Mechanical Forces in Tumor Angiogenesis

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Abstract

A defining hallmark of cancer and cancer development is upregulated angiogenesis. The vasculature formed in tumors is structurally abnormal, not organized in the conventional hierarchical arrangement, and more permeable than normal vasculature. These features contribute to leaky, tortuous, and dilated blood vessels, which act to create heterogeneous blood flow, compression of vessels, and elevated interstitial fluid pressure. As such, abnormalities in the tumor vasculature not only affect the delivery of nutrients and oxygen to the tumor, but also contribute to creating an abnormal tumor microenvironment that further promotes tumorigenesis. The role of chemical signaling events in mediating tumor angiogenesis has been well researched; however, the relative contribution of physical cues and mechanical regulation of tumor angiogenesis is less understood. Growing research indicates that the physical microenvironment plays a significant role in tumor progression and promoting abnormal tumor vasculature. Here, we review how mechanical cues found in the tumor microenvironment promote aberrant tumor angiogenesis. Specifically, we discuss the influence of matrix stiffness and mechanical stresses in tumor tissue on tumor vasculature, as well as the mechanosensory pathways utilized by endothelial cells to respond to the physical cues found in the tumor microenvironment. We also discuss the impact of the resulting aberrant tumor vasculature on tumor progression and therapeutic treatment.

Keywords

VE-cadherin · VEGF · Matrix stiffness · MMP · Contractility · Fluid shear stress · Interstitial pressure · Mechanotransduction · Mechanosensitivity · Barrier function

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6.1 Introduction

Like normal tissue, tumor tissue requires an adequate supply of nutrients and oxygen provided by blood vessels to meet metabolic needs, remove waste products, and survive. To meet these needs

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during tumor growth, blood vessels are developed through angiogenesis, the sprouting of new blood vessels from existing blood vessels [1, 2]. In normal tissue, the initiation of angiogenesis, known as the angiogenic switch, is tightly regulated; however, during tumor progression the appropriate balance of pro- and anti-angiogenic cues is lost, and the angiogenic switch is almost always activated [1]. Notably, while nutrient requirements can differ between tumor types and during tumor progression, the generation of a tumor blood supply is a rate-limiting step in solid tumor growth [3]. Consequently, solid tumors develop vasculature with many abnormal features [2, 4]. Solid tumor vasculature is exceptionally variable in size, shape, as well as architecture and is not organized in the conventional hierarchical arrangement found in normal tissue [5, 6]. This is due to the abnormal properties acquired by tumor endothelial cells [7, 8]. In the blood vessels of mouse mammary carcinomas, tumor endothelial cells have been shown to be poorly connected, grow on top of one another, and project into the lumen of the vessels [9]. Additionally, in many different types of solid tumors, the tumor vessel walls contain many openings, widened cell-cell junctions, and irregular or deficient basement membrane coverage [9–11]. Together, these abnormal features contribute to create hyperpermeable, tortuous, and dilated blood vessels, which generate heterogeneous blood flow and limited perfusion throughout the tumor.

A principal determinant of phenotypic differences found in tumors is the surrounding microenvironment [7]. Endothelial cells of recently formed blood vessels in the tumor are subjected to distinct extracellular signals including hypoxia, low pH, a deregulated and disorganized extracellular matrix (ECM), mechanical stresses, and soluble mediators released by surrounding tumor and stromal cells. Angiogenesis is tightly controlled by numerous chemical and mechanical signaling events, and these differences in extracellular cues have a profound effect on the formation of new capillaries. As such, the abnormal features of the tumor vasculature are believed to result from the disproportionate balance of pro- and anti-angiogenic cues found in the tumor microenvironment. Overexpression of vascular endothelial growth factor (VEGF) and other pro-angiogenic growth factors within the tumor microenvironment has been extensively investigated as major contributing factors in the formation of abnormal tumor vasculature. However, recent work have indicated that mechanical cues and forces within the tumor microenvironment play an important role in promoting a tumor vasculature phenotype [12].

Understanding the components of the tumor stroma such as the vasculature, has become key to understanding tumor growth and progression [3]. The tumor vasculature has been demonstrated to not only influence tumor growth but also be instrumental in facilitating metastasis and creating an irregular tumor microenvironment that assists in tumor progression [6, 7, 13]. This chapter will provide an overview of the mechanical cues and forces found in the tumor microenvironment and discuss their respective impact on tumor angiogenesis and promoting abnormal tumor vasculature. The mechanosensory pathways that are employed by endothelial cells to respond to mechanical stimuli, specifically aberrant mechanosensory pathways found in tumor endothelial cells, will be reviewed. Finally, this chapter will briefly discuss the clinical impact of abnormal tumor vasculature and its influence on cancer treatment.

6.2 Mechanical Cues in the Tumor Microenvironment

In the past few decades, there has been an increasing interest on how physical and mechanical cues in the tumor microenvironment influence cancer cells and cancer progression. As tumors stimulate neovascularization and angiogenesis to meet growth needs, the tumor vasculature is exposed to a mechanically abnormal and highly heterogeneous microenvironment (Fig. 6.1a). A critical component of the tumor microenvironment is the ECM, which is a complex three-dimensional assembly of macromolecules and interconnected cell-scale fibers with distinct physical and biomechanical properties [14–17]. The

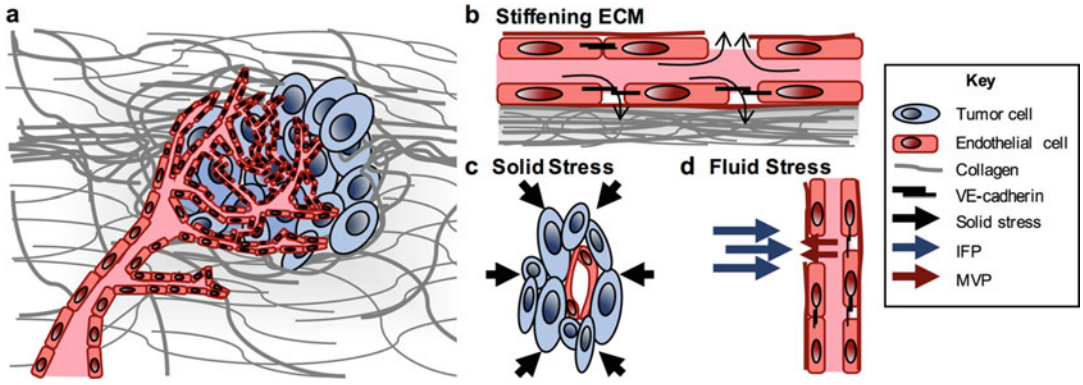

Fig. 6.1 Mechanical cues in the tumor microenvironment influence tumor angiogenesis. (**a**) To meet nutrient needs, tumors upregulate angiogenesis and produce a vasculature network. The resulting tumor vasculature has many abnormal characteristics and is highly disordered. (**b**) In the tumor microenvironment, stiffening of the ECM modulates cell-cell junctions and localization of VE-cadherin, which results in disrupted barrier function and increased permeability. (**c**) Growth-induced solid stress from ECM deposition and proliferating stromal and cancer cells causes tumor vessel compression. (**d**) Elevated interstitial fluid pressure (IFP) in the tumor often exceeds that of the microvascular pressure (MVP), causing limited perfusion and disrupting flow patterns

ECM determines the mechanical properties of a tissue as well as provides a dynamic and bioactive structure that fundamentally controls cell behavior through chemical and mechanical signals [17]. Tight regulation of the ECM is essential to maintaining tissue homeostasis, and abnormal ECM dynamics contribute to many pathological conditions, including cancer [18–20].

6.2.1 Increased Matrix Stiffness During Tumor Progression

During solid tumor progression, the ECM commonly becomes deregulated and disorganized, creating solid tumor tissue with heterogeneous three-dimensional matrix features, organization, rigidity, and composition [14, 21–23]. Such changes to the ECM can significantly alter biochemical properties, alter cell response to growth factors, and disrupt cell behaviors [14–16, 24, 25]. Notably, increased ECM stiffness and density, caused primarily from increased collagen deposition and increased crosslinking within the stroma during the progression of many solid tumors, have been demonstrated to be cell-instructive and involved in promoting a malignant phenotype [14, 26–28]. Compared to normal tissue, many solid tumors are markedly stiffer (Table 6.1).

In vascular biology, the ECM drives capillary morphogenesis by providing necessary organization cues to endothelial cells [63]. Endothelial cell capillary-like network formation is influenced by ECM concentration [64–66], ECM composition [67, 68], as well as matrix stiffness [69–72]. Collectively, these and other studies clearly demonstrate the important role of the ECM in directing endothelial cell network formation. Compared to normal endothelial cells, tumor endothelial cells are exposed to a highly mechanically heterogeneous and abnormal microenvironment [14, 21, 73]. These abnormal physical cues in the tumor microenvironment continuously alter cell-ECM force balances that can influence tumor endothelial gene expression and cell behavior [74–77]. Indeed, tumor endothelial cells are notably phenotypically different from normal endothelial cells, and the tumor endothelium displays distinct gene expression profiles from the normal epithelium [78]. Tumor endothelial cells also demonstrate constant expression of endothelial activation, enhanced pro-adhesion and angiogenic properties, upregulated cell survival pathways, as well as altered mechanosensitivity [79, 80]. After isolation from

Table 6.1 Mechanical properties of normal and tumor tissue

Tissue	State	Stiffness	Interstitial fluid pressure	Solid stress	References
Breast	Normal	0.4–2.0 kPa	0.0–3.0 mmHg		[26, 29–35]
	Breast carcinoma	4.0–12.0 kPa	4.0–53.0 mmHg	75.1–142.5 mmHg	
Lung	Normal	10.0 kPa	−7.0 mmHg		[31, 35–38]
	Lung carcinoma	25.0–35.0 kPa	1.0–27.0 mmHg	–	
Brain	Normal	0.26–0.49 kPa	0.0 mmHg		[35, 39–45]
	Glioblastoma	7.0–26.0 kPa	−0.5–15.0 mmHg	1.56 mmHg	
Liver	Normal	0.3–0.6 kPa	−2.2 mmHg		[46–48]
	Hepatoma	1.6–20.0 kPa	0.0–30.0 mmHg	–	
Colorectal	Normal	0.9–4.0 kPa	14.0 mmHg		[32, 35, 44, 49–53]
	Colorectal carcinoma	7.5–30.0 kPa	16.0–45.0 mmHg	7.5 mmHg	
Kidney	Normal	2.0 kPa	6.0 mmHg		[32, 45, 54, 55]
	Renal cell carcinoma	13.0 kPa	38.0 mmHg	–	
Skin	Normal	35.0–300.0 kPa	−2.0–0.4 mmHg		[31, 32, 35, 45, 56, 57]
	Metastatic melanoma	400.0 kPa	0.0–60.0 mmHg	–	
Pancreatic	Normal	1.0 kPa	8.0 mmHg		[44, 58, 59]
	Pancreatic ductal adenocarcinoma	2.0–4.0 kPa	75.0–130.0 mmHg	52.5 mmHg	
Bone	Normal	2.0–14.0 GPa	2.9 mmHg		[30, 34, 60–62]
	Osteosarcoma	>689 MPa	35.5 mmHg	35.3–48.3 mmHg	

tumors, tumor endothelial cell maintained these properties in cell culture, indicating a persistent alteration in phenotype. As such, tumor endothelial cells may be phenotypically adapted to stiffer ECM conditions in the tumor microenvironment by undergoing reprogramming of signaling pathways, possibly causing some of their aberrant functions [6].

Recent work has identified that altering matrix mechanics alone can induce a tumor vasculature phenotype. Increasing three-dimensional collagen stiffness without altering matrix architecture via nonenzymatic glycation increased angiogenic outgrowth and vascular branching density of in vitro endothelial cell spheroids, creating a morphology reminiscent of tumor vasculature [12]. Other methods of increasing collagen matrix stiffness in vitro have demonstrated comparable increases in angiogenic response in stiffer matrices (Table 6.2) [81–85]. Similar modulation of angiogenic outgrowth and branching by ECM stiffness was observed in vivo. In a MMTV-PyMT mouse tumor model[1] [86], β-aminopropionitrile (BAPN), a lysyl oxidase inhibitor, was used to modulate the stiffness of mammary tumors from approximately 4.5 kPa in control mice to 3 kPa in BAPN-treated mice. It was shown that decreasing matrix stiffness via BAPN treatment significantly reduced the extent of angiogenesis and vascular branching density within tumors. Changes in matrix stiffness were also revealed to modulate endothelial cell-cell junctional properties and endothelial cell permeability both in vitro and in vivo [12]. Notably, the changes observed in vascular phenotype were due solely

[1]The MMTV-PyMT transgenic mouse model is widely used to study mammary tumor progression and metastasis. In the MMTV-PyMT model, mammary gland-specific expression of the polyoma middle T antigen (PyMT) oncogene driven by the upstream mouse mammary tumor virus (MMTV) long terminal repeat promoter results in mammary epithelium transformation and rapid development of multifocal mammary adenocarcinomas and metastatic lesions.

Table 6.2 The effects of matrix stiffening on angiogenesis in three-dimensional in vitro models

Matrix	Method of altering matrix stiffness	Stiffness (kPa)	Angiogenic response	References
Collagen	Nonenzymatic glycation with ribose	~0.18–0.50	Increasing matrix stiffness resulted in increased angiogenic outgrowth and branching density	[12]
Collagen	Nonenzymatic glycation with glucose-6-phosphate	–	Decreased sprouting, but increased branching and tortuosity in crosslinked gels.	[81]
Collagen	Transglutaminase	0.45–0.89	Increasing matrix stiffness resulted in increased angiogenic sprouting, invasion, and remodeling	[82]
Collagen	Varying oligomer: monomer ratio	~0.06–0.26	Increasing stiffness increased network length, branching, and vascularized area	[83]
Collagen	EDC/NHS	–	Increased crosslinking resulted in increased capillary number and spoke-like vessel structure	[84]
Collagen	Varying pH of polymerization solution	~5–20	Thicker, deeper capillary networks on more rigid three-dimensional collagen gels. Formation of large lumen on rigid gels compared to flexible gels	[85]

to mechanical alterations to the ECM. For endothelial cells cultured on compliant (0.2 kPa) or stiff (10 kPa) polyacrylamide substrates, stiffer matrices impaired barrier function and localization of vascular endothelial cadherin (VE-cadherin), contributing to increased vessel permeability (Fig. 6.1b). Endothelial cells on stiffer matrices demonstrated punctate VE-cadherin and β-catenin positive endothelial cell-cell junctions, as well as stress-mediated localization of tight junction protein zona occludens 1 (ZO-1) that matched VE-cadherin. In vivo staining of VE-cadherin, β-catenin, and ZO-1 also demonstrated changes in junctional architecture in stiffer tumors. Interestingly, the altered vascular phenotype and increased angiogenic response required upregulation of matrix metalloproteinase (MMP) activity, specifically membrane-type 1 MMP (MT1-MMP). This finding suggests MMPs play an important role in promoting angiogenesis [12]. MMP activity has been shown to be important for ECM degradation and basement membrane remodeling during angiogenesis. MT-MMPs in particular are able to provide addition control over degradation events by providing spatial control of matrix degradation at the cell membrane surface [87]. Previous work has identified MT1-MMP activation is dependent on cell contractility and matrix stiffness [88]. Together, these findings demonstrate the importance of changing ECM cues during tumor progression, chiefly increased matrix stiffness, in promoting aberrant tumor vasculature.

6.2.2 Physical Forces in the Tumor Microenvironment

In addition to changes to the stromal ECM during tumorigenesis, solid tumors are also exposed to physical forces during tumor progression. As physical forces grow during solid tumor growth, increased tension in the tissue impacts not only tumor growth, but it also deforms the tumor vasculature [89]. These mechanical forces found in the tumor microenvironment can be categorized as solid or fluid stresses.

Solid stress is defined as the combined mechanical forces from the non-fluid, structural components of the tumor, predominantly cancer cells, various host cells, and the ECM [89]. Within solid tumors, solid stress is significantly elevated due to elevated cell and

matrix densities (Table 6.1). Solid stresses accumulate as the tumor tissue becomes stiffer than the normal surrounding tissue and the constrained production of mechanical forces by tumor components dislocates the surrounding normal tissue [89]. Furthermore, as cancer and stromal cells proliferate and migrate through the ECM, growth-induced solid stresses are generated and transmitted through the ECM [34]. Interestingly, the total solid stresses in the tumor are compressive in the interior of the tumor, but forces are compressive in the radial direction and tensile in the circumferential direction at the tumor-host interface [34, 90]. The ECM components of the tumor stroma, notably collagen, can also help to transmit these forces across the tumor and to surrounding host tissue. Tumor-associated collagen signatures including dense collagen, tense collagen fibers, and aligned collagen fibers have been identified in tumors and are associated with tumor progression [21]. Collagen fibers are extraordinarily stiff in tension and offer tensile strength to tissue and can also supply solid stress when highly contractile cancer cells apply forces to them [89]. Long-range stress transmission (250–1000 μm) between cells in fibrous matrices is well appreciated [91–93]. Tension-driven fiber alignment, fiber stiffness, as well as fiber strain-hardening all permit and facilitate long-range mechanical interactions [94]. Notably, the range of these mechanical interactions increases with increasing cellular polarization and contractility [94]. Tumor stromal cells such as fibroblasts have been shown to be highly contractile and generate tensional forces by contraction of the surrounding matrix. Tissue tension, such as that generated by activated fibroblasts, has been demonstrated to influence vascular growth. Ingrowth and expansion of vascular tissue are associated with and directed by tissue contraction, where endothelial cells outgrow along the direction of tensional forces [95, 96]. Such translocation of functional vascular formations into tissue has previously been described for tumor-induced neovascularization of mouse cornea [97] and in human dermal wound healing models [98]. These data help to establish the concept of biomechanical regulation of tissue vascularization.

Fluid stress in the tumor microenvironment is the combined forces exerted by the fluid components of the tumor, namely, the microvascular fluid pressure, interstitial fluid pressure, and shear stress, applied by the blood flow and interstitial flow [89]. Within tumors, elevated interstitial fluid pressure from leaking blood vessels and ineffective intratumor lymphatics leads to abnormal tumor vasculature due to the resulting transmural pressure (Table 6.1) [10, 90, 99]. In both experimental and human solid tumors, interstitial fluid pressure has been reported to commonly range from 4 to 60 mmHg in neoplastic regions [32, 35, 42, 100] and has been reported as high as 130 mmHg in mouse pancreatic ductal adenocarcinomas [59]. The subsequent abnormal structure of the tumor microvasculature increases geometric and viscous resistances to blood flow, further contributing to aberrant flow and limited perfusion in tumor tissue [89]. Aberrant flow in the tumor vasculature is significant and can influence endothelial cell function. Distinct flow patterns in the different regions of normal vessels are important in regulating molecular and morphological differences needed for endothelial cell specialization [101]. Flow and shear stresses have a well-established effect on endothelial cells. Fluid shear stress enacts signaling cascades that influence endothelial morphology as well as trigger remodeling of vascular networks [102]. Precisely, fluid shear stresses affect vascular endothelial growth factor receptor (VEGFR) conformational changes [103], tubule formation [104], and barrier function [105] and ultimately direct endothelial morphogenesis and sprout formation [106, 107]. Basal-to-apical transendothelial flow has also been demonstrated to induce an invasive phenotype through focal adhesion kinase (FAK)-mediated signaling and extensive endothelial cell-cell junction remodeling [108]. Endothelial cells lining tumor vessels are subjected to such transendothelial pressure and flow, and these findings are in agreement with early observations that tumor angiogenesis emerges predominately from the

venous side of the circulation [109]. Together, these data demonstrate that fluid stresses not only influence tumor vessel perfusion but also contribute to abnormal vessel structure and function.

Collectively, solid and fluid stresses in the tumor microenvironment act to compress tumor vessels and significantly alter blood flow through the tumor. Growth-induced solid stress in solid tumors has been reported to commonly range from 10 to 142 mmHg [89, 90], while interstitial fluid pressure within tumor tissue has been reported to commonly range from about 4 to 60 mmHg (Table 6.1) [32, 35, 42, 100, 110]. Together, these forces act to compress blood vessels in the tumor, causing limited perfusion and hypoxia throughout the tumor tissue (Fig. 6.1c, d). Notably, solid stress in the tumor, rather than increased interstitial fluid pressure, has been identified to be the predominant cause of vessel compression [90, 110]. Removal of the mechanical forces in solid tumor tissue can recover some of the aberrant features of the tumor vasculature. Depletion of the structural components that contribute to solid stress in the tumor microenvironment – cancer cells, fibroblasts, or collagen – significantly reduces solid stress and improves perfusion through the tumor tissue in breast, pancreatic, and melanoma tumor models [34]. Together, the physical forces that accumulate during tumor growth considerably impact vessel architecture, permeability, and perfusion. Better understanding of these physical forces, and their influence on tumorigenesis, will be important for improving therapeutic treatment.

6.3 Mechanosensory Pathways in Tumor Angiogenesis

Conventionally, biochemical signals have been believed to serve as the principal means that signaling pathways are activated in endothelial cells; however, mechanical forces have more recently also been demonstrated to regulate endothelial cell phenotype and function. Recent work has shown that mechanical forces control endothelial cell proliferation, survival, migration, and ECM remodeling, all of which play prominent roles in angiogenesis [111, 112]. Dynamic cellular response to mechanical forces is essential to vascular biology. For instance, fluid shear stress from blood flow plays a critical role in regulating vessel morphogenesis, sprouting, and barrier function [113, 114]. To convert mechanical forces and biophysical cues into intracellular biochemical signaling cascades, endothelial cells employ an interconnected system of mechanosensors to sense and respond to mechanical cues. These mechanosensors include the actin cytoskeleton, integrins, cell-cell adhesion receptors, receptor tyrosine kinases, and other membrane proteins including ion channels and G-protein-coupled receptors (Table 6.3). Often in cancer, and in tumor endothelial cells specifically, many of these mechanosensory pathways become deregulated and/or malfunction leading to abnormal tumor endothelial cell function.

6.3.1 The Actin Cytoskeleton and Integrins

The actin cytoskeleton and integrins act as principal mechanotransducers in cells. Early experiments identified molecular connections between integrins, cytoskeletal filaments, and nuclear scaffolds, where exogenous force on integrins caused cytoskeletal filament reorientation, nuclei distortion, and nucleoli redistribution [115]. The cytoskeleton serves as the load-bearing architecture of the cell as well as a mechanical coupler to the ECM. As such, the cytoskeleton is vital to cellular response to environmental cues [116–118]. Adhesion proteins, known as integrins, serve as the main receptors that mediate the connection of the cytoskeleton to the surrounding ECM. ECM components bind to integrins that are linked intracellularly to the actin cytoskeleton. Mechanical stresses distributed throughout the ECM then converge on integrins [117]. The short cytoplasmic tail of integrins enable intracellular signaling cascades in response to mechanical cues, which can regulate various cell functions including cell survival, proliferation,

Table 6.3 Prominent mechanosensory pathways in tumor angiogenesis

Mechanosensor	Location	Mechanical activation	Relevant function	Role in tumor angiogenesis	References
PECAM1	Adherens junctions, apicolateral membrane	Fluid shear stress, circumferential strain	Phosphorylated in response to mechanical forces, transactivates VEGFR	Important in changes to cytoskeletal architecture. Activates VEGFR and downstream signaling events	[114, 146, 147, 149, 151]
VE-cadherin	Adherens junctions	Fluid shear stress, circumferential strain	Transmembrane scaffolding of PECAM1 and VEGFR2/3. Important in maintaining barrier function	Disrupted VE-cadherin endothelial cell-cell junctions are observed in stiff environments and tumor vasculature	[12, 113, 114, 152, 156]
VEGFR2	Adherens junctions, apical membrane	Fluid shear stress, circumferential strain	Shear stress causes ligand-independent phosphorylation, activates MAPK/PI3K/Akt	Elevated expression in tumor blood vessels. Involved in tumor EC barrier integrity. Major signal transducer for angiogenesis	[147, 149, 157–159]
VEGFR3	Adherens junctions, apical membrane	Fluid shear stress, circumferential strain	Shear stress causes ligand-independent phosphorylation, activates MAPK/PI3K/Akt.	Involved in maintaining tumor EC barrier integrity. Inhibition reduces vascular density	[149, 160, 161]
Integrins	Basal adhesion complexes	Fluid shear stress, cell-ECM stress	Shear stress causes downstream activation by PI3K to regulate cell orientation. Important in sensing and applying cell-ECM stresses	Inhibition of α1β1 and α2β1, α5β1, as well as αvβ5 and αvβ3 suppress tumor angiogenesis. αvβ3 and αvβ5 integrin expression linked to grade of neuroblastoma	[117, 126, 130–133, 141]
Actin cytoskeleton	Cortical plasma membrane, cytoplas-mic, perinuclear	Fluid shear stress, circumferential strain, cell-ECM stress	Fluid shear stress causes filament deformations. Inhibition blocks many responses to mechanical cues	Tumor endothelial cells demonstrate increased cellular contractility and aberrant mechanosensitivity	[80, 113, 116–118]
TRPV4	Apical membrane	Fluid shear stress, circumferential strain	Regulates mechanosensitivity and Rho/ROCK activity	Tumor endothelial cells have reduced TRPV4 expression, leading to aberrant Rho/ROCK mechanosensitivity	[122, 174–176]
EP2	Apical/basal membrane	Fluid shear stress, cell-ECM stress	Induces VEGF expression via ERK2/JNK1 activation	Released from cancer cells to elicit a pro-angiogenic response	[178, 179, 181]
S1PR	Apical/basal membrane	Fluid shear stress, cell-ECM stress	Activation leads to Rac-Cdc42 signaling and correlates with ERK1 and ERK2 activation	Important role in regulating endothelial cell cytoskeletal structure, migration, capillary-like network formation, and vascular maturation	[178, 179, 182]
PAR1	Apical/basal membrane	Fluid shear stress, cell-ECM stress	Modulates Rho GTPase activity	Influences endothelial cell permeability. PAR1 expression increased in cancer	[178, 179, 183, 184]

and migration [119–121]. In endothelial cells, 130 pN force exerted on integrins has been demonstrated to elicit Rho-mediated cytoskeletal tension [122], which precedes both stress fiber and focal adhesion formations [123]. Recent work has implicated changes in cell mechanics in the pathogenesis of many diseases, including cancer. Cancer cells exhibit significantly distinct mechanical properties compared to their non-tumorigenic counterparts. As such, disruption of cytoskeletal regulation has been linked to cancer progression. Alterations to cytoskeletal organization as well as upregulation of cytoskeletal scaffolding proteins and signaling circuits contribute to an altered mechanical state and have been tied to tumorigenesis [124]. Cancer cells are associated with increased contractility, where cellular traction stresses increase with increasing metastatic potential in breast, prostate, and lung cancer models [125]. Similarly, many integrin signaling pathways are exploited in cancer to support tumor progression. Together, these alterations manipulate cell function in order to better manipulate the host microenvironment and provide abundant vasculature to the tumor to support tumor growth [126].

Changes to the ECM during tumor progression, such as ECM stiffening, are sensed through the cytoskeleton and integrin receptors. ECM stiffening causes enhanced integrin-mediated Rho/Rho-associated protein kinase (ROCK) activity and contraction in tumor epithelial cells [26, 127] as well as tumor endothelial cells [80]. Abnormal Rho-mediated sensing of mechanical forces has been suggested to contribute toward the aberrant behaviors observed in tumor endothelial cells that produce structural abnormalities [80]. Tumor endothelial cells have abnormal mechanosensitivity to uniaxial cyclic strain transmitted through the ECM [80], which has been shown to be mediated by dynamic regulation of Rho activity and cytoskeletal tension [128]. Interestingly, tumor endothelial cells also displayed thicker stress fibers, stronger adhesion strength, enhanced cytoskeletal tension, and constitutively high baseline activity of Rho and ROCK. However, normal and tumor endothelial cells express comparable levels of active β1 and β3 integrins, indicating these observations are a result of higher intrinsic Rho- and ROCK-dependent cytoskeletal tension [80]. These differences in response to mechanical cues between normal and tumor endothelial cells suggest that the abnormal mechanical and structural components of the tumor microenvironment may cause tumor endothelial cells to gradually obtain an altered phenotype. Such alteration in mechanosensitivity may additionally enable tumor endothelial cells to spread and form capillary networks over a wider range of matrix stiffness compared to normal endothelial cells [80].

Specific integrins have been demonstrated to contribute to not only angiogenesis but also tumor angiogenesis and tumor progression [129–131]. Expression of α1β1 and α2β1 integrins is upregulated by VEGF in endothelial cells [132], and combined antagonism of α1β1 and α2β1 reduces tumor growth and tumor angiogenesis of human squamous cell carcinoma xenografts [133]. The α5β1 integrin is selectively expressed in angiogenic vasculature and is necessary for proper angiogenesis [131, 134]. Endothelial cells undergoing angiogenesis upregulate αvβ3 and αvβ5 integrins in order to facilitate growth and survival of newly forming vessels [126, 135]. Cytokine-dependent pathways of angiogenesis have been shown to have a necessity for αv integrins. Integrin αvβ3 is necessary for angiogenic pathways activated by basic fibroblast growth factor (bFGF) or tumor necrosis factor α (TNF-α), and integrin αvβ5 is necessary for angiogenic pathways activated by VEGF or transforming growth factor α (TGF-α) [136]. Specifically, the αvβ5 integrin pathway downstream of VEGF causes activation of FAK and Src kinase [137]. Many of these pro-angiogenic factors have been implicated in promoting tumor angiogenesis [3]. The αvβ3 integrin has also been demonstrated to be required for angiogenesis [138], as well as associate with VEGFR2 and be involved with VEGFR2 recycling events [126]. Consequently, binding of αvβ3 to its corresponding ECM ligands has been shown to increase VEGF

signaling [139, 140]. Moreover, αvβ3 and αvβ5 integrins are selectively expressed in tumor vasculature [130]. Integrin αvβ3 is highly expressed on angiogenic vessels of malignant breast carcinoma [141], and the level of expression of αvβ3 and αvβ5 integrins in tumor endothelial cells has been tied to the grade of malignancy in neuroblastoma [142]. Inhibition of αvβ3 suppressed angiogenesis and reduced tumor growth of breast carcinoma in a severe combined immunodeficient (SCID) mouse/human chimeric model [141] as well as resulted in tumor reduction in human clinical trials [143]. Combined inhibition of αvβ3 and αvβ5 integrins also significantly reduced growth of human melanoma xenografts in SCID mice [144]. Integrin α6β4 signaling has likewise been demonstrated to be involved in cancer cell invasion and selectively expressed in tumor vasculature. Specifically, integrin α6β4 is involved in the promotion and onset of the invasive phase of pathological angiogenesis. The β4 substrate domain promotes bFGF- and VEGF-mediated angiogenesis and regulates angiogenic sprouting by promoting nuclear translocation of activated ERK and NF-κB as endothelial cells migrate [129]. Furthermore, melanoma, lung, lymphoma, and fibrosarcoma tumors in mice carrying targeted deletion of the signaling portion of the integrin β4 subunit had significant reduction in tumor size and microvascular density compared to wild-type mice, indicating the β4 substrate domain promotes tumor angiogenesis [129]. Together, these data demonstrate the role of cytoskeletal- and integrin-mediated mechanosensory pathways in facilitating tumor angiogenesis.

6.3.2 Cell-Cell Adhesion Receptors

Endothelial cells form mechanical connections to neighboring cells through a multiprotein cell-cell adhesion structure known as adherens junctions. Adherens junctions are important in endothelial monolayer integrity, contact inhibition of growth, and apoptosis [145, 146]. Within adherens junctions is a mechanosensory complex comprised of platelet endothelial cell adhesion molecule 1 (PECAM1), VE-cadherin, and VEGFR2/3. Within this complex, PECAM1 directly transmits mechanical force, VE-cadherin acts as an adaptor, and VEGFR2 activates biochemical signaling (Fig. 6.2) [113, 147]. Notably, small GTPase activity is required for the functioning of this mechanosensory complex [148].

PECAM1 is a transmembrane immunoglobulin family protein that participates in homophilic adhesion at cell-cell junctions. In response to mechanical stimuli, PECAM1 triggers Src-mediated activation of a Src family kinase, possibly the Src family tyrosine kinase Fyn, which phosphorylates and activates VEGFR2 [147, 149]. PECAM1 is vital to proper vascular development, and PECAM polymorphisms have been linked to pathological vessels [150]. PECAM1 and VE-cadherin-based adhesions are essential for flow-induced integrin activation, and PECAM1-VE-cadherin mechanosensory response has been thought to be dependent on direct force exerted on PECAM1 [146]. Focal adhesion growth and adaptive cellular stiffening in endothelial cells occur due to integrin-dependent RhoA activation from force transduction via PECAM1. Furthermore, local mechanical stimulation of PECAM1 has been demonstrated to elicit a global cellular response, specifically force-dependent activation of PI3K and RhoA activity [151]. Together, this mechanochemical signaling response enables changes to cytoskeletal architecture and adaptive cytoskeletal stiffening.

VE-cadherin assists the association of PECAM1 and VEGFR2 through its transmembrane domain to stimulate downstream activity of VEGFR in response to mechanical activation of PECAM1 [113]. VE-cadherin also plays an important role in sensing and responding to changes in matrix stiffness. Specifically, VE-cadherin modulates cytoskeletal mechanics in response to changes in matrix stiffness through small Rho GTPases [152]. Comparable to integrin-mediated changes in contractility, cadherin-mediated increases in contractility are actin-dependent. As such, endothelial cell-cell

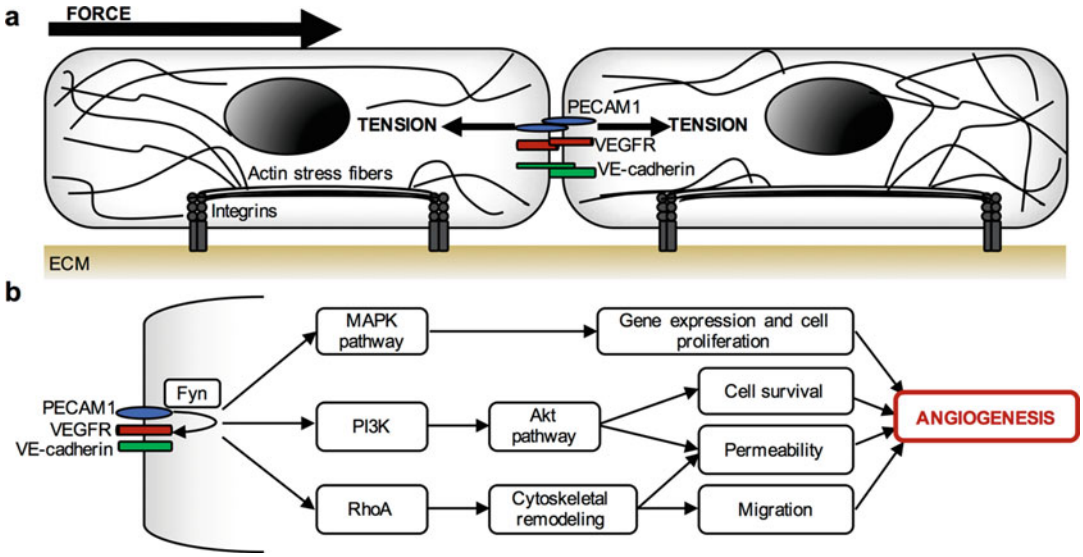

Fig. 6.2 The PECAM1, VE-cadherin, and VEGFR mechanosensory complex utilized by endothelial cells in response to mechanical forces. (**a**) In response to force, tension is applied to PECAM1, followed by VE-cadherin-assisted association of PECAM1 and VEGFR. (**b**) PECAM1 triggers Src-mediated activation of the Src family kinase Fyn, which phosphorylates and activates VEGFR2/3. VEGFR2/3 activates RhoA, PI3K, MAPK, and Akt signaling cascades that influence endothelial cell function and promote angiogenesis

junction integrity is maintained by VE-cadherin. In quiescent endothelial cell networks, VE-cadherin is localized linearly beside cell-cell borders to form continuous, stable adherens junctions, while VE-cadherin is organized in short linear structures perpendicular to cell-cell borders in endothelial cells with reduced network integrity [153]. In response to increased matrix stiffness, disruption of VE-cadherin-mediated cell-cell junctions results in disrupted barrier integrity and increased endothelial cell monolayer permeability in both in vitro and ex vivo models [154]. Such disruptions are also observed in tumor neovasculature. Aberrant tumor vessels demonstrate decreased levels of junctional VE-cadherin, which contributes to lowered barrier tightness and increased vascular permeability [155, 156]. However, cell contractility increases with matrix stiffness, and inhibition of Rho-mediated cell contractility has been demonstrated to decrease VE-cadherin cell-cell separation distance and restore monolayer integrity [154] as well as normalize tumor endothelial cell behavior [80]. These data demonstrate the importance of mechanical cues on VE-cadherin function and cell-cell and cell-matrix connectivity.

6.3.3 VEGFRs and VEGF Signaling

VEGFR signaling is critical for normal endothelial cell migration, proliferation, and angiogenesis. VEGFRs are transmembrane receptor tyrosine kinases (RTKs) that mediate most of the angiogenic effects of VEGF. VEGF-induced activation of RhoA is necessary for endothelial cell cytoskeleton reorganization and migration, and these changes are also accompanied by the formation of small cell-cell openings that contribute to increased permeability [157]. In response to shear stress, VEGFR2 undergoes rapid induction and nuclear translocation, followed by ligand-independent phosphorylation that causes activation of MAPK, PI3K, and Akt signaling pathways that are involved in promoting angiogenesis (Fig. 6.2) [149, 158, 159]. VEGFR2 phosphorylation is additionally accompanied by VEGFR2 membrane clustering and downstream signaling [158]. Cyclic strain prompts dissocia-

tion of VEGFR2 from VE-cadherin, which can increase vascular permeability [113]. Similarly, VEGFR3 has recently been recognized as a member of this mechanosensory complex [149], and has been suggested to be involved in maintaining endothelial barrier integrity during tumor angiogenesis [160]. Antibody inference of VEGFR3 function significantly reduced tumor growth of lung, pancreatic, renal, colon, and prostate tumor xenografts in immunocompromised mice. Notably, the blood vessel density was decreased and the amount of hypoxic and necrotic tissue was increased in these anti-VEGFR3 treated tumors [161]. Depletion of VEGFR2 and/or VEGFR3 leads to significantly diminished endothelial cell response to mechanical cues. More specifically, depletion of either VEGFR significantly lessened shear-induced integrin activation and cell alignment as well as weakly reduced PI3K and AKT signaling; however, all effectors were strongly inhibited through depletion of both VEGFRs [149].

ECM stiffness influences VEGFR expression and vascular development in vitro and in vivo. GATA2 and VEGFR2 expression is increased with increasing substrate stiffness, where GATA2 mediates p190RhoGAP-dependent control of VEGFR2 expression [162]. Matrix stiffness has also been demonstrated to alter cell response to growth factors. Substrate stiffness has recently been shown to modify the coordinated actions of VEGF-matrix binding that is critical for VEGF internalization [163]. In endothelial cells, VEGF-induced changes in stress fiber organization and contractile response are mediated by VEGFR2 and ROCK signaling [157, 164]. Elevated expression of VEGFRs has also been linked to many cancers. For example, VEGFR2, the predominant receptor tyrosine kinase that mediates VEGF signaling and VEGF-mediated angiogenesis, has been identified in bladder, brain, breast, cervical, colon, endometrial, gastric, head and neck, hepatocellular, lung, melanoma, mesothelioma, multiple myeloma, myeloid leukemia, esophageal, ovarian, pancreatic, prostate, renal cell carcinoma, squamous, and thyroid human cancers [165]. In many of these tumors, VEGFR expression has been correlated with either poor survival, disease progression, and/or recurrence [165]. This increased VEGFR expression has been seen on both tumor cells and endothelial cells. Notably, compared to normal blood vessels, the expression of VEGFR1 (FLT1) as well as VEGFR2 (KDR) is enhanced in tumor blood vessels [166–168]. These data suggest that cell response to growth factor signaling is closely linked to matrix stiffness, and altered sensitivity may play an important role in tumor angiogenesis.

6.3.4 Membrane Proteins

The cell membrane offers a large target for external mechanical forces to act upon, and as such mechanosensitive ion channels present in the membrane serve as one of the earliest responses to mechanical force and changes to the microenvironment. As key operators of cell signaling, ion channels have been implicated in tumorigenesis and have altered expression in tumor cells as well as stromal and endothelial cells [169]. Recent work has demonstrated that the transient receptor potential (TRP) ion channel superfamily is linked with an array of cancers [170], and abnormal TRP ion channel function can cause sustained proliferation, evasion of growth suppressors, and resistance to cell death [171, 172].

TRP channels have also been identified to be critical to endothelial cell function, and TRP ion channel malfunction and/or dysregulation is associated with endothelial cell dysfunction including disruption of angiogenic competence and barrier maintenance [173]. Specifically, transient receptor potential vanilloid 4 (TRPV4) has been shown to regulate tumor angiogenesis and tumor endothelial cell function by modulating cellular mechanosensitivity. Tumor endothelial cells demonstrate reduced TRPV4 expression correlated with aberrant mechanosensitivity toward ECM stiffness. Together, these changes in TRPV4 expression lead to increased migration and abnormal angiogenesis [174]. Loss of TRPV4 in TPRV4 knockout endothelial cells leads to significantly increased proliferation, migration, and basal Rho activation reminiscent of tumor-derived

endothelial cells [175]. Further, the absence of TRPV4 in TRPV4 knockout mice was found to result in increased vascular density, increased vessel diameter, and reduced pericyte coverage within lung carcinoma tumors compared to wild-type mice – all principle characteristics of abnormal tumor angiogenesis [174]. Either overexpression or pharmacological activation of TRPV4 or pharmacological inhibition of the downstream Rho/ROCK pathway was able to normalize tumor vasculature, reduce tumor growth, and improve cancer therapy of lung tumors in a mouse model [174, 175]. These findings provide further support that aberrant Rho/ROCK mechanosensitivity is a significant contributor to abnormal tumor endothelial cell function. Interestingly, some data also suggests that integrins and mechanosensitive ion channels are well connected [122]. Cyclic strain to endothelial cells causes activation of TRPV4, which then activates supplementary integrins and triggers downstream cytoskeletal reorganization [176]. While TRPV4 has been the most studied TRP channel in tumor angiogenesis, other TRP superfamily channels have been implicated as contributors of abnormal tumor angiogenesis as well [169]. These data further demonstrate the role of abnormal mechanosensory pathways in tumor endothelial cell function and tumor angiogenesis.

The large family of cell-surface G-protein-coupled receptors (GPCRs) have additionally been identified as contributors of tumor angiogenesis and aberrant tumor endothelial cell function. Normally, GPCRs are activated when an extracellular ligand binds to or induces an active conformation. However, fluid shear stress and increased membrane tension have also been reported to induce conformational transitions and activation of GPCRs in endothelial cells, suggesting GPCRs are involved in mediating mechanochemical signaling in endothelial cells [177]. Many GPCRs are overexpressed in various cancers. During tumor progression, cancer cells frequently take over the natural physiological functions of GPCRs to proliferate, evade immune detection, invade surrounding tissue and metastasize, as well as increase angiogenesis [178]. The GPCRs prostaglandin E2 (PGE2) receptor EP2, sphingosine-1 phosphate receptors (S1PRs), and protease-activated receptor 1 (PAR1) have all been strongly implicated in eliciting a pro-angiogenic response in breast, head and neck, colon, non-small-cell lung, and prostate cancers [178–180]. The release of PGE2 from tumor cells, due to unregulated expression of COX2, stimulates expression of EP2 receptors on endothelial cells and induces VEGF expression via ERK2/JNK1 activation [181]. S1PR1 activation has been linked to endothelial cell survival, chemotactic motility, and capillary-like network formation as well as release of pro-angiogenic cytokines from tumor cells [182]. PAR1 activation has been shown to modulate Rho GTPase activity and play an important role in endothelial adherens junction disassembly and vascular permeability [178, 183]. Notably, PAR1 expression is directly correlated with invasiveness of breast cancer, where highly metastatic human breast cell lines and breast carcinoma biopsy specimens express high levels of PAR1 [184]. Taken together, these GPCRs provoke a pro-angiogenic response in tumors via activation of a network of small GTPases, Akt, and MAPK signaling that stimulates endothelial cell migration, survival, and growth.

6.4 Clinical Impact of Abnormal Tumor Vasculature

6.4.1 Impaired Barrier Function and Delivery of Chemotherapeutics

Together, the mechanical forces found in tumors work to produce a functionally abnormal tumor vasculature with impaired barrier function. Solid tumor vasculature is often leaky with a defective endothelium. Indeed, the tumor vasculature is characterized by its defective endothelial monolayer, large intercellular openings and holes, and abnormal sprouts that all work to impair barrier function [6]. Normal endothelial cells form uniform monolayers; however, tumor endothe-

lial cells are irregular in shape and size, have cytoplasmic projections into the vessel lumen, and form an incomplete endothelium. Tumor blood vessels have large intracellular gaps between tumor endothelial cells, highlighted by transcellular holes, fenestra, and channels [6]. Additionally, high tumor endothelial cell motility and turnover may hinder the formation of intercellular junctions, further promoting larger intercellular openings [6]. Endothelial junctions are also highly dynamic and sensitive to extracellular stimuli. As such, VE-cadherin-based junctions are susceptible to continuous reorganization due to the dynamically changing tumor ECM and the aberrant mechanosensitivity of tumor endothelial cells [80]. Consequently, tumor blood vessel hyperpermeability and impaired barrier function arise due to the combined effects of tumor vessels lacking or having abnormal function of endothelial cells, pericytes, and/or basement membrane [185].

Leakiness of the tumor vasculature not only impacts tumor growth and metastasis but also has a profound impact on drug delivery to the tumor. Traditionally, vessel leakiness is believed to be due to overexpression of pro-angiogenic growth factors; however, emerging work has demonstrated that the physical environment plays an important role in impairing endothelial cell barrier integrity. Elevated ECM stiffness increases endothelial cell-cell junctional properties and endothelial permeability in vitro and in vivo [12]. Vessel compression due to mechanical forces in the tumor microenvironment causes large areas of the tumor to have limited perfusion and limited systemic administration of therapeutic agents [186–188]. Vessel compression along with the highly tortuous and disorganized arrangement of tumor blood vessels creates sluggish and heterogeneous blood flow, which can affect microvascular pressure [89, 189]. While accurate measurements of microvascular pressure are challenging to obtain, it has been reported that increased tumor interstitial fluid pressure is also accompanied by increased microvascular pressure [190]. For example, microvascular pressure in normal tissue is approximately 15 to 25 mmHg, while the microvascular pressure in tumor tissue has been reported to range from 5.5 to 34 mmHg in MCaIV mouse mammary carcinoma tumors [35]. Importantly, the elevated interstitial fluid pressure found in tumor tissue is often nearly as high as or can exceed microvascular pressure, eliminating pressure gradients across tumor vessels and inhibiting convective transport of drugs [89]. Combined, these factors severely limit the efficacy of traditional cancer treatments.

Efficient and uniform systemic delivery of cancer therapeutics is a critical challenge in cancer treatment. To increase the delivery and efficacy of therapeutics, an emerging cancer treatment strategy seeks to normalize the tumor vasculature [191]. The anti-angiogenic drug bevacizumab, an antibody targeted against VEGF, has been used in combination with chemotherapy and has produced a 5-month increase in survival in colorectal cancer patients [192]. Other preclinical studies have demonstrated that anti-angiogenic agents can increase perfusion and drug uptake in tumors [42, 193]. As such, this has led to many pro- and anti-angiogenic therapies that seek to restore normal vessel densities [194]; however, angiogenic signaling is robust and redundant, and inhibition of individual signaling molecules can be overcome by escape mechanisms [194, 195]. For example, initial response to anti-angiogenic therapies targeting the VEGF pathway is followed by a restoration of tumor progression. In both clinical and preclinical settings, emerging data describe that tumors develop either evasive resistance or intrinsic resistance to these treatments [196]. For these reasons, it is essential to pursue novel methods for tumor vasculature normalization, and targeting mechanical forces in the tumor and/or mechanosensory pathways may be one possible strategy.

6.4.2 Promotion of an Aggressive Tumor Phenotype

As the vasculature not only provides oxygen and nutrients but is also a conduit for the removal of waste products, abnormalities in the vasculature are a major contributor to other abnormalities that exist in the tumor microenvironment [6].

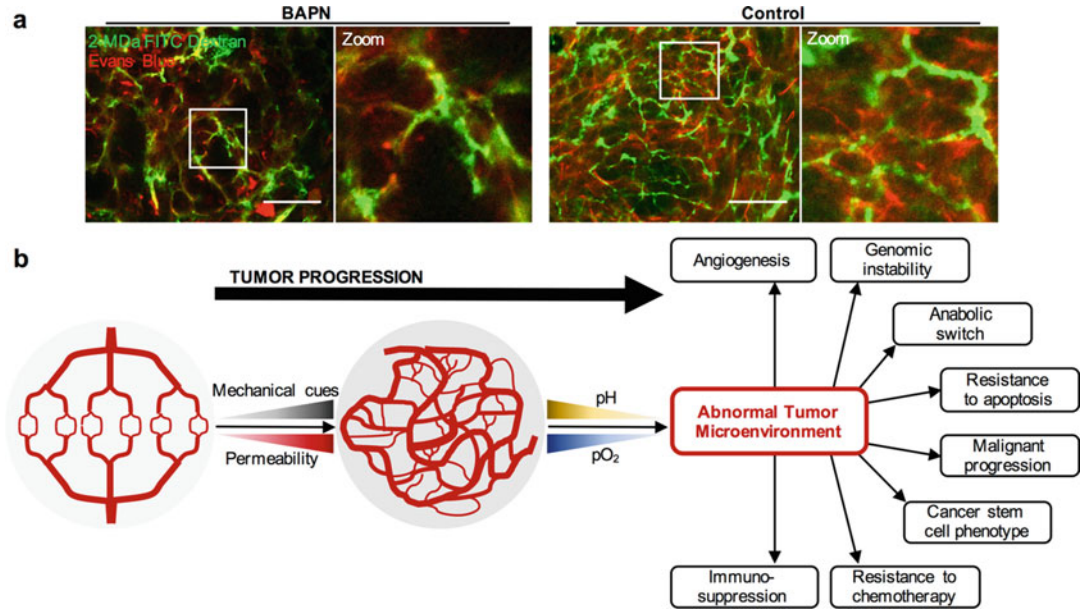

Fig. 6.3 Abnormal tumor vasculature that develops during tumor progression helps to promote an abnormal tumor microenvironment that promotes a more aggressive tumor phenotype. (**a**) In vivo tumors from MMTV-PyMT mice treated with BAPN to soften the tumor tissue or vehicle controls showing 2 MDa FITC-labeled vasculature (green) and extravasating Evans Blue (red) (scale bar = 150 μm). Control tumors demonstrate more abnormal vascular architecture and increased vascular permeability compared to softened tumors. (**b**) During tumor progression, increased mechanical cues in the tumor microenvironment contribute to creating abnormal tumor vasculature that is highly permeability and inefficient in delivering oxygen and nutrients. Limited diffusion in the tumor creates a hypoxic and acidic environment that not only promotes angiogenesis but also promotes genomic instability, an anabolic switch in metabolism, resistance to apoptosis, malignant progression, induction of a cancer stem cell phenotype, as well as resistance to many cancer therapies

Vascular abnormalities lead to a hypoxic and acidic tumor microenvironment [197]. It is well established that tumor blood vessels are heterogeneous in organization and structure, and tumor blood vessels are often more abundant at the tumor-host interface compared to more central regions of the tumor. Furthermore, vascular density has been reported to decrease during tumor progression [198]. As previously discussed, these heterogeneities and abnormal organization arise from changes to the ECM and accumulation of stresses during tumor progression. Consequently, the spatial disorganization and abnormal architecture of the tumor vasculature create diffusion-limited hypoxia throughout the tumor tissue as intercapillary distances often exceed 100–200 μm, the maximum nutrient and oxygen diffusion limits [2].

Such a harsh microenvironment was originally thought to starve the tumor and decrease cancer cell survival; however, it has been established that hypoxia helps to promote a more aggressive and difficult-to-treat tumor phenotype (Fig. 6.3). Specifically, the abnormal tumor microenvironment employs selective pressures that cause cancer cell populations to dynamically adapt [13]. Not only do cancer cells prosper in this harsh environment, but such selection pressures contribute to the propagation of cancer cells [6]. Hypoxia provokes proteome changes, induce pro-survival changes in gene expression, control the anabolic switch in central metabolism, as well as help to drive malignant progression through genomic changes in neoplastic cells [199, 200]. Additionally, a hypoxic and acidic microenvironment affects host immuneresponse. Hypoxia and

acidosis reprogram local macrophages into an immunosuppressive phenotype that helps cancer cells evade immune detection as well as diminishes the killing potential of immune effector cells within the tumor microenvironment [13].

Hypoxia also influences cancer cell response to radiation and many chemotherapeutics. This can occur through a variety of mechanisms [199]. The most widely occurring mechanisms of hypoxia-mediated resistance to cytotoxic therapy include extracellular acidification causing decreased drug uptake, resistance to apoptosis, and genomic instability that causes further mutagenesis of cancer cells. For many bio-reductive prodrugs that are intended to be metabolized, inadequate extravascular penetration of the drug significantly contributes to chemoresistance [199, 201]. Together, these findings indicate that abnormalities in the tumor vasculature help to make cancer treatments exceedingly challenging due to a rapidly altering cancer cell phenotype and resistance to many traditional therapies.

6.5 Conclusions

Mechanical forces in the tumor microenvironment play an important role in directing tumor growth and promoting abnormal tumor vasculature. Stiffening of the tumor ECM promotes abnormal branching patterns, vascular density, as well as increased endothelial cell-cell junctions and permeability, whereas mechanical stresses in the tumor compress tumor blood vessels and limit perfusion. Growing evidence indicates that such mechanical alterations in the tumor microenvironment help to alter tumor endothelial cell phenotype and mechanosensitivity. This abnormal mechanosensitivity is now being tied to deregulated or malfunctioning mechanosensors in tumor endothelial cells. While it is clear that the mechanical microenvironment mediates tumor angiogenesis, much work still remains to fully understand specific mechanosensory pathways utilized by endothelial cells to respond to aberrant mechanical cues. Identifying these pathways will better our understanding of mechanical regulation in tumor angiogenesis and provide new methods to tame the physical forces in tumors. Such findings will provide important understanding to how changes in the tumor microenvironment facilitate tumor progression and may present new therapeutic targets to normalize the tumor vasculature.

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