act like semipermeable membranes. Consequently, fluid movement across them depends on the net imbalance between the osmotic absorption pressure of the plasma proteins and the capillary hydraulic pressure generated by the heart beating (Levick & Michel 2010). The Starling principle has been revised to take into account the presence of the glycocalyx layer covering the luminal surface of ECs (Michel 1997, Weinbaum 1998).

Several models of transport through the interendothelial clefts have been proposed. We refer readers to Levick & Michel (2010) and Sugihara-Seki & Fu (2005) for a review of recent developments. Whereas the majority of these models are based on continuum approaches, it was recently suggested that a more suitable analysis must be based on the molecular nature of the fluid because of the comparable sizes of the mean intermolecular distance (approximately 0.3 nm) and the cleft width (18 nm) (Sugihara-Seki et al. 2008). We suggest that Starling’s model can be enhanced using recent advances in imaging and our enhanced knowledge of the glycocalyx structure. The development of multiscale computational models (Koumoutsakos 2005, Praprotnik et al. 2008), coupling, for example, the molecular structure of the glycocalyx with a continuum description of the flow, is highly suitable in this context.

It is important to note that in comparison with normal healthy vasculature, tumor-induced vessels have discontinuities in the endothelium, with several studies reporting that the pore size of tumor microvessel walls can vary between 100 and 780 nm in diameter (Hobbs et al. 1998; Yuan et al. 1994a,b). This may significantly affect the transport of fluid and solutes across the endothelium. Estimates of parameters for the Starling model applied to fluid exchange in tumor vasculature require detailed analysis and must take into account differences in tumor vascular architecture in comparison to normal ones.

Solute transport from the vasculature to cells has been largely modeled as passively transported elements with a flux proportional to the drug concentration. In advective transport, this can be modeled by Darcy’s law. The difference between solute transport inside the tumor and that in normal tissue was recently analyzed using computational models of diffusion based on high-resolution images. Baish et al. (2011) linked the vascular structure to the delivery of solutes. According to their analysis, the key factors are the maximum distance in the tissue from the nearest blood vessel and a measure of the shape of the spaces between vessels, which differ significantly between normal and tumor tissue.

7.3. Angiogenesis Models

Computational modeling of tumor-induced angiogenesis can be grouped into continuum and discrete models and traces back to the early methods proposed by Balding & McElwain (1985) and Stokes & Lauffenburger (1991). For a more detailed overview, we refer readers to reviews
by Qutub et al. (2009) and Peirce (2008), and references therein. A recent model in the field of continuum modeling is presented in the work of Bergdorf et al. (2010), who modeled the EC population by a density function that resolves the vascular branching patterns. Discrete models can be further categorized into cell-based models (Bauer et al. 2009, Merks et al. 2008) and lattice-based models in which tip cells migrate at discrete grid locations (Chaplain 2000). Milde et al. (2008) and Travasso et al. (2011) presented a hybrid modeling approach coupling a discrete tip-cell representation to a continuum description of the blood vessels. Most of these models consider VEGF released from a tumor source to diffuse through the ECM, establishing a chemical gradient. ECs lining the blood vessel wall react to this gradient and migrate toward the tumor, leading to an extension of the vascular network. Such models have been extended to include an explicit representation of the ECM and its guiding properties on EC migration (Bauer et al. 2009, Sun et al. 2005) and matrix-bound VEGF that can be cleaved from the ECM by MMPs released at the sprouting front (Milde et al. 2008).

McDougall et al. (2002) coupled a model for sprouting angiogenesis with a model for blood flow in the vascular network, assuming Poiseuille flow inside a network of connected pipes. This approach has been adapted to account for variability in blood viscosity and evolving capillary vessels that can dilate and constrict to study the transport of therapeutic agents inside the growing vasculature (Stephanou et al. 2006). More recently, the model was combined with a continuum model of tumor growth (Macklin et al. 2009). For an in-depth report on recent work in the modeling and simulation of vascularized tumors, we refer readers to Lowengrub et al. (2010), and references therein.

8. SUMMARY AND OUTLOOK

This review presents a fluid mechanics perspective on the growth, progression, and metastasis of cancer, along with an overview of related experimental diagnostics, computational modeling, and therapeutic techniques. Flow-mediated processes in cancer include abnormal molecular diffusion during tumor formation, EC migration, nutrient transport to tumors during angiogenesis, vascular remodeling due to flow-imparted stresses on the endothelium, IA, and tumor cell metastasis through the blood vasculature and the lymph node network. Moreover, transport phenomena are essential in therapeutic strategies that aim, for example, to effectively transport drugs to tumors and their cohorts or to regularize the tumor-induced vasculature to increase drug-delivery efficacy.

The integration of genomic investigations with studies of related flow processes presents a new perspective on how to gain insight into cancer and how to devise effective therapies. We suggest that this integration requires a dialogue between members of the communities of cancer medicine and biology, nanotechnology, and fluid mechanics. Even though there has been significant progress in realizing that fluid mechanics is an essential organizing principle for cancer, there is tremendous potential for further important contributions. This review is attempting a first, small step in making aspects of cancer that interface with fluid mechanics accessible to the broader fluid mechanics community.

Cancer involves tightly interacting processes that span spatial scales from the gene to the organ and timescales of microseconds (as in gene mutations) to decades (as pertinent to metastasis). There is a significant need to develop experimental, analysis, and simulation methods capable of systematically quantifying these interactions. Understanding cancer as a systemic disease, with an essential fluid mechanics component, will stimulate future studies that combine experimental and computational fluid mechanics with genetic and molecular investigations, as well as with advances in areas such as nanotechnology. We firmly believe that a future review on this subject will include a lengthy list of fluid mechanics contributions to understanding, diagnosing, and treating cancer.
FUTURE ISSUES

1. Progress is needed for the multiscale, spatiotemporal reconstruction of the tumor vasculature, microenvironment, and blood and lymphatic flow.
2. Multiscale computational models with predictive capabilities must be developed. The synergy of computational and experimental studies is imperative.
3. CTCs should be tracked to gain a better understanding of metastasis.
4. Investigators should focus on integrative studies of genetics with fluid and solid mechanics in cancer.
5. Guided flow-mediated delivery of therapeutic nanoparticles would help improve drug delivery to tumors.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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