Physical and Chemical Gradients in the Tumor Microenvironment Regulate Tumor Cell Invasion, Migration, and Metastasis

MADELEINE J. OUDIN¹ AND VALERIE M. WEAVER^{2,3,4,5}

¹*Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139*

²*Department of Surgery, Center for Bioengineering and Tissue Regeneration, University of California, San Francisco, San Francisco, California 94143*

³*UCSF Comprehensive Cancer Center, Helen Diller Family Cancer Research Center, University of California, San Francisco, San Francisco, California 94143*

⁴*Department of Anatomy, Department of Bioengineering and Therapeutic Sciences, and Department of Radiation Oncology, University of California San Francisco, San Francisco, California 94143*

⁵*Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research and The Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, San Francisco, California 94143*

Correspondence: Valerie.Weaver@ucsf.edu

Cancer metastasis requires the invasion of tumor cells into the stroma and the directed migration of tumor cells through the stroma toward the vasculature and lymphatics where they can disseminate and colonize secondary organs. Physical and biochemical gradients that form within the primary tumor tissue promote tumor cell invasion and drive persistent migration toward blood vessels and the lymphatics to facilitate tumor cell dissemination. These microenvironment cues include hypoxia and pH gradients, gradients of soluble cues that induce chemotaxis, and ions that facilitate galvanotaxis, as well as modifications to the concentration, organization, and stiffness of the extracellular matrix that produce haptotactic, alignotactic, and durotactic gradients. These gradients form through dynamic interactions between the tumor cells and the resident fibroblasts, adipocytes, nerves, endothelial cells, infiltrating immune cells, and mesenchymal stem cells. Malignant progression results from the integrated response of the tumor to these extrinsic physical and chemical cues. Here, we first describe how these physical and chemical gradients develop, and we discuss their role in tumor progression. We then review assays to study these gradients. We conclude with a discussion of clinical strategies used to detect and inhibit these gradients in tumors and of new intervention opportunities. Clarifying the role of these gradients in tumor evolution offers a unique approach to target metastasis.

Tumors are highly heterogeneous and this feature compromises treatment efficacy and promotes tumor cell dissemination and metastasis to reduce patient survival. Tumor heterogeneity is driven in part by genetic alterations induced through genomic instability and maintained by the evolutionary selection of these genetically modified cells (Alizadeh et al. 2015). Epigenetic, transcriptional, and posttranslational modifications also contribute significantly to tumor cell diversity. Furthermore, cancer develops within a complex tissue microenvironment that itself fosters tumor heterogeneity through clonal selection as well as via epigenetic reprogramming and modifications of the tumor phenotype. The tumor microenvironment is composed of cellular constituents that include cells of the vasculature, infiltrating immune cells, and resident fibroblasts, adipocytes and nerves, and a noncellular component composed of diverse soluble factors and a highly variable and evolving insoluble extracellular matrix (ECM) (Joyce and Pollard 2009). The composition and organization of the tumor microenvironment vary significantly within and across tumors.

One key feature of the noncellular microenvironment is the existence of local chemical and physical gradients that exert profound effects on the tumor phenotype, including its predilection to disseminate. In this regard, metastasis is facilitated by efficient local tumor cell invasion that is fostered by ECM and chemokine/cytokine/growth factor gradients which cooperate to promote the directional migration of the tumor cells toward the vasculature and lymphatics and facilitates their extravasation from the primary tumor site into the circulation and their intravasation into the distal tissue. In this review, we discuss the role of directional migration in cancer progression and metastasis in the context of local microenvironmental gradients that develop in the primary and metastatic tumor tissue. We begin by reviewing the generation of endogenous gradients within both the primary and metastatic tumor tissues. We then summarize some of the techniques currently used to study directional migration in cancer and discuss putative signaling mechanisms regulating directional migration in response to external cues and the intrinsic state of the tumor cell. We have

chosen to focus exclusively on methods applied to the study of cancer progression and mechanisms driving directional migration of tumor cells. Finally, we discuss existing approaches available to interfere with directional migration that could prevent tumor metastasis and outline potential future avenues for therapeutic development.

TUMOR HETEROGENEITY LEADS TO ENDOGENOUS GRADIENT GENERATION IN TUMORS

Local Gradients of Secreted Factors

Chemokines and growth factors (GFs) are key drivers of tumor cell invasion and facilitate tumor cell extravasation and intravasation to promote metastasis. Tumor cells secrete chemoattractants for macrophages, neutrophils, lymphocytes, fibroblasts, and mesenchymal stem cells that once recruited secrete chemokines and GFs that stimulate tumor cell migration (Roussos et al. 2011). The specific, directed migration of stromal cells into the tumor and the reciprocal persistent migration of the tumor cells into and through the stroma are reinforced by the restricted expression of specific receptors for each of the plethora of secreted factors. For example, tumor-associated macrophages (TAMs) release growth factors, including epidermal growth factor (EGF), that activate the EGFR receptor expressed on the breast tumor cells to stimulate and direct their migration (Wyckoff et al. 2004). The breast tumor cells in turn secrete colony stimulating factor 1 (CSF-1), which stimulates and recruits TAMs through TAM-specific expression of the CSF-1 receptor. This vicious feed-forward paracrine loop between the invading tumor cells and infiltrating macrophages ensures the persistent migration of the tumor cells toward the vasculature that is key for tumor cell dissemination and metastasis. The chemokine CXCL12, produced by pericytes and tumor cells, can also drive in breast tumor cell chemotaxis in vivo (Müller et al. 2001). Although the molecular details remain to be elaborated, several in vitro studies have attested to the existence of similar local reciprocal gradients of GFs such as EGF ligands, fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), transforming growth factor β (TGF- β), vascular endothelial growth factor (VEGF), and CSF-1, in a variety of tumor types, investigating how this interplay stimulates stromal cell infiltration to potentiate tumor cell migration and dissemination (Roussos et al. 2011). Interestingly, tumor cells themselves can also create local gradients to facilitate their directed migration as has been shown through their matrix metalloproteinase-mediated degradation of the ECM or as was recently illustrated for melanoma tumor cells that create their own lipid chemoattractant lysophosphatidic acid gradient to foster their migration (Muinonen-Martin et al. 2014; Tweedy et al. 2016). Furthermore, soluble gradients have been implicated in tissue tropism within the context of metastasis and distal organ colonization. Indeed, chemokines secreted by cancer-associated fibroblasts (CAFs) have been associated with local stromal

cells secreting a chemokine whose receptor is located on the tumor cell. For example, CAF-secreted CXCL12/SDF-1 drives metastasis of CXCR4-expressing breast cancer cells to the lymph nodes and lung (Müller et al. 2001), with CCL27 driving CCR10-dependent skin metastasis of melanoma and CCL21/CCL19 activating CCR7 to promote lymph node metastasis (Ben-Baruch 2008) (Figs. 1 and 2).

Changes in ECM Abundance and Organization

The ECM is a complex scaffold of proteins that provide structural support to the surrounding tissue. Increased deposition and accelerated post-translational modifications including cross-linking of the ECM are characteristic features of the tumor desmoplastic response that is associated with poor patient prognosis (Walker 2001). Recently, proteomics of the decellularized, soluble ECM fraction of the tumor tissue identified core matrix proteins and found that this signature contains as many as 274 proteins composed of glycoproteins, collagens, and proteoglycans (Naba et al. 2012). Intriguingly, using this approach and relying on analysis that uses species-specific peptides, it was revealed that many of the tumor ECM proteins including a number of abundant laminins (LAMA3, B3, C2) and Sned1 are secreted predominantly by the tumor cells, whereas other ECM components such as tenascin C (TNC) and vitronectin are secreted exclusively by resident fibroblasts and infiltrating stromal cells (Naba et al. 2014). Complementing these studies, advanced proteomic approaches have been able to profile the insoluble ECM proteins within the tumor, revealing distinct insoluble protein profiles within a tumor type that not only reflect the tumor stage but also the tumor genotype (Goddard et al. 2016; Laklai et al. 2016). Data revealed that the most aggressive pancreatic tumors that harbor SMAD4 mutations and that are associated with the shortest patient survival deposited quantities of collagen 12, TNC, and fibronectin (FN) nests surrounding the invading ductal lesions (Laklai et al. 2016). Overall, multiple proteomic studies have permitted researchers to conclude that the ECM is temporally modulated, spatially distinct, and influenced by the tumor genotype, and that its phenotype is dictated by both the tumor cells themselves as well as the resident and infiltrating stromal cells (Naba et al. 2014, 2016). For instance, FN, which is typically elevated in tumors, is highly heterogeneous with the highest levels found at the tissue periphery, near the vasculature and associated with thick collagen bundles (Astrof and Hynes 2009; Zhang et al. 2014; Miroshnikova et al. (in review)). Peripheral solid tumors are typically fibrotic and characterized by elevated reorganized collagens that are often lysyl oxidase cross-linked into bundles upon which tumor cells have been seen migrating (Wang et al. 2004; Wyckoff et al. 2006; Erler and Weaver 2009). Using two-photon imaging has made it possible to characterize tumor collagen organization, and using structured illumination polarized imaging informs on the degree of alignment

Figure 1. Endogenous gradients are present in primary breast tumors. Tumorigenesis is associated with changes in the tumor microenvironment that lead to the development of endogenous gradients at the single-cell level to whole tissue (summarized in part 1). pH: At the single-cell level, pH gradients develop between the intracellular and extracellular compartments. GF/Chemokine: Individual tumor cells can also respond to soluble gradients secreted by surrounding stromal cells. Stiffness: Tissue compression by the tumor and external forces generated by tumor cells or tumor cell-driven ECM remodeling of collagen can all lead to the development of endogenous stiffness gradients at the tumor-stroma interface. Electrical: Tumorigenesis is accompanied by changes in membrane potential of luminal structures. ECM: The ECM of tumor versus normal tissues changes and some ECM cues, in particular FN, are heterogeneously distributed throughout the tumor, leading to the development of endogenous gradients. Oxygen: It is well established that tumor tissues become hypoxic as they grow due to poor vascularization, leading to whole-tissue oxygen gradients. GF, growth factor; ECM, extracellular matrix; FN, fibronectin.

(Acerbi et al. 2015). These two approaches have revealed that the thick, highly aligned, fibrillar collagens typically observed in a primary tumor are the result of the dramatic reorganization of the relaxed, coiled short fibers found in a normal tissue (Levental et al. 2009). Provocatively, the organization of the tumor-associated fibrillar collagens is highly heterogeneous with prominent oriented fibers frequently localized to the invasive front, associated with invading blood vessels, and around the tumor periphery (Provenzano et al. 2006; Levental et al. 2009; Lopez et al. 2011; Acerbi et al. 2015). The clinical relevance of collagen reorganization at the primary tumor was emphasized by a retrospective study in which the presence of perpendicular collagen bundles in invasive human breast tumors was found to associate with poor patient outcome (Egeblad et al. 2010; Conklin and Keely 2012). Intriguingly, recent work revealed that several matricellular proteins, such as FN and TNC, can also accumulate in the premetastatic niche and may serve to attract and subsequently enhance tumor cell growth and survival, particularly because they are deposited as a highly endogenous gradient in the niche (Kaplan et al. 2005; Costa-Silva et al. 2015). Evidence to support this possibility was provided by experimental data showing that TNC is a major gene that is significantly up-regulated in breast tumor cells that preferentially colonize the lung (Minn et al. 2005).

Stiffness Gradients

Tumors are mechanically corrupted tissues whose physical attributes typically include high tumor solid stress that reflects the tumor genotype and is promoted by the expanding tumor cell mass, the elevated interstitial pressure, and the resistance exerted by the surrounding stiffened, fibrotic ECM (Padera et al. 2004; Paszek et al. 2005; Willipinski-Stapelfeldt et al. 2005). ECM stiffening reflects an increase in protein abundance; particularly of fibrillar proteins such as collagens I and III, as well as their reorganization and posttranslation modifications that include lysyl oxidase-mediated cross-linking (Erler and Weaver 2009; Levental et al. 2009; Egeblad et al. 2010; Cox et al. 2016). Although imaging elastography and unconfined compression analysis have consistently revealed an incremental increase in tissue stiffness as a function of tumor stage, grade atomic force microscopy (AFM) indentation analysis has also emphasized the heterogeneity of the ECM stiffness within a tumor (Evans et al. 2012; Plodinec et al. 2012; Yi et al. 2013; Fenner et al. 2014). For instance, AFM indentation revealed that the invasive front of human tumors is by far the stiffest (Acerbi et al. 2015) and determined that the vasculature within the core of the tumor is softer than the vasculature at the periphery (Lopez et al. 2011); possibly reflecting

Figure 2. Endogenous gradients at sites of distant metastases. Local changes in the tumor microenvironment contribute to colonization and the formation of distant metastases. Fibrotic tissues, such as in the lung or liver, show increased stiffness and increased colonization. Secretion of chemokines from local stromal cells present in secondary sites can promote extravasation to tumor cells. Accumulation of ECM proteins in the pre-metastatic niche also leads to the development of endogenous gradients of haptotactic cues. ECM, extracellular matrix; CTCs, circulating tumor cells.

differences in pericyte coverage (Ribeiro and Okamoto 2015). These distinct regions of ECM stiffness result in endogenous stiffness gradients that can foster tumor cell migration through a process termed durotaxis (Lo et al. 2000; Isenberg et al. 2009; Plotnikov et al. 2012). Interestingly, two common organs of secondary metastasis are the liver and the lung (Wynn 2008; Steeg 2016), both of which suffer from age, disease, and obesity-mediated chronic fibrosis (Zeisberg and Kalluri 2013). Chronic liver and lung fibrosis are characterized by tissue stiffening through elevated levels of fibrillar-type collagens, TNC and FN, and more proteoglycan and lysyl oxidase-mediated cross-linking, which would comprise a strong stiffness differential gradient toward which disseminated tumor cells would be attracted (Levental et al. 2009; Chang et al. 2017).

Establishment of Hypoxia

Tumors are hypoxic (Wilson and Hay 2011) as a result of insufficient or dysfunctional vasculature that is often leaky, heterogeneous, and tortuous. This irregular branching and abnormal shunting lead to heterogeneous blood flow (Carmeliet and Jain 2011), which in turn creates endogenous hypoxia gradients within the tumor that can include extremely low pO_2 levels in some areas of the tumor (Li et al. 2007). The tumors adapt to the reduced oxygen by adjusting their intrinsic metabolism, which creates a layered metabolite gradient within the tissue. Thus, vascular heterogeneity creates gradients of extracellular oxygen levels or pO_2 , which alter the intravascu-

lar pO_2 and the metabolic consumption rate of the tumor and stromal cells within the tissue. The combination of these events creates pO_2 and metabolite gradients within the tissue that can have a profound effect on tumor cell behavior.

Inversion of Normal pH Gradient

In differentiated epithelial cells, the intracellular pH (pH_i , 7.2) is lower than the extracellular pH (pH_e , 7.4). However, in a tumor tissue the pH can change dramatically depending on the pO_2 and metabolite availability, resulting in a reversal of the normal pH gradient (Webb et al. 2011), with cancer cells developing a higher pH_i of 7.4 and much lower pH_e of 6.5–7.1 (Stüwe et al. 2007; Gallagher et al. 2008; Hashim et al. 2011). Changes in pH in tumor cells can occur as a consequence of up-regulation of expression and/or activity of ion pumps and transporters, such as H^+ ATPases (Martinez-Zaguilan et al. 1993), Na^+-H^+ exchanger NHE1 of the SLC9A family (McLean et al. 2000), and monocarboxylate- H^+ efflux cotransporters MCT1 and MCT4 or the SLC16A family (Pinheiro et al. 2008; Chiche et al. 2012) that drive H^+ efflux, leading to a high pH_e . Furthermore, high activity of carbonic anhydrases CAIX and CAXII driven by hypoxia can accelerate the hydration of extracellular CO_2 to HCO_3^- and H^+ (Pastorekova et al. 2006; Chiche et al. 2009; Swietach et al. 2009). Finally, reduced blood flow and subsequent oxygen depletion at the early “carcinoma in situ” stage will drive tumor cells to undergo a metabolic switch toward a glycolytic phenotype (Gatenby and Gillies 2004). The increased flux of carbons resulting from glycolysis leads to an acidification of the extracellular pH of the tumors that is particularly pronounced in luminal structures (Barathova et al. 2008). Importantly, similar to pO_2 and metabolite gradients, tumors can also develop pH gradients that can influence their behavior and additionally contribute to tumor heterogeneity.

Alterations in Electrical Fields

Electrical signaling plays a fundamental role in cell and tissue homeostasis. The membrane potential of a cell is maintained by the unequal distribution of ions across the plasma membrane, which creates voltage differences between the cytoplasm and the extracellular environment (Yang and Brackenbury 2013). In epithelial tissues, naturally occurring electrical fields (EFs) are maintained by the spatial organization of ion pumps, with the apical domain of the cell being especially enriched in Na^+ channels and Cl^- transporters, and the basolateral domain containing Na^+-K^+ ATPases (McCaig et al. 2009). The generation of ionic gradients leads to extracellular ionic current flow and the establishment of endogenous voltage gradients. Normal epithelial cells are highly polarized; however, the membrane potential of tumor cells is often depolarized, and this difference results in a drop in transepithelial potential difference (James et al. 1956; Faupel et al. 1997). Depolarized tumor cells display in-

creased intracellular Na^+ levels, stable K^+ levels, and Cl^- and Ca^{2+} influx (Yang and Brackenbury 2013). Tumor depolarization results in an extracellular voltage gradient between normal and tumor cells (Cuzick et al. 1998) that can vary widely within one tumor and differs between tumor types. For example, across the luminal wall of rat prostate glands, a steady direct current electrical field of 500 mV/mm exists, with a transepithelial electrical potential difference of -10 mV, the lumen being negatively charged (Szatkowski et al. 2000; Djamgoz et al. 2001). In contrast, in the mammary duct, the transepithelial electrical potential is $+30$ mV, with duct lumen being positively charged (Pu et al. 2007).

TECHNIQUES TO STUDY DIRECTIONAL MIGRATION

The transwell or Boyden chamber assay (Boyden 1962) is the original assay used to study directional migration, in which cells are plated on a porous filter membrane in between two cell culture chambers. By layering a soluble or ECM cue in only one of the two chambers, it is possible to study directional migration through the filter toward a cue of interest. Although this assay has unequivocally enhanced our understanding of directional migration, the approach is fraught with many limitations that include most importantly an inability to control the local environment and to generate gradient slopes. Furthermore, the technique does not easily lend itself to live imaging. In recent years, the convergence of bioengineering and cancer research has helped facilitate the development of more accurate and reliable methods to

study directional migration, both in vitro and in vivo (see Fig. 3).

Micropipette/Micropatterning Assays for Local Two-Dimensional Gradients In Vitro

The first directional migration assay that was developed to generate local quantifiable gradients involved the use of a micromanipulator-controlled micropipette, loaded with a given concentration of a soluble cue that was slowly released in proximity to a two-dimensional (2D)-plated cell. In this assay, micropipettes are placed within 1–2 cell diameters of each cell, and a pump is used to control the flow of the chemoattractant out of the pipette and, in turn, the gradient (Mouneimne et al. 2004; Soon et al. 2005). This type of assay has been used successfully to examine carcinoma cell responses to soluble GF gradients (Carmona et al. 2016). However, this method is low-throughput, technically tedious, and requires expensive equipment. Moreover, the gradients generated by the micropipette approach are transient, making it difficult to quantify chronic cellular changes, and lend themselves only to the study of the effect of soluble cues on cell behavior. To address this issue, 2D micropatterning has been developed to study durotactic gradients. Although these 2D durotactic gradients have enjoyed varying degrees of success, they are notoriously technically challenging and thus not easily adapted to most cancer cell biology laboratories. To facilitate these studies, new durotactic gradient assays have been developed using mechanically tunable 2D polymer hydrogels conjugated with different ECM ligands (Roca-Cusachs et al. 2013). In these assays, stiffness gradients are generated by vary-

In vitro				In vivo	
Assay	Chemotaxis	Chemotaxis Durotaxis	Chemotaxis Haptotaxis Durotaxis Alignotaxis Electrotaxis	Chemotaxis Haptotaxis	Chemotaxis Haptotaxis
	2D+3D	2D	2D+3D		
Pro	- easy setup - commercially available	- controlled local gradients - live imaging coupled to IF	- stable gradients - easy manipulation - cheap - recreate complex environments	- physiological tumor environment - 6 conditions at once	- physiological tumor environment
Con	- no stable gradient - little environmental control	- expensive setup - low throughput	- no biochemical analysis, requires microscopy	- expensive setup - not commercially available	- requires IVI - expensive - not commercially available - low throughput

Figure 3. In vitro and in vivo methods to study directional migration: commonly used directional migration assays as well as pros and cons associated with each assay. In vitro study of directional migration relies on three main types of assays: the traditional transwell assay, microneedles, or micropatterning and microfluidics. To study in vivo directional migration, the main assays currently used are the needle collection assays and implantable devices. IF, immunofluorescence; IVI, intervertebral implant.

ing the cross-linking density of polyacrylamide hydrogels (Kuo et al. 2012), controlled by the amount of the cross-linker or light, for photoinitiated polymerization of gels (Sunyer et al. 2012). Alternately, a three-dimensional (3D) durotaxis gradient can be generated using a novel 3D collagen/FN/basement membrane hydrogel bioreactor that rapidly induces a 3D stiffness gradient by varying the angle of uniaxial stretch (Cassereau et al. 2015).

New Advances in Microfluidics

Advances in microfluidic technology have paved the way for its application to the study of directional migration. These tailor-made microfluidic devices permit precise spatiotemporal control over the microenvironment the cells are in and the gradients toward which cells will migrate. At present the vast majority of these microfluidic devices use polydimethyl-siloxane (PDMS), which facilitates the generation of microfabricated patterns and structures. Microfluidic devices are easily adopted in a cell biology platform and have the added benefit that they are reasonably cheap to generate. They also use small volumes of reagents, can be easily manipulated, and lend themselves to high-resolution time-lapse imaging (Bersini et al. 2014). To this end, microfluidic devices have been used to study the impact of soluble gradients of growth factors on cancer cell migration with good success (Li and Lin 2011). Some of the newer generation microfluidic devices incorporate pumps that provide a continuous flow of the soluble gradient and permit intermittent fluid analysis (Wu et al. 2012), whereas others generate long-term stable gradients and have revealed that subtle differences in gradients can exert significant biological effects (Suraneni et al. 2012). Stable ECM haptotactic gradients have also been developed using microfluidics and used to study 2D and 3D responses to multiple ECM cues such as FN, laminin, and vitronectin (Chan et al. 2014; Oudin et al. 2016a). Microfluidic channel width can also be used to directly modulate collagen alignment. In these iterations, a flow-based 3D microchannel assay that plated collagen in wide channels (3 mm) produced a random matrix, whereas when the collagen was added to a narrow channel (1 mm), it produced an aligned collagen matrix (Riching et al. 2014). Finally, microfluidics have significantly improved the study of galvanotaxis, which requires stable, integrated, tightly sealed cell culture chambers to maintain the provision of multiple stable electric fields (Huang et al. 2009; Li and Lin 2011).

Visualizing and Measuring Directional Migration In Vivo

Generating any sustained gradient—whether it is a soluble factor, a mechanical constraint, or a biochemical cue—within an endogenous tumor microenvironment *in vivo* is extremely challenging. One assay developed to study tumor cell migration responses to local gradients *in vivo* is the needle collection or *in vivo* invasion assay. In these assays, stainless steel needles containing an attractant are

placed within an endogenous tumor into an anesthetized mouse that has developed a genetically engineered or a xenografted tumor (Hernandez et al. 2009). Intravital imaging studies in mice containing needles with EGF have shown that cells enter the needle by active migration only (Wyckoff et al. 2004). Since its inception this assay has been used to study both chemotactic and haptotactic responses (Oudin et al. 2016a). More recently, a new microscale device was developed which incorporates multiple reservoirs in the delivery system to permit the time-resolved delivery of several compounds. This new device permits the slow diffusion of drugs over time (Jonas et al. 2015). These devices can be placed on the edges of tumors and, coupled with intravital imaging, can permit real-time imaging of the tumor cells response to large sustained local gradients *in vivo* (Oudin et al. 2016a). Other implantable devices have been developed, such as the iNanivid (Williams et al. 2016b), to visualize cell responses *in vivo* directly, and these approaches are likely to become far more powerful in the near future. Overall, *in vivo* directional migration studies are technically difficult, require specific noncommercially available equipment, and are expensive. Nevertheless, they serve to validate the importance of microenvironmental gradients as a key regulator of the tumor phenotype.

MECHANISMS OF DIRECTIONAL MIGRATION

Directional migration favors the invasion of tumor cells into the parenchyma and their metastatic dissemination and colonization. The basic mechanisms driving cell migration, as well as the different modes a cell can use to migrate, have been described in detail elsewhere (Friedl and Wolf 2003; Petrie et al. 2009). Directional migration occurs through a series of defined steps that include cell polarization via asymmetric protrusion formation, focal adhesion formation and adhesion, and cell contraction leading to rear end detachment. Here, we focus specifically on mechanisms that have been shown to be involved in the directed migration of cancer cells and that have been implicated in cancer progression. The pathways described below have been summarized in Figure 4.

Chemotaxis

Numerous soluble cues drive chemotaxis by stimulating actin polymerization through RhoGTPases through the cells' cognate receptor tyrosine kinase (RTK) receptors or G α i protein-coupled receptors (GPCRs) depending on the ligand (for review, see Roussos et al. 2011). In breast cancer cells, multiple GFs such as EGF, heparin-binding (HB)-EGF, TGF- α , insulin-like growth factor (IGF), and hepatocyte growth factor (HGF), but not PDGF and heregulin, induce strong protrusion responses that are predicted to drive 3D invasion *in vitro* (Meyer et al. 2012). Interestingly, the *in vivo* invasion assay revealed that EGF, TGF- α , and CSF-1, but not heregulin, VEGF α , fibroblast growth factor 1 (FGF-1), and PDGF, can induce the directed invasion of PyMT tumor cells in

Figure 4. Cellular mechanisms known to drive directional migration: a summary of some of the main receptor families and signaling pathways that have been involved in the multiple modes of directional migration. These data are explained and cited in the text in more detail. It is important to note that some modes of directional migration, like galvanotaxis and pH-mediated migration, have their own individual cell surface receptors that facilitate responses to these cues. However, some of the intracellular signaling pathways mediating the responses overlap with other modes of migration. Chemotaxis, haptotaxis, and durotaxis have all been shown to require overlapping cell surface receptors. Similarly, many of the intracellular pathways, such as kinases and actin-myosin machinery, are required for response to a variety of different cues. VGSC, voltage-gated sodium channel; EGFR, epidermal growth factor receptor; GPCR, G-protein-coupled receptor; RTK, receptor tyrosine kinase; PLCγ, phospholipase C γ; TGF-β, transforming growth factor β.

mammary tumors formed in polyoma middle T (PyMT)-mouse mammary tumor virus (MMTV) mice and identified a set of core signaling pathways including those that influence Rho/ROCK activity, the capping protein cofilin, and the Arp2/3 pathway (Wang et al. 2004; Wyckoff et al. 2004; Patsialou et al. 2012). These studies also identified the actin regulatory protein Mena, a member of the Ena/VASP family whose alternately spliced isoform Mena^{INV} has been strongly implicated in breast metastasis as well as poor patient prognosis (Goswami et al. 2009; Roussos et al. 2010; Gertler and Condeelis 2011; Oudin et al. 2016a). Importantly, Mena^{INV} deregulates the phosphatase PTP1B to enhance RTK phosphorylation and potentiate actin polymerization via the phospholipase Cγ (PLCγ)–cofilin pathway greatly potentiating the tumor cells' response to chemotactic GFs such as EGF, IGF, and HGF (Philippart et al. 2008; Hughes et al. 2015). Chemotaxis downstream from GPCRs also contributes to tumor cell migration. For instance, GIV/Girdin, a non-receptor guanine nucleotide exchange factor (GEF) that triggers trimeric G-protein activation, binds to RTKs to promote metastasis (Garcia-Marcos et al. 2015), likely by fostering chemotaxis in response to insulin through enhanced Akt signaling. Similarly, chemokines such as CXCL12 (or SDF-1) can also drive chemotaxis via their cognate chemokine receptors.

Haptotaxis

The characteristic increase and heterogeneity in the distribution of the newly deposited and remodeled ECM protein in the tumor is consistent with the notion that haptotaxis contributes to the heterogeneity of the tumor

phenotype. Haptotaxis is defined as the directed migration of cells on gradients of substrate-bound cues, in which cells are migrating on or within an ECM (Wu et al. 2012). In this respect, tumor haptotaxis toward gradients of the glycoprotein FN has been well studied. Studies using models of triple-negative breast cancer revealed that the actin binding protein Mena promotes FN haptotaxis through its ability to directly interact with the FN receptor integrin α5β1 (Oudin et al. 2016a). In this work, levels of the invasive isoform of Mena, Mena^{INV}, whose expression associates with a poor breast cancer patient outcome, drove tumor cell haptotaxis toward high concentrations of FN by increasing focal adhesion kinase (FAK)-dependent outside-in signaling by enhancing inside-out signaling through ECM remodeling. The concept was validated in vivo using an implantable device to create FN gradients and intravital imaging to monitor and quantify effects on tumor cell migration. Importantly, these studies showed not only that FN can function as a bona fide directional cue, but also that a haptotaxis phenotype fosters tumor metastasis. Interestingly, in metastatic melanoma, loss of the tumor suppressor LKB1 and its downstream target, the MARK family kinases, compromises FN haptotaxis (Chan et al. 2014). Moreover, inserting a P29S mutation into the proinvasion, prometastatic Rac1 molecule also abrogates fibroblast haptotaxis in vitro (King et al. 2016). Therefore, these data suggest context-specific effects of FN haptotaxis during tumor progression.

In addition to the haptotactic behavior of FN, Boyden chamber experiments have attested to the haptotactic potential of thrombospondin for human melanoma (Tarabozetti et al. 1987). Given that thrombospondin is a glycoprotein localized at basement membrane and vessel

walls, this raises the possibility that circulating melanoma cells could feasibly encounter gradients of thrombospondin that could facilitate their intravasation into secondary organs to form metastatic lesions. Similarly, vitronectin, an ECM component involved in melanoma metastasis and present in serum, can act as haptotactic cue for A2058 melanoma cells by ligating $\alpha v \beta 3$ integrin to promote the tyrosine phosphorylation of paxilin (Aznavorian et al. 1996). Collagen, one of the most abundant ECM components in solid tumors, can also drive haptotaxis. Indeed, pancreatic tumor cells can drive haptotaxis in response to collagen by binding $\alpha 2 \beta 1$ integrin to enhance FAK activity (Lu et al. 2014). GIV/Girdin, which promotes metastasis, is a nonreceptor GEF that triggers trimeric G-protein activation that binds to RTKs (Garcia-Marcos et al. 2015) and also activates integrins to promote haptotaxis toward collagen (Leyme et al. 2015).

Durotaxis

Cells preferentially migrate toward and remain associated with a stiffer ECM through a process termed durotaxis (Lo et al. 2000). Over the past several years, the original observations defining durotaxis have been elaborated upon using 2D and 3D natural and synthetic biomaterials with ECM stiffness gradients and have illustrated how cells persistently migrate up a stiffness gradient (Isenberg et al. 2009; Plotnikov et al. 2012; Cassereau et al. 2015). The importance of durotaxis to malignancy has also been shown by a series of *in vitro* studies using 3D hydrogels with calibrated stiffness and *in vivo* experiments in transgenic mouse models in which ECM rigidity was reduced using cross-linking inhibitors or enhanced through ECM cross-linking (for review, see Kai et al. 2016). These studies showed that a stiffened ECM promotes breast and squamous cell invasion by promoting integrin focal adhesions to enhance growth factor and GPCR signaling that activates RhoGTPases, Wnt, mitogen-activated protein (MAP), and phosphoinositide 3-kinases (PI3Ks) (Paszek et al. 2005; Levental et al. 2009). The relevance of ECM stiffness to breast tumor metastasis was further demonstrated by transgenic mouse studies in which collagen cross-linking and ECM stiffness were manipulated. These studies identified TGF- β and microRNAs that target tumor suppressors like the PI3K repressor phosphatase and tensin homolog (PTEN) as critical molecular modulators of the durotactic phenotype (Pickup et al. 2013; Mouw et al. 2014). Recently, a role for ECM rigidity in promoting pancreatic tumor progression and glioblastoma aggression that expand upon *in vitro* studies was demonstrated (Ulrich et al. 2009; Laklai et al. 2016; Miroshnikova et al. 2016). Building upon these observations data generated using MDA-MB-231 mesenchymal breast cancer cells defined an integrin-associated myosin-dependent molecular mechanism that regulates cellular rigidity sensing downstream from RTK signaling that is corrupted in tumors through AXL and ROR2 kinase perturbations (Lo et al. 2000; Hoffman et al. 2011; Plotnikov et al. 2012; Raab et al. 2012; Yang et al. 2016).

Alignotaxis

The reorganization of collagen from curly, coiled fibers to strait aligned fibers promotes the directed, rapid, and persistent migration of tumor cells, which we describe as alignotaxis. Alignotaxis was illustrated by studies conducted using the metastatic MDA-MB-231 breast cancer cells (Provenzano et al. 2008). Furthermore, aligned collagen fibers that are assembled through coordinated tumor colony interactions foster the rapid and directed migration of tumor cells (Shi et al. 2013). Consistently, intravital imaging studies in breast tumor models revealed that tumor cells located in close proximity to collagen fibers migrate faster than those located within the core of the tumor mass (Wang et al. 2002). Using fluorescently tagged myosin light chain (MLC), one study showed that MLC is recruited to the protrusions of migrating cells, with second-harmonic generation (SHG) imaging showing concomitant deformation in collagen fibers at the site of the protrusion (Wyckoff et al. 2006). Computational modeling followed by experimental validation showed that migratory persistence is enhanced by collagen fiber alignment because it limits the number of protrusions and restricts their formation to the direction of the alignment (Riching et al. 2014). Additional studies revealed that collagen fiber alignment can predict cell motility, relative to the pore size and density (Fraleley et al. 2015), and that an increase in protrusion frequency, persistence, and lengthening along the direction of the fibers depends on FAK and Rac1 signaling (Carey et al. 2016). Indeed, preventing collagen alignment by inhibiting the activity of the collagen cross-linker LOXL2 (Grossman et al. 2016), or through knockdown of the proteoglycan syndecan1 (Yang et al. 2011), but not by inhibiting protease activity (matrix metalloproteinases [MMPs] and serine and cysteine proteases), reduced the directional migration of tumor cells *in vitro* and *in vivo* (Wyckoff et al. 2006; Levental et al. 2009; Rubashkin et al. 2014). Thus compelling *in vitro* and *in vivo* data attest to the importance of alignotaxis as a regulator of the local invasion of tumor cells.

Galvanotaxis

Galvanotaxis (or electrotaxis) describes the directional movement driven by different EFs. Multiple studies have illustrated a correlation between the metastatic potential of a tumor cell and its sensitivity to galvanotaxis (Djamgoz et al. 2001), including the galvanotaxis-mediated directional migration of prostate cancer cells (Djamgoz et al. 2001), breast cancer (Fraser et al. 2005), lung adenocarcinoma (Sun et al. 2012), and glioblastoma cells (Huang et al. 2016). Interestingly, although prostate, lung, and glioblastoma multiforme (GBM) tumor cells show cathodal galvanotaxis, the breast tumor cells migrate toward the anode, suggesting that different tumor microenvironments might contribute to EF-driven invasion. Although the primary stimulus differs, galvanotaxis likely exploits many of the same cytoskeletal pathways that mediate chemotaxis, haptotaxis, and durotaxis

(Mycielska and Djamgoz 2004). For instance, EFs increase intracellular Ca^{2+} to induce actin polymerization and actomyosin contractility, which are key for the formation of localized cellular protrusions that permit directional migration (Mycielska and Djamgoz 2004). More recently, voltage-gated Na^{+} channels (VGSCs) were shown to control the galvanotaxis behavior of tumor cells, and their levels correlated with the tumor cells metastatic potential (Djamgoz et al. 2001; Fraser et al. 2005). More recently, A549 lung carcinoma galvanotaxing toward a cathode polarized their EGFRs and assembled filamentous actin cables in the direction of the cathode. These galvanotaxing cells also showed oriented ERK and Akt signaling that lined up with the EF and apparently was critical for directional migration (Yan et al. 2009). Consistently, one study showed that the galvanotaxis of brain tumor initiating cells from three distinct GBM subtypes also depended upon the oriented activity of PI3K and ERK signaling (Huang et al. 2016). Although these findings suggest galvanotaxis can promote the directed migration of tumor cells, data clearly suggest that the nature of the behavior including the anodal versus the cathodal effect is context-dependent and likely cell type-dependent.

pH

The tumor-associated reversal of the normal pH gradient can profoundly influence intracellular signaling and modify the extracellular microenvironment to influence cell migration. Thus, the abnormally elevated intracellular pH_i in the tumor cell can activate several intracellular signaling pathways linked to integrins, ion channel activity, and actin polymerization that will promote cell proliferation and migration. For instance, several guanine nucleotide exchange factors, such as DBS, that stimulate CDC42 become activated when the pH rises (Grillo-Hill et al. 2015). Other molecules that bind to actin and promote polymerization, such as cofilin (Frantz et al. 2008), profilin (McLachlan et al. 2007), and talin (Srivastava et al. 2008), also have increased activity at higher pH_i. The extrusion of H^{+} from tumor cells is accompanied by an influx of water and subsequent osmotic swelling and opening of aquaporin channels (Saadoun et al. 2005), effects which contribute to driving cell migration. Consistently, the low pH_e induced in tumor cells can significantly alter the ECM to create a prometastatic environment (Webb et al. 2011). Indeed, the lower pH_e in the tumor microenvironment can activate multiple proteases such as MMP3 and MMP9 (Johnson et al. 2000), which result in ECM remodeling to foster the creation of migratory tracks and the release of ECM-bound soluble factors that stimulate cell motility.

STRATEGIES TO TARGET DIRECTIONAL MIGRATION AND PREVENT METASTASIS

Exploiting Directional Migration to Predict Metastasis

The ability to predict which patients' tumor has a high probability of metastasis would provide critical clinical

insight to guide treatment and would likely influence the patient outcome. In this regard, a number of commercially available tests have recently been developed to estimate metastatic risk in cancer patients. One such test is the MammaPrint DX, which is a 70-gene signature that can identify breast cancer patients who are most likely to develop metastasis within 5 years of diagnosis with reasonable accuracy (van't Veer et al. 2002; van de Vijver et al. 2002). Similarly, the 15-gene signature DecisionDx-UM test is able to accurately predict which patients with uveal melanoma are most likely to metastasize and has been tested in large multicenter trials with excellent results (Harbour 2014). Exploiting the concept of tumor gradients as key regulators of tumor migration and metastasis is the derivation of a new metastasis biomarker that quantifies the number of tumor microenvironment of metastasis (TMEM) sites within a tumor. TMEMs are a tripartite structure composed of a macrophage, a Mena-expressing tumor cell, and an endothelial cell that result from and reflect chemotactic gradients within the tumor (Robinson et al. 2009). Recent clinical work suggests that as the number of TMEMs within an $\text{ER}^{+}/\text{PR}^{+}$ breast tumor increases, metastasis increases; hence, patient prognosis decreases. The success of these new approaches to predict patient prognosis emphasizes the potential utility of developing directional migration biomarkers to predict metastatic disease (Rohan et al. 2014).

Blocking Cell Surface Receptors and Signaling to Impede Directional Migration

Therapies targeting the main chemotactic signaling pathways such as GFs/RTK and chemokine/GPCR have been approved for patient administration, with many more in clinical trials. Although most of these are aimed at inhibiting tumor growth and angiogenesis, they have the potential to impact metastasis-relevant processes. Yet, despite the fact that a role of EGF chemotaxis has been well established in breast cancer metastasis in mouse models, EGFR inhibitors have had mixed clinical efficacy (Crown et al. 2012). Small-molecule tyrosine kinase inhibitors (TKIs) such as gefitinib or erlotinib have failed to show any significant improvement in the triple-negative breast cancer (TNBC) patient outcome over standard-of-care chemotherapy (Baselga et al. 2005). The monoclonal EGFR antibody cetuximab showed only a modest increase in the overall response rate and progression-free survival relative to cisplatin (Baselga et al. 2013). Inhibitors targeting other surface RTKs that can modulate directional migration are currently in clinical trials for breast cancer, such as Met, IGF1R, FGFR, and ERBB3, and await verification of their utility (Jin and Mu 2015). Many molecular mechanisms are proposed to account for the failure of these promising RTK targets, including the expansion of genetically mutated tumor cells and reliance on parallel survival pathways.

Integrins are the main cell surface receptors that allow cells to sense, respond to, and reorganize their tumor mi-

croenvironment and cells use integrins for haptotaxis, chemotaxis, and durotaxis. However, although in vitro and preclinical studies have shown promising results (Schaffner et al. 2013), many of the integrin targeting clinical trials have ultimately failed (Desgrosellier and Cheresch 2010). The high-affinity anti- $\alpha 5 \beta 1$ antibody volociximab stabilized disease in metastatic renal cell carcinoma (Conti et al. 2013); however, another monoclonal $\alpha 5 \beta 1$ integrin antibody PF-04605412 had no effect on a variety of solid tumors and has consequently been discontinued (Mateo et al. 2014). A phase 3 trial in KRas wild-type metastatic colorectal cancer found no improvement with abituzumab, an anti- αv integrin antibody, relative to standard of care (Élez et al. 2015). Similarly, the integrin antagonist cilengitide did not improve the outcome in glioblastoma patients compared with radiotherapy, and the development of this drug was also discontinued (Stupp et al. 2014). Again the reason for these clinical failures remains unclear and are likely complex, including cross talk with diverse RTK signaling pathways.

An alternative to directly targeting cell surface receptors is identifying and targeting the signaling pathways that become activated downstream from cell surface receptors that mediate responses to multiple directional cues. Examples of such downstream targets toward which potent clinical inhibitors could be directed include FAK and Src kinase. Several clinical trials are ongoing that treat tumors by targeting FAK, although conclusive results are yet to be obtained. Interestingly, multiple preclinical studies have shown that inhibition of FAK may synergize well with therapies targeting other pathways or components of the microenvironment. Indeed, inhibition of FAK with VS-4718 delayed tumor progression in the

p48-Cre;LSL-Kras^{G12D};Trp53^{fllox/+} KPC model for human pancreatic ductal adenocarcinoma (PDAC) and also rendered these tumors more sensitive to T-cell immunotherapy and PD-1 antagonists' PDAC (Jiang et al. 2016). In metastatic melanoma, treatment with a FAK inhibitor prevented resistance to Braf inhibitors caused by fibroblast-mediated ECM deposition, which activated integrin signaling in tumor cells (Hirata et al. 2015).

Galvanotaxis, regulated mainly by VGSCs, can drive directional migration that contributes to metastasis (Fraser et al. 2005), suggesting that blocking VGSC activity can be a strategy to block metastasis. Indeed, inhibition of VGSCs by tetrodotoxin has been used in a prostate cancer in vivo model to reduce metastatic burden (Yildirim et al. 2012). Targeting pathways that allow cells to respond to EFs may be useful in targeting directional migration. Elucidating the mechanisms driving directional migration in response to multiple cues has increased our understanding of the pathways to target to inhibit metastasis. However, it is important to note how challenging it is to develop antimetastatic therapies from a clinical perspective (for a review, see Steeg 2016).

Disrupting the Gradient

An alternate approach to target and prevent directional migration in tumors is by directly disrupting the endogenous gradients that drive tumor cell invasion (Fig. 5). Toward this objective, several stromal cells have been identified that can contribute to the generation of local growth factor or chemokine gradients that drive directional migration in tumors. The same stromal compart-

Figure 5. Strategies to target directional migration. We have identified four strategies to use directional migration to target tumor growth, local invasion, dissemination, and colonization (outlined in the four boxes in this figure). First, studying the mechanisms driving directional migration can lead to the development prognostic markers that can be used to predict likelihood of disease progression. Second, identifying the core signaling pathways that drive the cell responses to endogenous gradients will allow for use of already available drugs or the development of drugs against new targets. Third, the physical and biochemical properties of gradients can be leveraged to facilitate local drug delivery and drug release. Fourth, strategies to directly disrupt the gradient can be developed to remove the local cues that promote aggressive tumor phenotypes in the first place. TMEM, tumor microenvironment of metastasis; GF, growth factor; ECM, extracellular matrix.

ment also plays an important role in the deposition of ECM, as well as its organization, and thereby regulates haptotaxis, alignotaxis, and durotaxis in tumors (Conklin and Keely 2012; Naba et al. 2012). Macrophages in particular, which secrete promigratory growth factors, were identified as viable therapeutic targets in cancer. However, despite these provocative findings, depletion of macrophages, through genetic or pharmacological perturbations, does reduce tumor cell motility and intravasation in breast cancer tumors (Wyckoff et al. 2004; Harney et al. 2015). Nevertheless, several pharmacological agents are currently in clinical trials that should block macrophage precursor recruitment, deplete tumor-associated macrophages, or reprogram their function that might prove more efficacious (Williams et al. 2016a). Inhibition of the CSF-1 receptor on macrophages in particular offers one promising strategy to reduce directed cell migration and tumor dissemination and is currently in clinical trials in advanced refractory breast or prostate cancer.

Oriented fibrillary collagen can promote directional migration in tumors through alignotaxis and durotaxis, suggesting strategies to prevent collagen alignment and stiffening, and may provide a viable therapeutic strategy to prevent tumor progression and metastasis. Consistent with this concept, several preclinical studies have attested to the utility of targeting collagen-processing enzymes to inhibit malignancy *in vivo*. For instance, inhibition of LOX through pharmacological inhibition or injection with lysyl oxidase (LOX)-neutralizing antibodies reduced tumor metastasis from solid tumors (Levental et al. 2009; Cox et al. 2013; Pickup et al. 2013). Similarly, studies using function-blocking antibodies to inhibit lysyl oxidase-like 2 (LOXL2) reduced collagen alignment and decreased tumor burden in a breast tumor xenograft (Grossman et al. 2016); these antibodies have been associated with invasiveness and poor outcome in breast cancer (Ahn et al. 2013). Higher LOXL2 levels are also associated with tumor invasion and poor outcome in pancreatic cancer patients and showed some efficacy in mouse models of PDAC (Park et al. 2016). However, when a LOXL2 inhibitor simtuzamab (Gilead) was tested in combination with gemcitabine in a cohort of previously untreated advanced pancreatic cancer patients, there was no observed significant clinical benefit over placebo. Alternately, FN is required for LOX-dependent collagen cross-linking, and treatment of mice with liver fibrosis with a peptide that blocks FN fibril formation (pUR4) significantly decreased their fibrosis, suggesting this type of treatment may be useful to reduce ECM accumulation in solid tumors (Altrock et al. 2015). Similarly, mice treated with the antifibrotic agent pirfenidone showed a significant reduction in lung metastasis (Takai et al. 2016).

Intriguingly, galvanotaxis has been extensively characterized in the context of wound healing (Zhao et al. 2006), and studies have shown that the movement of epithelial sheets can be easily and tightly controlled through manipulation of electric currents (Cohen et al. 2014). Such findings suggest that application of low-magnitude EFs could locally manipulate cell migration within tis-

sues, particularly in the context of nonhealing and chronic wounds, and could be developed as a noninvasive approach to control the local invasive behavior of primary tumors (Wu and Lin 2014).

Taking Advantage of the Gradient

Chemotherapy remains the standard of care for most cancers despite its nonspecificity and high toxicity. One approach is to concentrate the chemical and target its delivery through microfabricated nanoparticles generated with diverse polymeric carriers that can be functionalized to specifically target the tumor itself by exploiting unique properties of the tumor microenvironment (Kwon et al. 2015). In this case, the inverted pH gradient present in a variety of solid tumors that acidifies the extracellular pH has been exploited by developing pH-sensitive carrier systems that permit optimal cargo release only in the presence of the low pH within the tumor (Tian and Bae 2012). For example, a layer-by-layer pH-responsive sheddable nanoparticle shell exists in which the neuronal layers that encapsulate the drug are only shed in response to the low pH in the tumor, exposing the nanoparticle surface loaded with a chemotherapeutic agent that can be taken up by the cells (Poon et al. 2011). Similarly, the hypoxic regions of the tumor have been leveraged to optimize therapeutic delivery to tumor regions using multiple mechanisms (Patel and Sant 2016). One such example is provided by a bioactive prodrug, such as the N-oxide drug tirapazamine (TPZ), which induces DNA damage, works synergistically with chemotherapy and radiation therapy, and is only activated under hypoxia (von Pawel et al. 2000). These data suggest that the exploitation of endogenous gradients (hypoxia, pH) within a tumor could be used to enhance drug-targeting strategies that would potentiate tumor treatment and reduce many undesirable side effects.

CONCLUSION AND OUTSTANDING QUESTIONS

How Do Different Gradients Affect Each Other?

Out of necessity, most cancer studies focus on the tumor cells responses to a single cue. Yet, *in vivo*, tumor cells simultaneously face a variety of endogenous gradients, many of which interact positively and negatively with each other (Fig. 6). This raises the important point that to understand tumor evolution it will be critical to design studies that can interrogate these complex physical and chemical interactions. For example, changes in ECM deposition and organization simultaneously promote haptotaxis, alignotaxis, and durotaxis. Furthermore, a recent study showed that GBM galvanotaxis proceeds toward the anode on a poly-L-ornithine/laminin 2D surface but switches to a cathode bias when the tumor cells are assayed with a 3D hyaluronic acid and collagen gel (Huang et al. 2016). This underscores the importance of using *in vitro* assays that can accurately model multiple aspects of the tumor microenvironment to gain a clear and accurate

Figure 6. Potential for cross talk in response to the abundance of cues provided by the tumor microenvironment. The changes in local microenvironments that occur as a consequence of tumor formation lead to the generation of multiple endogenous gradients simultaneously, which each can promote their own mode of directional migration. However, it is not clear that changes in one cue can affect another and that common signaling pathways might regulate responses to multiple cues. The *inset* highlights the potential for cross talk in the context of directional migration in response to extracellular matrix (ECM) cues: given that both ECM amount and structure can affect stiffness, haptotaxis, durotaxis, and alignotaxis and could each occur simultaneously, and it is likely that pathways driving one response may be involved in the other. The ECM is well known to act as a reservoir for growth factor (GF), and several signaling pathways have been shown to be involved in response to both chemotactic and haptotactic cues. Hypoxia has been shown to drive changes in ECM via up-regulation of Hif1 α , implying that endogenous hypoxia gradients could lead to the generation of haptotactic gradients. Hypoxia leads to the development of an acidic environment, which in turn leads to matrix metalloproteinase (MMP) activation, which is known to degrade and reorganize ECM. Therefore, the establishment of pH gradients could affect response to ECM gradients. Finally, changes in cellular electrical potential, in part mediated by voltage-gated sodium channels (VGSCs), can affect cell adhesion, as well as drive H⁺ efflux, suggesting that galvanotaxis may affect responses to ECM gradients and the maintenance of pH gradient.

understanding of the role of directional migration of tumors *in vivo*.

How Does One Cell Integrate Multiple Directional Cues?

The same cell surface receptors and signaling pathways have been shown to regulate directional responses to multiple cues, suggesting that there may be core signaling pathways that coordinate directional responses in several contexts. For example, the invasive isoform of the actin regulator Mena^{INV} drives haptotaxis to high FN gradients (Oudin et al. 2016a) while also sensitizing cells to low EGF concentrations (Hughes et al. 2015). Interestingly, when breast tumor cells are incubated with both cues, expression of Mena^{INV} renders them hyperinvasive, driving synergy between the signaling pathways downstream from both integrins and RTKs, leading to an increased migratory response that is stronger than each cue alone

(Oudin et al. 2016b). Altogether, these studies highlight the importance of studying how different cues cooperate mechanistically to promote metastasis.

Attraction versus Repulsion?

In this review, we focused our discussion on how attractive cues promote local invasion. However, repulsive cues can also contribute to directional cell migration as has been consistently shown for neural guidance in the developing brain. For instance, the soluble ligand Slit and its transmembrane receptor Roundabout (Robo) are well-established regulators of axon growth (Tessier-Lavigne and Goodman 1996); recent work suggests that this pathway may also play a role in cancer (Mehlen et al. 2011). This raises the intriguing possibility that repulsion may also play a role in modulating tumor cell guidance and metastasis.

How Do Current Therapies Affect Directional Migration?

Tumor cells that are particularly sensitive to physical and chemical directional cues are often the same cells that are intrinsically highly metastatic. This observation merits investigation because such heightened sensitivity could influence their treatment response and promote their dissemination. In this regard, Mena^{INV}, which drives chemotaxis and haptotaxis (Hughes et al. 2015; Oudin et al. 2016a), also promotes resistance to paclitaxel, a chemotherapeutic drug that is standard of care for the treatment of metastatic breast cancer (Oudin et al. 2017).

ACKNOWLEDGMENTS

This work was supported by K99-CA207866-01 to M.J.O. from the National Cancer Institute (NCI), the National Institutes of Health (NIH) NCI R01 grants CA138818-01A1, CA192914-01, CA174929-01, and CA085482 and 1U01CA202241-01 to V.M.W., and a Department of Defense (DOD) Breast Cancer Research Program (BCRP) grant BC122990 to V.M.W.

We apologize to the authors whose work we were unable to cite because of space limitations.

REFERENCES

- Acerbi I, Cassereau L, Dean I, Shi Q, Au A, Park C, Chen YY, Liphardt J, Hwang ES, Weaver VM. 2015. Human breast cancer invasion and aggression correlates with ECM stiffening and immune cell infiltration. *Integr Biol (Camb)* **7**: 1120–1134.
- Ahn SG, Dong SM, Oshima A, Kim WH, Lee HM, Lee SA, Kwon S-H, Lee J-H, Lee JM, Jeong J, et al. 2013. LOXL2 expression is associated with invasiveness and negatively influences survival in breast cancer patients. *Breast Cancer Res Treat* **141**: 89–99.
- Alizadeh AA, Aranda V, Bardelli A, Blanpain C, Bock C, Borowski C, Caldas C, Califano A, Doherty M, Elsner M, et al. 2015. Toward understanding and exploiting tumor heterogeneity. *Nat Med* **21**: 846–853.
- Altrock E, Sens C, Wuerfel C, Vasel M, Kawelke N, Dooley S, Sottile J, Nakchbandi IA. 2015. Inhibition of fibronectin deposition improves experimental liver fibrosis. *J Hepatol* **62**: 625–633.
- Astrof S, Hynes RO. 2009. Fibronectins in vascular morphogenesis. *Angiogenesis* **12**: 165–175.
- Aznavorian S, Stracke ML, Parsons J, McClanahan J, Liotta LA. 1996. Integrin $\alpha v \beta 3$ mediates chemotactic and haptotactic motility in human melanoma cells through different signaling pathways. *J Biol Chem* **271**: 3247–3254.
- Barathova M, Takacova M, Holotnakova T, Gibadulinova A, Ohradanova A, Zatovicova M, Hulikova A, Kopacek J, Parkkila S, Supuran CT, et al. 2008. Alternative splicing variant of the hypoxia marker carbonic anhydrase IX expressed independently of hypoxia and tumour phenotype. *Br J Cancer* **98**: 129–136.
- Baselga J, Albanell J, Ruiz A, Lluch A, Gascón P, Guillém V, González S, Sauleda S, Marimón I, Tabernero JM, et al. 2005. Phase II and tumor pharmacodynamic study of gefitinib in patients with advanced breast cancer. *J Clin Oncol* **23**: 5323–5333.
- Baselga J, Gómez P, Greil R, Braga S, Climent MA, Wardley AM, Kaufman B, Stemmer SM, Pêgo A, Chan A, et al. 2013. Randomized phase II study of the anti-epidermal growth factor receptor monoclonal antibody cetuximab with cisplatin versus cisplatin alone in patients with metastatic triple-negative breast cancer. *J Clin Oncol* **31**: 2586–2592.
- Ben-Baruch A. 2008. Organ selectivity in metastasis: Regulation by chemokines and their receptors. *Clin Exp Metastasis* **25**: 345–356.
- Bersini S, Jeon JS, Moretti M, Kamm RD. 2014. In vitro models of the metastatic cascade: From local invasion to extravasation. *Drug Discov Today* **19**: 735–742.
- Boyden S. 1962. The chemotactic effect of mixtures of antibody and antigen on polymorphonuclear leucocytes. *J Exp Med* **115**: 453–466.
- Carey SP, Goldblatt ZE, Martin KE, Romero B, Williams RM, Reinhart-King CA. 2016. Local extracellular matrix alignment directs cellular protrusion dynamics and migration through Rac1 and FAK. *Integr Biol (Camb)* **8**: 821–835.
- Carmeliet P, Jain RK. 2011. Principles and mechanisms of vessel normalization for cancer and other angiogenic diseases. *Nat Rev Drug Discov* **10**: 417–427.
- Carmona G, Perera U, Gillett C, Naba A, Law A-L, Sharma VP, Wang J, Wyckoff J, Balsamo M, Mosis F, et al. 2016. Lamellipodin promotes invasive 3D cancer cell migration via regulated interactions with Ena/VASP and SCAR/WAVE. *Oncogene* **35**: 5155–5169.
- Cassereau L, Miroshnikova YA, Ou G, Lakins J, Weaver VM. 2015. A 3D tension bioreactor platform to study the interplay between ECM stiffness and tumor phenotype. *J Biotechnol* **193**: 66–69.
- Chan KT, Asokan SB, King SJ, Bo T, Dubose ES, Liu W, Berginski ME, Simon JM, Davis IJ, Gomez SM, et al. 2014. LKB1 loss in melanoma disrupts directional migration toward extracellular matrix cues. *J Cell Biol* **207**: 299–315.
- Chang TT, Thakar D, Weaver VM. 2017. Force-dependent breaching of the basement membrane. *Matrix Biol* **57–58**: 178–189.
- Chiche J, Ilc K, Laferriere J, Trottier E, Dayan F, Mazure NM, Brahimi-Horn MC, Pouyssegur J. 2009. Hypoxia-inducible carbonic anhydrase IX and XII promote tumor cell growth by counteracting acidosis through the regulation of the intracellular pH. *Cancer Res* **69**: 358–368.
- Chiche J, Le Fur Y, Vilmen C, Frassinetti F, Daniel L, Halestrap AP, Cozzzone PJ, Pouyssegur J, Lutz NW. 2012. In vivo pH in metabolic-defective Ras-transformed fibroblast tumors: Key role of the monocarboxylate transporter, MCT4, for inducing an alkaline intracellular pH. *Int J Cancer* **130**: 1511–1520.
- Cohen DJ, James Nelson W, Maharbiz MM. 2014. Galvanotactic control of collective cell migration in epithelial monolayers. *Nat Mater* **13**: 409–417.
- Conklin MW, Keely PJ. 2012. Why the stroma matters in breast cancer: Insights into breast cancer patient outcomes through the examination of stromal biomarkers. *Cell Adh Migr* **6**: 249–260.
- Conti A, Santoni M, Amantini C, Burattini L, Berardi R, Santoni G, Cascinu S, Muzzonigro G. 2013. Progress of molecular targeted therapies for advanced renal cell carcinoma. *Biomed Res Int* **2013**: 419176.
- Costa-Silva B, Aiello NM, Ocean AJ, Singh S, Zhang H, Thakur BK, Becker A, Hoshino A, Mark MT, Molina H, et al. 2015. Pancreatic cancer exosomes initiate pre-metastatic niche formation in the liver. *Nat Cell Biol* **17**: 816–826.
- Cox TR, Bird D, Baker AM, Barker HE, Ho MWY, Lang G, Erler JT. 2013. LOX-mediated collagen crosslinking is responsible for fibrosis-enhanced metastasis. *Cancer Res* **73**: 1721–1732.
- Cox TR, Gartland A, Erler JT. 2016. Lysyl oxidase, a targetable secreted molecule involved in cancer metastasis. *Cancer Res* **76**: 188–192.
- Crown J, O'Shaughnessy J, Gullo G. 2012. Emerging targeted therapies in triple-negative breast cancer. *Ann Oncol* **23**(Suppl 6): vi56–vi65.
- Cuzick J, Holland R, Barth V, Davies R, Faupel M, Fentiman I, Frischbier HJ, LaMarque JL, Merson M, Sacchini V, et al. 1998. Electropotential measurements as a new diagnostic modality for breast cancer. *Lancet* **352**: 359–363.

- Desgrosellier JS, Cheresh DA. 2010. Integrins in cancer: Biological implications and therapeutic opportunities. *Nat Rev Cancer* **10**: 9–22.
- Djamgoz MBA, Mycielska M, Madeja Z, Fraser SP, Korohoda W. 2001. Directional movement of rat prostate cancer cells in direct-current electric field: Involvement of voltage gated Na⁺ channel activity. *J Cell Sci* **114**: 2697–2705.
- Egeblad M, Rasch MG, Weaver VM. 2010. Dynamic interplay between the collagen scaffold and tumor evolution. *Curr Opin Cell Biol* **22**: 697–706.
- Élez E, Kocáková I, Höhler T, Martens UM, Bokemeyer C, Van Cutsem E, Melichar B, Smakal M, Csősz T, Topuzov E, et al. 2015. Abituzumab combined with cetuximab plus irinotecan versus cetuximab plus irinotecan alone for patients with KRAS wild-type metastatic colorectal cancer: The randomised phase I/II POSEIDON trial. *Ann Oncol* **26**: 132–140.
- Erler JT, Weaver VM. 2009. Three-dimensional context regulation of metastasis. *Clin Exp Metastasis* **26**: 35–49.
- Evans A, Whelehan P, Thomson K, McLean D, Brauer K, Purdie C, Baker L, Jordan L, Rauchhaus P, Thompson A. 2012. Invasive breast cancer: Relationship between shear-wave elastographic findings and histologic prognostic factors. *Radiology* **263**: 673–677.
- Faupel M, Vanel D, Barth V, Davies R, Fentiman IS, Holland R, Lamarque JL, Sacchini V, Schreer I. 1997. Electropotential evaluation as a new technique for diagnosing breast lesions. *Eur J Radiol* **24**: 33–38.
- Fenner J, Stacer AC, Winterroth F, Johnson TD, Luker KE, Luker GD. 2014. Macroscopic stiffness of breast tumors predicts metastasis. *Sci Rep* **4**: 5512.
- Fraley SI, Wu P-H, He L, Feng Y, Krisnamurthy R, Longmore GD, Wirtz D. 2015. Three-dimensional matrix fiber alignment modulates cell migration and MT1-MMP utility by spatially and temporally directing protrusions. *Sci Rep* **5**: 14580.
- Frantz C, Barreiro G, Dominguez L, Chen X, Eddy R, Condeelis J, Kelly MJS, Jacobson MP, Barber DL. 2008. Cofilin is a pH sensor for actin free barbed end formation: Role of phosphoinositide binding. *J Cell Biol* **183**: 865–879.
- Fraser SP, Diss JKI, Chioni A-M, Mycielska ME, Pan H, Yamaci RF, Pani F, Siwy Z, Krasowska M, Grzywna Z, et al. 2005. Voltage-gated sodium channel expression and potentiation of human breast cancer metastasis. *Clin Cancer Res* **11**: 5381–5389.
- Friedl P, Wolf K. 2003. Tumour-cell invasion and migration: Diversity and escape mechanisms. *Nat Rev Cancer* **3**: 362–374.
- Gallagher FA, Kettunen MI, Day SE, Hu D-E, Ardenkjaer-Larsen JH, Zandt R, Jensen PR, Karlsson M, Golman K, Lerche MH, et al. 2008. Magnetic resonance imaging of pH in vivo using hyperpolarized ¹³C-labelled bicarbonate. *Nature* **453**: 940–943.
- Garcia-Marcos M, Ghosh P, Farquhar MG. 2015. GIV/Girdin transmits signals from multiple receptors by triggering trimeric G protein activation. *J Biol Chem* **290**: 6697–6704.
- Gatenby RA, Gillies RJ. 2004. Why do cancers have high aerobic glycolysis? *Nat Rev Cancer* **4**: 891–899.
- Gertler F, Condeelis J. 2011. Metastasis: Tumor cells becoming MENacing. *Trends Cell Biol* **21**: 81–90.
- Goddard ET, Hill RC, Barrett A, Betts C, Guo Q, Maller O, Borges VF, Hansen KC, Schedin P. 2016. Quantitative extracellular matrix proteomics to study mammary and liver tissue microenvironments. *Int J Biochem Cell Biol* **81**: 223–232.
- Goswami S, Philippart U, Sun D, Patsialou A, Avraham J, Wang W, Di Modugno F, Nistico P, Gertler FB, Condeelis JS. 2009. Identification of invasion specific splice variants of the cytoskeletal protein Mena present in mammary tumor cells during invasion in vivo. *Clin Exp Metastasis* **26**: 153–159.
- Grillo-Hill BK, Choi C, Jimenez-Vidal M, Barber DL. 2015. Increased H⁺ efflux is sufficient to induce dysplasia and necessary for viability with oncogene expression. *Elife* **4**: e03270.
- Grossman M, Ben-Chetrit N, Zhuravlev A, Afik R, Bassat E, Solomonov I, Yarden Y, Sagi I. 2016. Tumor cell invasion can be blocked by modulators of collagen fibril alignment that control assembly of the extracellular matrix. *Cancer Res* **76**: 4249–4258.
- Harbour JW. 2014. A prognostic test to predict the risk of metastasis in uveal melanoma based on a 15-gene expression profile. *Methods Mol Biol* **1102**: 427–440.
- Harney AS, Arwert EN, Entenberg D, Wang Y, Guo P, Qian B-Z, Oktay MH, Pollard JW, Jones JG, Condeelis JS. 2015. Real-time imaging reveals local, transient vascular permeability, and tumor cell intravasation stimulated by TIE2hi macrophage-derived VEGFA. *Cancer Discov* **5**: 932–943.
- Hashim AI, Zhang X, Wojtkowiak JW, Martinez GV, Gillies RJ. 2011. Imaging pH and metastasis. *NMR Biomed* **24**: 582–591.
- Hernandez L, Smirnova T, Wyckoff J, Condeelis J, Segall JE. 2009. In vivo assay for tumor cell invasion. *Methods Mol Biol* **571**: 227–238.
- Hirata E, Girotti MR, Viros A, Hooper S, Spencer-Dene B, Matsuda M, Larkin J, Marais R, Sahai E. 2015. Intravital imaging reveals how BRAF inhibition generates drug-tolerant microenvironments with high integrin β 1/FAK signaling. *Cancer Cell* **27**: 574–588.
- Hoffman BD, Grashoff C, Schwartz MA. 2011. Dynamic molecular processes mediate cellular mechanotransduction. *Nature* **475**: 316–323.
- Huang C-W, Cheng J-Y, Yen M-H, Young T-H. 2009. Electrotaxis of lung cancer cells in a multiple-electric-field chip. *Biosens Bioelectron* **24**: 3510–3516.
- Huang Y-J, Hoffmann G, Wheeler B, Schiapparelli P, Quinones-Hinojosa A, Searson P, et al. 2016. Cellular microenvironment modulates the galvanotaxis of brain tumor initiating cells. *Sci Rep* **6**: 21583.
- Hughes SK, Oudin MJ, Tadtros J, Neil J, Del Rosario A, Joughin BA, Ritsma L, Wyckoff J, Vasile E, Eddy R, et al. 2015. PTP1B-dependent regulation of receptor tyrosine kinase signaling by the actin-binding protein Mena. *Mol Biol Cell* **26**: 3867–3878.
- Isenberg BC, DiMilla PA, Walker M, Kim S, Wong JY. 2009. Vascular smooth muscle cell durotaxis depends on substrate stiffness gradient strength. *Biophys J* **97**: 1313–1322.
- James AM, Ambrose EJ, Lowick JH. 1956. Differences between the electrical charge carried by normal and homologous tumour cells. *Nature* **177**: 576–577.
- Jiang H, Hegde S, Knolhoff BL, Zhu Y, Herndon JM, Meyer MA, Nywening TM, Hawkins WG, Shapiro IM, Weaver DT, et al. 2016. Targeting focal adhesion kinase renders pancreatic cancers responsive to checkpoint immunotherapy. *Nat Med* **22**: 851–860.
- Jin X, Mu P. 2015. Targeting breast cancer metastasis. *Breast Cancer (Auckl)* **9**: 23–34.
- Johnson LL, Pavlovsky AG, Johnson AR, Janowicz JA, Man CF, Ortwine DF, Purchase CF, White AD, Hupe DJ. 2000. A rationalization of the acidic pH dependence for stromelysin-1 (Matrix metalloproteinase-3) catalysis and inhibition. *J Biol Chem* **275**: 11026–11033.
- Jonas O, Landry HM, Fuller JE, Santini JT, Baselga J, Tepper RI, Cima MJ, Langer R. 2015. An implantable microdevice to perform high-throughput in vivo drug sensitivity testing in tumors. *Sci Transl Med* **7**: 284ra57.
- Joyce JA, Pollard JW. 2009. Microenvironmental regulation of metastasis. *Nat Rev Cancer* **9**: 239–252.
- Kai FB, Laklai H, Weaver VM. 2016. Force matters: Biomechanical regulation of cell invasion and migration in disease. *Trends Cell Biol* **26**: 486–497.
- Kaplan RN, Riba RD, Zacharoulis S, Bramley AH, Vincent L, Costa C, MacDonald DD, Jin DK, Shido K, Kerns SA, et al. 2005. VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. *Nature* **438**: 820–827.
- King SJ, Asokan SB, Haynes EM, Zimmerman SP, Rotty JD, Alb JG, Tagliatela A, Blake DR, Lebedeva IP, Marston D, et al. 2016. Lamellipodia are crucial for haptotactic sensing and response. *J Cell Sci* **129**: 2329–2342.

- Kuo C-HR, Xian J, Brenton JD, Franze K, Sivanian E. 2012. Complex stiffness gradient substrates for studying mechanotactic cell migration. *Adv Mater* **24**: 6059–6064.
- Kwon EJ, Lo JH, Bhatia SN. 2015. Smart nanosystems: Bio-inspired technologies that interact with the host environment. *Proc Natl Acad Sci* **112**: 14460–14466.
- Laklai H, Miroshnikova YA, Pickup MW, Collisson EA, Kim GE, Barrett AS, Hill RC, Lakins JN, Schlaepfer DD, Mouw JK, et al. 2016. Genotype tunes pancreatic ductal adenocarcinoma tissue tension to induce matricellular fibrosis and tumor progression. *Nat Med* **22**: 497–505.
- Levental F, Yu H, Kass L, Lakins JN, Egeblad M, Erler JT, Fong SFT, Csiszar K, Giaccia A, Weninger W, et al. 2009. Matrix crosslinking forces tumor progression by enhancing integrin signaling. *Cell* **139**: 891–906.
- Leyme A, Marivin A, Perez-Gutierrez L, Nguyen LT, Garcia-Marcos M. 2015. Integrins activate trimeric G proteins via the nonreceptor protein GIV/Girdin. *J Cell Biol* **210**: 1165–1184.
- Li J, Lin F. 2011. Microfluidic devices for studying chemotaxis and electrotaxis. *Trends Cell Biol* **21**: 489–497.
- Li X-F, Carlin S, Urano M, Russell J, Ling CC, O'Donoghue JA. 2007. Visualization of hypoxia in microscopic tumors by immunofluorescent microscopy. *Cancer Res* **67**: 7646–7653.
- Lo CM, Wang HB, Dembo M, Wang YL. 2000. Cell movement is guided by the rigidity of the substrate. *Biophys J* **79**: 144–152.
- Lopez JJ, Kang I, You W-K, McDonald DM, Weaver VM. 2011. In situ force mapping of mammary gland transformation. *Integr Biol* **3**: 910–921.
- Lu J, Zhou S, Siech M, Habisch H, Seufferlein T, Bachem MG. 2014. Pancreatic stellate cells promote haptotaxis of cancer cells through collagen I-mediated signalling pathway. *Br J Cancer* **110**: 409–420.
- Martinez-Zaguilan R, Lynch RM, Martinez GM, Gillies RJ. 1993. Vacuolar-type H(+)ATPases are functionally expressed in plasma membranes of human tumor cells. *Am J Physiol* **265**: C1015–C1029.
- Mateo J, Berlin J, De Bono JS, Cohen RB, Keedy V, Mugundu G, Zhang L, Abbattista A, Davis C, Gallo Stampino C, et al. 2014. A first-in-human study of the anti- $\alpha 5 \beta 1$ integrin monoclonal antibody PF-04605412 administered intravenously to patients with advanced solid tumors. *Cancer Chemother Pharmacol* **74**: 1039–1046.
- McCaig CD, Song B, Rajnicek AM. 2009. Electrical dimensions in cell science. *J Cell Sci* **122**: 4267–4276.
- McLachlan GD, Cahill SM, Girvin ME, Almo SC. 2007. Acid-induced equilibrium folding intermediate of human platelet profilin. *Biochemistry* **46**: 6931–6943.
- McLean LA, Roscoe J, Jorgensen NK, Gorin FA, Cala PM. 2000. Malignant gliomas display altered pH regulation by NHE1 compared with nontransformed astrocytes. *Am J Physiol Cell Physiol* **278**: C676–C688.
- Mehlen P, Delloye-Bourgeois C, Chédotal A. 2011. Novel roles for Slits and netrins: Axon guidance cues as anticancer targets? *Nat Rev Cancer* **11**: 188–197.
- Meyer AS, Hughes-Alford SK, Kay JE, Castillo A, Wells A, Gertler FB, Lauffenburger DA. 2012. 2D protrusion but not motility predicts growth factor-induced cancer cell migration in 3D collagen. *J Cell Biol* **197**: 721–729.
- Minn AJ, Gupta GP, Siegel PM, Bos PD, Shu W, Giri DD, Viale A, Olshen AB, Gerald WL, Massague J. 2005. Genes that mediate breast cancer metastasis to lung. *Nature* **436**: 518–524.
- Miroshnikova YA, Mouw JK, Barnes JM, Pickup MW, Lakins JN, Kim Y, Lobo K, Persson AI, Reis GF, McKnight TR, et al. 2016. Tissue mechanics promote IDH1-dependent HIF1 α -tenascin C feedback to regulate glioblastoma aggression. *Nat Cell Biol* **18**: 1336–1345.
- Mouneimne G, Soon L, DesMarais V, Sidani M, Song X, Yip S-C, Ghosh M, Eddy R, Backer JM, Condeelis J. 2004. Phospholipase C and cofilin are required for carcinoma cell directionality in response to EGF stimulation. *J Cell Biol* **166**: 697–708.
- Mouw JK, Yui Y, Damiano L, Bainer RO, Lakins JN, Acerbi I, Ou G, Wijekoon AC, Levental KR, Gilbert PM, et al. 2014. Tissue mechanics modulate microRNA-dependent PTEN expression to regulate malignant progression. *Nat Med* **20**: 360–367.
- Muinenon-Martin AJ, Susanto O, Zhang Q, Smethurst E, Fallor WJ, Veltman DM, Kalna G, Lindsay C, Bennett DC, Sansom OJ, et al. 2014. Melanoma cells break down LPA to establish local gradients that drive chemotactic dispersal. *PLoS Biol* **12**: e1001966.
- Müller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, McClanahan T, Murphy E, Yuan W, Wagner SN, et al. 2001. Involvement of chemokine receptors in breast cancer metastasis. *Nature* **410**: 50–56.
- Mycielska ME, Djamgoz MBA. 2004. Cellular mechanisms of direct-current electric field effects: Galvanotaxis and metastatic disease. *J Cell Sci* **117**: 1631–1639.
- Naba A, Clauser KR, Hoersch S, Liu H, Carr SA, Hynes RO. 2012. The matrisome: In silico definition and in vivo characterization by proteomics of normal and tumor extracellular matrices. *Mol Cell Proteomics* **11**: M111.014647.
- Naba A, Clauser KR, Lamar JM, Carr SA, Hynes RO. 2014. Extracellular matrix signatures of human mammary carcinoma identify novel metastasis promoters. *Elife* **3**: e01308.
- Naba A, Clauser KR, Ding H, Whittaker CA, Carr SA, Hynes RO. 2016. The extracellular matrix: Tools and insights for the “omics” era. *Matrix Biol* **49**: 10–24.
- Oudin MJ, Jonas O, Kosciuk T, Broye LC, Guido BC, Wyckoff J, Riquelme D, Lamar JM, Asokan SB, Whittaker C, et al. 2016a. Tumor cell-driven extracellular matrix remodeling drives haptotaxis during metastatic progression. *Cancer Discov* **6**: 516–531.
- Oudin MJ, Miller MA, Klazen JAZ, Kosciuk T, Lussiez A, Hughes SK, Tadros J, Bear JE, Lauffenburger DA, Gertler FB. 2016b. MenaINV mediates synergistic cross-talk between signaling pathways driving chemotaxis and haptotaxis. *Mol Biol Cell* **27**: 3085–3094.
- Oudin MJ, Barbier L, Schäfer C, Kosciuk T, Miller MA, Han S, Jonas O, Lauffenburger DA, Gertler F. 2017. Mena confers resistance to Paclitaxel in triple-negative breast cancer. *Mol Cancer Ther* **16**: 143–155.
- Padera TP, Stoll BR, Tooredman JB, Capen D, di Tomaso E, Jain RK. 2004. Pathology: Cancer cells compress intratumour vessels. *Nature* **427**: 695.
- Park JS, Lee J-H, Lee YS, Kim JK, Dong SM, Yoon DS. 2016. Emerging role of LOXL2 in the promotion of pancreas cancer metastasis. *Oncotarget* **7**: 42539–42552.
- Pastorekova S, Parkkila S, Zavada J. 2006. Tumor-associated carbonic anhydrases and their clinical significance. *Adv Clin Chem* **42**: 167–216.
- Paszek MJ, Zahir N, Johnson KR, Lakins JN, Rozenberg GI, Gefen A, Reinhart-King CA, Margulies SS, Dembo M, Boettiger D, et al. 2005. Tensional homeostasis and the malignant phenotype. *Cancer Cell* **8**: 241–254.
- Patel A, Sant S. 2016. Hypoxic tumor microenvironment: Opportunities to develop targeted therapies. *Biotechnol Adv* **34**: 803–812.
- Patsialou A, Wang Y, Lin J, Whitney K, Goswami S, Kenny PA, Condeelis JS. 2012. Selective gene-expression profiling of migratory tumor cells in vivo predicts clinical outcome in breast cancer patients. *Breast Cancer Res* **14**: R139.
- Petrie RJ, Doyle AD, Yamada KM. 2009. Random versus directionally persistent cell migration. *Nat Rev Mol Cell Biol* **10**: 538–549.
- Philipp U, Roussos ET, Oser M, Yamaguchi H, Kim H-D, Giampieri S, Wang Y, Goswami S, Wyckoff JB, Lauffenburger DA, et al. 2008. A Mena invasion isoform potentiates EGF-induced carcinoma cell invasion and metastasis. *Dev Cell* **15**: 813–828.
- Pickup MW, Laklai H, Acerbi I, Owens P, Gorska AE, Chytil A, Aakre M, Weaver VM, Moses HL. 2013. Stromally derived

- lysyl oxidase promotes metastasis of transforming growth factor- β -deficient mouse mammary carcinomas. *Cancer Res* **73**: 5336–5346.
- Pinheiro C, Longatto-Filho A, Ferreira L, Pereira SMM, Etlinger D, Moreira MAR, Jubé LF, Queiroz GS, Schmitt F, Baltazar F. 2008. Increasing expression of monocarboxylate transporters 1 and 4 along progression to invasive cervical carcinoma. *Int J Gynecol Pathol* **27**: 568–574.
- Plodinec M, Loparic M, Monnier CA, Obermann EC, Zanetti-Dallenbach R, Oertle P, Hyotyla JT, Aebi U, Bentes-Alj M, Lim RYH, et al. 2012. The nanomechanical signature of breast cancer. *Nat Nanotechnol* **7**: 757–765.
- Plotnikov SV, Pasapera AM, Sabass B, Waterman CM. 2012. Force fluctuations within focal adhesions mediate ECM-rigidity sensing to guide directed cell migration. *Cell* **151**: 1513–1527.
- Poon Z, Chang D, Zhao X, Hammond PT. 2011. Layer-by-layer nanoparticles with a pH-sheddable layer for in vivo targeting of tumor hypoxia. *ACS Nano* **5**: 4284–4292.
- Provenzano PP, Eliceiri KW, Campbell JM, Inman DR, White JG, Keely PJ. 2006. Collagen reorganization at the tumor-stromal interface facilitates local invasion. *BMC Med* **4**: 38.
- Provenzano PP, Inman DR, Eliceiri KW, Trier SM, Keely PJ. 2008. Contact guidance mediated three-dimensional cell migration is regulated by Rho/ROCK-dependent matrix reorganization. *Biophys J* **95**: 5374–5384.
- Pu J, McCaig CD, Cao L, Zhao Z, Segall JE, Zhao M. 2007. EGF receptor signalling is essential for electric-field-directed migration of breast cancer cells. *J Cell Sci* **120**: 3395–3403.
- Raab M, Swift J, Dingal PCDP, Shah P, Shin J-W, Discher DE. 2012. Crawling from soft to stiff matrix polarizes the cytoskeleton and phosphoregulates myosin-II heavy chain. *J Cell Biol* **199**: 669–683.
- Ribeiro AL, Okamoto OK. 2015. Combined effects of pericytes in the tumor microenvironment. *Stem Cells Int* **2015**: 868475.
- Riching KM, Cox BL, Salick MR, Pehlke C, Riching AS, Ponik SM, Bass BR, Crone WC, Jiang Y, Weaver AM, et al. 2014. 3D collagen alignment limits protrusions to enhance breast cancer cell persistence. *Biophys J* **107**: 2546–2558.
- Robinson BD, Sica GL, Liu Y-F, Rohan TE, Gertler FB, Condeelis JS, Jones JG. 2009. Tumor microenvironment of metastasis in human breast carcinoma: A potential prognostic marker linked to hematogenous dissemination. *Clin Cancer Res* **15**: 2433–2441.
- Roca-Cusachs P, Sunyer R, Trepas X. 2013. Mechanical guidance of cell migration: Lessons from chemotaxis. *Curr Opin Cell Biol* **25**: 543–549.
- Rohan TE, Xue X, Lin H-M, D'Alfonso TM, Ginter PS, Oktay MH, Robinson BD, Ginsberg M, Gertler FB, Glass AG, et al. 2014. Tumor microenvironment of metastasis and risk of distant metastasis of breast cancer. *J Natl Cancer Inst* **106**: djul136.
- Roussos ET, Wang Y, Wyckoff JB, Sellers RS, Wang W, Li J, Pollard JW, Gertler FB, Condeelis JS. 2010. Mena deficiency delays tumor progression and decreases metastasis in polyoma middle-T transgenic mouse mammary tumors. *Breast Cancer Res* **12**: R101.
- Roussos ET, Condeelis JS, Patsialou A. 2011. Chemotaxis in cancer. *Nat Rev Cancer* **11**: 573–587.
- Rubashkin MG, Cassereau L, Bainer R, DuFort CC, Yui Y, Ou G, Paszek MJ, Davidson MW, Chen YY, Weaver VM. 2014. Force engages vinculin and promotes tumor progression by enhancing PI3K activation of phosphatidylinositol (3,4,5)-triphosphate. *Cancer Res* **74**: 4597–4611.
- Saadoun S, Papadopoulos MC, Hara-Chikuma M, Verkman AS. 2005. Impairment of angiogenesis and cell migration by targeted aquaporin-1 gene disruption. *Nature* **434**: 786–792.
- Schaffner F, Ray AM, Döntenwill M. 2013. Integrin $\alpha 5 \beta 1$, the fibronectin receptor, as a pertinent therapeutic target in solid tumors. *Cancers (Basel)* **5**: 27–47.
- Shi Q, Ghosh RP, Engelke H, Rycroft CH, Cassereau L, Sethian JA, Weaver VM, Liphardt JT. 2013. Rapid disorganization of mechanically interacting systems of mammary acini. *Proc Natl Acad Sci* **111**: 658–663.
- Soon L, Mouneimne G, Segall J, Wyckoff J, Condeelis J. 2005. Description and characterization of a chamber for viewing and quantifying cancer cell chemotaxis. *Cell Motil Cytoskeleton* **62**: 27–34.
- Srivastava J, Barreiro G, Groscurth S, Gingras AR, Goult BT, Critchley DR, Kelly MJS, Jacobson MP, Barber DL. 2008. Structural model and functional significance of pH-dependent talin-actin binding for focal adhesion remodeling. *Proc Natl Acad Sci* **105**: 14436–14441.
- Steeg PS. 2016. Targeting metastasis. *Nat Rev Cancer* **16**: 201–218.
- Stupp R, Hegi ME, Gorlia T, Erridge SC, Perry J, Hong YK, Aldape KD, Lhermitte B, Pietsch T, Grujicic D, et al. 2014. Cilengitide combined with standard treatment for patients with newly diagnosed glioblastoma with methylated MGMT promoter (CENTRIC EORTC 26071-22072 study): A multicentre, randomised, open-label, phase 3 trial. *Lancet Oncol* **15**: 1100–1108.
- Stüwe L, Müller M, Fabian A, Waning J, Mally S, Noël J, Schwab A, Stock C. 2007. pH dependence of melanoma cell migration: Protons extruded by NHE1 dominate protons of the bulk solution. *J Physiol* **585**: 351–360.
- Sun Y-S, Peng S-W, Lin K-H, Cheng J-Y. 2012. Electrotaxis of lung cancer cells in ordered three-dimensional scaffolds. *Bio-microfluidics* **6**: 14102–14102-14.
- Sunyer R, Jin AJ, Nossal R, Sackett DL. 2012. Fabrication of hydrogels with steep stiffness gradients for studying cell mechanical response. *PLoS One* **7**: e46107.
- Suraneni P, Rubinstein B, Unruh JR, Durnin M, Hanein D, Li R. 2012. The Arp2/3 complex is required for lamellipodia extension and directional fibroblast cell migration. *J Cell Biol* **197**: 239–251.
- Swietach P, Patiar S, Supuran CT, Harris AL, Vaughan-Jones RD. 2009. The role of carbonic anhydrase 9 in regulating extracellular and intracellular pH in three-dimensional tumor cell growths. *J Biol Chem* **284**: 20299–20310.
- Szatkowski M, Mycielska M, Knowles R, Kho AL, Djamgoz MBA. 2000. Electrophysiological recordings from the rat prostate gland in vitro: Identified single-cell and transepithelial (lumen) potentials. *BJU Int* **86**: 1068–1075.
- Takai K, Le A, Weaver VM, Werb Z. 2016. Targeting the cancer-associated fibroblasts as a treatment in triple-negative breast cancer. *Oncotarget* **7**: 82889–82901.
- Tarabozetti G, Roberts DD, Liotta LA. 1987. Thrombospondin-induced tumor cell migration: Haptotaxis and chemotaxis are mediated by different molecular domains. *J Cell Biol* **105**: 2409–2415.
- Tessier-Lavigne M, Goodman CS. 1996. The molecular biology of axon guidance. *Science (80-)* **274**: 1123–1133.
- Tian L, Bae YH. 2012. Cancer nanomedicines targeting tumor extracellular pH. *Colloids Surf B Biointerfaces* **99**: 116–126.
- Tweedy L, Susanto O, Insall RH. 2016. Self-generated chemotactic gradients—Cells steering themselves. *Curr Opin Cell Biol* **42**: 46–51.
- Ulrich TA, de Juan Pardo EM, Kumar S. 2009. The mechanical rigidity of the extracellular matrix regulates the structure, motility, and proliferation of glioma cells. *Cancer Res* **69**: 4167–4174.
- van de Vijver MJ, He YD, van't Veer LJ, Dai H, Hart AA, Voskuil DW, Schreiber GJ, Peterse JL, Roberts C, Marton MJ, et al. 2002. A gene-expression signature as a predictor of survival in breast cancer. *N Engl J Med* **347**: 1999–2009.
- van't Veer L, Dai H, van de Vijver M, He YD, Hart AA, Mao M, Peterse HL, van der Kooy K, Marton MJ, Witteveen AT, et al. 2002. Gene expression profiling predicts clinical outcome of breast cancer. *Nature* **415**: 530–536.
- von Pawel J, von Roemeling R, Gatzemeier U, Boyer M, Elisson LO, Clark P, Talbot D, Rey A, Butler TW, Hirsh V, et al. 2000. Tirapazamine plus cisplatin versus cisplatin in advanced non-small-cell lung cancer: A report of the international CATA-PULT I study group. Cisplatin and Tirapazamine in subjects

- with advanced previously untreated non-small-cell lung tumors. *J Clin Oncol* **18**: 1351–1359.
- Walker RA. 2001. The complexities of breast cancer desmoplasia. *Breast Cancer Res* **3**: 143–145.
- Wang W, Wyckoff JB, Frohlich VC, Oleynikov Y, Hüttelmaier S, Zavadil J, Cermak L, Bottinger EP, Singer RH, White JG, et al. 2002. Single cell behavior in metastatic primary mammary tumors correlated with gene expression patterns revealed by molecular profiling. *Cancer Res* **62**: 6278–6288.
- Wang W, Goswami S, Lapidus K, Wells AL, Wyckoff JB, Sahai E, Singer RH, Segall JE, Condeelis JS. 2004. Identification and testing of a gene expression signature of invasive carcinoma cells within primary mammary tumors. *Cancer Res* **64**: 8585–8594.
- Webb BA, Chimenti M, Jacobson MP, Barber DL. 2011. Dysregulated pH: A perfect storm for cancer progression. *Nat Rev Cancer* **11**: 671–677.
- Williams CB, Yeh ES, Soloff AC. 2016a. Tumor-associated macrophages: Unwitting accomplices in breast cancer malignancy. *NPJ Breast Cancer* **2**: 15025.
- Williams JK, Entenberg D, Wang Y, Avivar-Valderas A, Padgen M, Clark A, Aguirre-Ghiso JA, Castracane J, Condeelis JS. 2016b. Validation of a device for the active manipulation of the tumor microenvironment during intravital imaging. *Intra-Vital* **5**: e1182271.
- Willipinski-Stapelfeldt B, Riethdorf S, Assmann V, Woelfle U, Rau T, Sauter G, Heukeshoven J, Pantel K. 2005. Changes in cytoskeletal protein composition indicative of an epithelial-mesenchymal transition in human micrometastatic and primary breast carcinoma cells. *Clin Cancer Res* **11**: 8006–8014.
- Wilson WR, Hay MP. 2011. Targeting hypoxia in cancer therapy. *Nat Rev Cancer* **11**: 393–410.
- Wu J, Lin F. 2014. Recent developments in electrotaxis assays. *Adv Wound Care* **3**: 149–155.
- Wu C, Asokan SB, Berginski ME, Haynes EM, Sharpless NE, Griffith JD, Gomez SM, Bear JE. 2012. Arp2/3 is critical for lamellipodia and response to extracellular matrix cues but is dispensable for chemotaxis. *Cell* **148**: 973–987.
- Wyckoff J, Wang W, Lin EY, Wang Y, Pixley F, Stanley ER, Graf T, Pollard JW, Segall J, Condeelis J. 2004. A paracrine loop between tumor cells and macrophages is required for tumor cell migration in mammary tumors. *Cancer Res* **64**: 7022–7029.
- Wyckoff JB, Pinner SE, Gschmeissner S, Condeelis JS, Sahai E. 2006. ROCK- and myosin-dependent matrix deformation enables protease-independent tumor-cell invasion in vivo. *Curr Biol* **16**: 1515–1523.
- Wynn TA. 2008. Cellular and molecular mechanisms of fibrosis. *J Pathol* **214**: 199–210.
- Yan X, Han J, Zhang Z, Wang J, Cheng Q, Gao K, Ni Y, Wang Y. 2009. Lung cancer A549 cells migrate directionally in DC electric fields with polarized and activated EGFRs. *Bioelectromagnetics* **30**: 29–35.
- Yang M, Brackenbury WJ. 2013. Membrane potential and cancer progression. *Front Physiol* **4**: 185.
- Yang N, Mosher R, Seo S, Beebe D, Friedl A. 2011. Syndecan-1 in breast cancer stroma fibroblasts regulates extracellular matrix fiber organization and carcinoma cell motility. *Am J Pathol* **178**: 325–335.
- Yang B, Lieu ZZ, Wolfenson H, Hameed FM, Bershadsky AD, Sheetz MP. 2016. Mechanosensing controlled directly by tyrosine kinases. *Nano Lett* **16**: 5951–5961.
- Yi A, Moon WK, Cho N, Chang JM, Bae MS, Kim SJ, Han W, Park IA. 2013. Association of tumour stiffness on sonoelastography with axillary nodal status in T1 breast carcinoma patients. *Eur Radiol* **23**: 2979–2987.
- Yildirim S, Altun S, Gumushan H, Patel A, Djamgoz MBA. 2012. Voltage-gated sodium channel activity promotes prostate cancer metastasis in vivo. *Cancer Lett* **323**: 58–61.
- Zeisberg M, Kalluri R. 2013. Cellular mechanisms of tissue fibrosis. 1. Common and organ-specific mechanisms associated with tissue fibrosis. *Am J Physiol Cell Physiol* **304**: C216–C225.
- Zhang Y, Lin Z, Foolen J, Schoen I, Santoro A, Zenobi-Wong M, Vogel V. 2014. Disentangling the multifactorial contributions of fibronectin, collagen and cyclic strain on MMP expression and extracellular matrix remodeling by fibroblasts. *Matrix Biol* **40**: 62–72.
- Zhao M, Song B, Pu J, Wada T, Reid B, Tai G, Wang F, Guo A, Walczysko P, Gu Y, et al. 2006. Electrical signals control wound healing through phosphatidylinositol-3-OH kinase- γ and PTEN. *Nature* **442**: 457–460.

Cold Spring Harbor Symposia on Quantitative Biology

Physical and Chemical Gradients in the Tumor Microenvironment Regulate Tumor Cell Invasion, Migration, and Metastasis

Madeleine J. Oudin and Valerie M. Weaver

Cold Spring Harb Symp Quant Biol 2016 81: 189-205 originally published online April 19, 2017
Access the most recent version at doi:[10.1101/sqb.2016.81.030817](https://doi.org/10.1101/sqb.2016.81.030817)

References

This article cites 165 articles, 43 of which can be accessed free at:
<http://symposium.cshlp.org/content/81/189.full.html#ref-list-1>

Creative Commons License

This article is distributed under the terms of the
<http://creativecommons.org/licenses/by-nc/4.0/>, which permits reuse and
redistribution, except for commercial purposes, provided that the original
author and source are credited.

Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in
the box at the top right corner of the article or [click here](#).

To subscribe to *Cold Spring Harbor Symposia on Quantitative Biology* go to:
<http://symposium.cshlp.org/subscriptions>