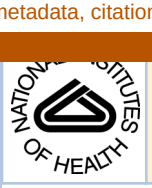

Published in final edited form as:

Trends Pharmacol Sci. 2013 May ; 34(5): 283–289. doi:10.1016/j.tips.2013.03.001.

Targeting tumor cell motility as a strategy against invasion and metastasis

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Abstract

Advances in diagnosis and treatment have rendered most solid tumors largely curable if they are diagnosed and treated before dissemination. However, once they spread beyond the initial primary location, these cancers are usually highly morbid, if not fatal. Thus current efforts focus on both limiting initial dissemination and preventing secondary spread. There are two modes of tumor dissemination – invasion and metastasis – each leading to unique therapeutic challenges and likely driven by distinct mechanisms. However, these two forms of dissemination utilize some common strategies to accomplish movement from the primary tumor, establishment in an ectopic site, and survival therein. The adaptive behaviors of motile cancer cells provide an opening for therapeutic approaches if we understand the molecular, cellular, and tissue biology that underlie them. Herein we review the signaling cascades and organ reactions that lead to dissemination, as these are non-genetic in nature, focusing on cell migration as the key to tumor progression. In this context, the cellular phenotype will also be discussed because the modes of migration are dictated by quantitative and physical aspects of the cell motility machinery.

Keywords

Tumor dissemination; migration; survival; proliferation; dormancy

Basic mechanisms of tumor dissemination

Tumors of solid organs (carcinomas, sarcomas and CNS tumors) kill patients mainly by dissemination from the primary site. Surgical and radiological advances have rendered localized cancers largely manageable, if not curable. However, once the cells migrate beyond the primary site into adjacent or distant tissue, the cells are difficult to extirpate. This dissemination may take two forms: (i) localized invasion throughout the tissue (especially for glioblastoma cerebri) and into the adnexia (most carcinomas), or (ii) metastatic dissemination (Table 1).

These two modes of dissemination require distinct sets of cellular behaviors, some of which are shared and others are distinct (Figure 1). In localized invasion, the tumor cell must acquire properties (i) enabling at least partial separation from the primary mass, (ii) recognition and (iii) reorganization of the barrier matrices, (iv) active migration through these matrices, and (v) survival in the adjacent tissues [1]. The invasive tumor then exists as a

physical extension of the primary mass, with the cancer cells moving into the tissue in a syncytial manner.

The steps of the second mode of dissemination, that of metastatic seeding have been described [2]. Initially, carcinoma cells must acquire properties that allow at least partial, if not complete separation from the original mass to escape through boundary matrices and intravasate into a conduit [3]. Although there are copious clinical correlations and experimental animal studies showing that acquisition of mesenchymal-associated phenotypes and markers promote this escape, it is still possible that epithelioid carcinoma cells occasionally do reach the conduits and may provide for metastatic loci [4, 5]. Survival during transit via vasculature is important because the shear stresses and lack of supportive signals from adhesive sites challenge these cells; still, the rapid nature of this dissemination and the mesenchymal properties probably aid in viable cells reaching target organs. At the metastatic locale the cells must recognize the endothelial cells, initiate separation from this monolayer, and then extravasate first onto and then through the basement membrane to gain access to the tissue parenchyma, a process that has been captured in experimental settings [6]. What occurs next is known only by extrapolation because metastatic nodules are not clinically evident until weeks to years later. For rapidly presenting tumor nodules, it is assumed that the carcinomas continue to proliferate as in the primary site. However, for the delayed emergences, it is still open as to whether there is balanced proliferation and death/apoptosis, or individual cell dormancy, although the latter has been shown to be more statistically probable via advanced computer modeling systems [7]. Lastly, there is emerging evidence that the carcinoma cells may undergo a phenotypic switch towards a more epithelioid cell to establish metastases [5, 8–11], although this may revert again to a mesenchymal phenotype as the metastasis becomes clinically evident [12].

It is assumed mainly by extrapolation that the cell aspect of invasion is syncytial, whereas metastasis occurs as escape of singular cells. The histopathological findings of invasive tumors often retaining cell-cell connections and singular metastatic tumor cells are what led orignally to these assumptions, but these observations are post-hoc and may represent convergence of cells in the case of presumed syncytial invasion and death of co-disseminated cells in metastatic seeding. Experimental tracking of melanoma cell invasion into matrices suggest mainly mass movement of communicating cells though there are singular cells extending ahead of the front [13, 14]. Others have found breast cancer cells breaking away indivdiually prior to hematologous metastasis [15], and circulating tumor cells (CTC) in patients are found to be singular [16, 17]. Based on these and other observations, it is generally assumed that the two modes of dissemination utilize and even require quantitative, if not qualitative differences in cell separation, but this is not likely as absolute as presented in simplified descriptions. The key for this discussion is that whether as single cells or as a group, motility is a rate-limiting process in tumor dissemination [3].

Tumors utilize both modes of dissemination, invasion and metastasis, to cause morbidity and mortality, but select tumor types show a predilection for one or the other. For example, bladder carcinoma and glioblastoma multiformes are primarily invasive, whereas breast and lung carcinomas are initially metastatic; melanoma is invasive as a prelude metastasis [9, 18]. Thus, future therapies must account for both modes of cancer cell motility to be truly effective in limiting tumor progression. As such, there are two cell properties common to invasion and metastasis. The first is the epithelial to mesenchymal transition (EMT) that loosens the primary tumor cell mass. However, targeting this dedifferentiation is questionable because of its seemingly transient nature. The phenotypic plasticity to a more mesenchymal phenotype is reversed in the metastatic site, indicating facile adaptation by these cells [11, 19, 20]. This would require treatments to capture all migratory cancer cells before seeding at the metastatic site, a requirement that is unlikely to be met in most cases.

Additionally, the syncytial migration of localized invasion may occur with cells expressing a partial epithelial phenotype [21]. The second shared characteristic is growth factor-induced cell motility [1]. This common requirement may hold a key to limiting tumor progression and turning cancer from a progressively lethal disease into a manageable chronic condition. This brief review should allow for the reader to discern the aspects of cell motility so as to derive approaches to targeting this process.

Motility cascade

Tumors move under two stimuli, a basal motility from adhesion receptors and a faster rate from soluble growth factors. Growth factor receptor-mediated motility is a major driver of tumor cell dissemination via invasion or metastasis [1, 15]. Thus, understanding the key molecular controls of this behavior should provide novel targets to limit initial or secondary dissemination. Solid tumors produce both autocrine and paracrine factors that, in turn, generate reciprocal paracrine signaling networks which actuate motility machinery [22]. It is possible to examine tumor cell movement even in the absence of exogenously-added signals. Although far from optimal in deciphering the richness of the tumor microenvironment, these controlled contextual situations allow for parsing of key molecular switches.

The best understood mode of induced tumor cell migration is that of mesenchymal motility which is seen as cells move across stiff substrata (Box 1). However, *in vivo,* tumor cells move through matrices in three dimensions. Emerging research indicates that tumor cells vacillate between mesenchymal motility and a less-adherent amoeboid-like motility [13, 22]. Still, the findings from mesenchymal motility have been validated in the more complex context of organismal dissemination in animal models. Numerous studies have shown that targeting individual intracellular effectors of motility as defined in 2D contexts can limit tumor invasion and metastasis in animal models [1, 3]. More importantly, the key signaling nexus of transcellular contractility/cell-substratum adhesion [23] has been shown to govern 3D migration and integrate matrix stiffness [24, 25].

Box 1

Key molecular switches during factor-induced mesenchymal motility

Tumor cells move under the influence of autocrine and paracrine signaling. This cell migration can be characterized as a persistent random walk in the absence of attractant concentration gradients, and as a biased persistent random walk in the presence of such gradients. In either case, two key descriptors of the migration behaviors are the translational speed (distance traveled per time) and the directional persistence (average time interval between major changes in direction). These properties can be measured experimentally using individual-cell videotracking methods, and have been shown to be influenced by environmental stimuli such as soluble growth factors and insoluble extracellular matrix components [65, 66]. In turn, cell migration speed and directional persistence result from a highly integrated set of biophysical processes underlying locomotion At a minimum, these biophysical processes include lamellipodal (and/or filopodal) membrane protrusion, cell/substratum attachment intracellular force generation, and detachment of cell/substratum adhesions [67] (Figure I). Overriding the haptokinetic controls of adhesion are key targetable molecular switches (in italics) that impose a faster but less persistent motility. Inhibiting any one of these will limit cell motility and tumor invasion in experimental models [68, 69]. However, coordination of these processes into cell translocation further requires a biophysical asymmetry between the effective front of the cell and its rear, so that attachment can remain strong at the front while detachment occurs at the rear. Thus, the key nodes for intervention were sought by unbiased systems biology approaches. A decision tree analysis found myosin-light chain

and PKCδ activation status as key [23], a finding supported by principle component analyses [24]. These suggest that the ratio of adhesion to transcellular contrality governs mesenchymal motility.

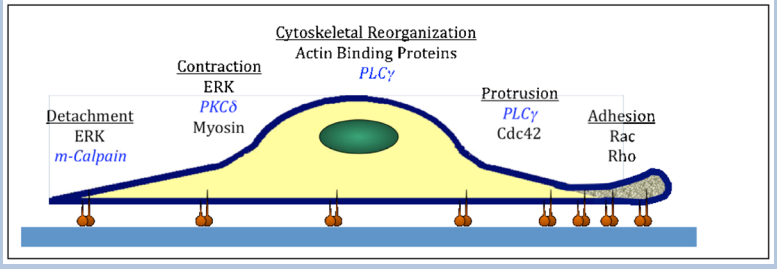

Figure I.
During mesenchymal migration the specific steps of cytoskeletal reorganiztion, lamellipodial protrusion, new forward adhesions, transcellular contractility, and rear detachment are controlled by key signaling nexi. The key integrating molecules for each process are shown, in black for those operative during both adhesion- and growth factor-driven motility, whereas those only activated during the enhanced movement promoted by growth factor receptor activation are shown in blue italics.

To move *in vivo*, cancer cells must break away from the primary tumor mass and remodel the extracellular matrix (ECM). In a 3D environment, the force of protrusive actin polymerization drives cells into spindle-shaped mesenchymal morphology that can allow cell migration, and this process requires proteases in dense ECM [26, 27]. A hallmark of tumor cell invasion is upregulation of proteolytic enzymes generated both by migrating cells and the stromal cells [28]. Our appreciation of proteases in cancer progression has progressed from an early simplified conception of dissolving barrier matrices to one of not only judicious 'loosening' of matrices, primarily for syncytial invasion [29], but also of releasing or modulating pro-motility signals [30]. In addition to providing space for invasive cells to move forward, even minimal proteolytic processing changes the matrix composition, which in turn alters tumor cell migration [31]. This matrix degradation is required to a greater extent during syncytial migration or even migration of individual cells in a mesenchymal mode [32].

Depending on the 3D context, some tumor cells can alternatively invade without a requirement for proteolytic activity. They can move through the ECM without its degradation by either following natural cleavage planes or acquiring a rounded morphology and using actin contractile force to generate amoeboid bleb-like protrusions that push and squeeze cells through the ECM [26, 27] (Figure 2). During amoeboid motility, little matrix proteolysis is needed as the cells 'bleb' though tight passages in the matrices; this is noted *in vitro* by imaging and suggested *in vivo* by finding rounded cells in the process of traversing barrier matrices (Figure 2). Migration is possible in the absence of proteolysis if both the porosity of the matrix and the cell body deformability can support it. Molecular and structural characteristics of both tissue microenvironment and cell behavior determine whether cells will migrate collectively or individually in mesenchymal or amoeboid mode. ECM stiffness, fiber orientation, density and gap size provide parameters that modulate cell adhesion and cytoskeletal organization [33]. Although mesenchymal migration is dependent on alternative pushing and pulling cycles, amoeboidal migration is equally mechanically complex and combines stronger pushing with less adhesive pulling of the substrate.

Tumor cell migration involves alteration between cellular states [33]. Intracellular switches that control cytoskeletal tension, and thus cell shape, regulate the conversion between mesenchymal and rounded migration; targetable molecules involve the balance between the

Rac pathway that promotes cell spreading and the Rho/ROCK signaling axis that leads to cellular contraction [26] Silencing of ROCK pathway induces the transition from amoeboidal to mesenchymal invasiveness [34], whereas silencing of Rac induces the opposite shift [35]. In addition, active Rac negatively regulates Rho/ROCK signaling [36] and inhibits cell rounding, whereas active Rho/ROCK limits Rac activity that inhibits cell extensions [34]. Therefore, anti-motility strategies must block both modes of movement.

The signaling pathways that actuate the cell motility in tumor cells do show altered levels of expression, but individual genes are not usually mutated; this is likely as activation of one step but not the others in the migration process would lead to un-coordinated and inefficient motility. As both modes of mesenchymal and ameboid motility are interchangeable and arise in response to cues from the microenvironment [37], the signaling systems must be intact and functional. Furthermore, this same switching mechanism is used in physiological processes such as wound healing.

Extracellular regulators of migration

Many studies have explored the extrinsic controls of tumor cell migration. At first, researchers focused on soluble signals, such as growth factors, which could drive tumor dissemination. There are extensive recent reviews of these aspects [1, 22, 38], thus these will not be reviewed here. Briefly, many of the classic growth factors, with those of the epdiermal growth factor (EGF) and hepatocyte growth factor (HGF) families at the forefront, have been shown to promote cell migration. This raised hope that targeting such factors or their receptors might be an avenue for rationally targeting cancers. Some limited clinical advances have been made with growth factor inhibitors (e.g. of the EGF receptor), these have mainly focused on limiting cell proliferation or driving cell death when such receptors are mutated or over-expressed. The reason for the limited successes and recurrent resistance is the redundancy of the receptor pathways and the fact that often these signals occur in epigenetic or contextual situations in which the cancer cell is not intrinsically predisposed to be dependent on any one given signal. Thus, strategies to target common intracellular signals may be more effective.

Chemokines are a second group of soluble signaling proteins which were originally investigated as a secondary class of signals in immuno-regulation [39]; more recently, they have been examined in the context of cancer [40]. This association occurred after increasing appreciation that these ligand-receptor systems functioned in all cells, and were crucial to terminating wound repair [41] and to inducing angiogenesis [42]. Chemokines that signal via CXCR4 [43] can promote cancer cell metastasis by increasing cell motility via both autocrine and paracrine signaling loops [44–46]. One of the chemokine networks that has come to the fore of carcinoma motility investigation is that of the CXCR3 receptor, which physiologically functions as an immune cell chemoattractant and an adherent cell inhibitor of locomotion. This receptor enhances tumor cell motility *in vitro*[47–49] and metastasis *in vivo*[50, 51]. Although this might suggest that these ligands or the cognate CXCR3 receptor may be a target for limiting spread, this is a two-edged sword because this signaling system limits the movement of more differentiated cells. The switch to pro-motogenic signaling occurs via a change in the splice variant from the adherent cell CXCR3B to the immune cell CXCR3A isoform [52]. Thus, even usual ‘stop’ signals may become ‘go’ signals, requiring the targeting of the basic motility mechanisms.

Emerging evidence has pointed to active signaling by intrinsic matrix components through growth factor receptors as major drivers in tumor progression; this is in contrast to the well-described haptokinesis, deriving on physical considerations, driven through classical matrix receptors. The cancer situation resembles a wound state in which the ‘immature’

extracellular matrix presents numerous pro-motility domains while lacking the anti-migratory small leucine rich proteoglycans (SLRP) [53, 54], thus representing a 'wound that won't heal'. Central to these pro-motility matrices are the matrix components tenascin-C and laminin [55], molecules that contain EGF-like repeats that bind at low affinity but high avidity to the EGF receptor to preferentially drive motility [56]. Additionally, the decrease in the SLRP (such as decorin) [57] leads to unbalanced signaling which promotes tumor invasion [13]. Although such molecules might be considered candidates for therapies, the redundancy aspect again argues against such a straightforward strategy.

Although matrix remodeling may occur only to a limited extent in amoeboid migration, proteases can nonetheless still play an important role in tumor cell migration by altering signaling elements. Many pro-motility growth factors either are produced as pro-factors that require proteolytic processing for release (including all EGF receptor ligands) or activation (HGF is an example), or which are sequestered in the matrix requiring mobilization (such as the heparin-binding members of the FGF family or IGF-1 bound to its sequestration molecules). In these cases, the proteases liberate the signals to act in paracrine mode, with asymmetric expression of membrane-tethered and secreted proteinases providing a signaling gradient for directional motility. Additionally, many of these same proteases can provide access to cryptic signaling elements in the matrix, such as tenascin-C. Anti-motility chemokines are also affected by proteases. These soluble peptides are inactivated by proteolysis, thus contributing to a pro-migratory environment[58]. For instance, the CXCR3-activating CXCL11/IP-9 is abrogated by a number of MMPs present during tumor cell invasion [59].

Potential interventional opportunities

Given the central role of induced migration in tumor progression, therapies aimed at limiting this behavior should be an obvious goal. This can be attempted by targeting signals upregulated in specific cancers (such as select MMPs), molecules common to many cancers (such as tenascin-C), or the common nodal points for the cell phenotype. Targeting specific molecules over-expressed in a specific cancer offers the opportunity to tailor the therapy to individual patients and hopefully limit toxicity. The same avoidance of toxicity can justify targeting general tumor progress ion-associated factors, such as tenascin-C. However, both of these approaches are limited mainly by the redundancy of these pro-migratory signals, suggesting that abrogation of one would be of limited or short-lived benefit. Such a rebound phenomenon has been noted for receptor tyrosine kinase inhibitors such as Herceptin and erlotinib [60]; this issue would be especially relevant for maintenance therapies that are needed to limit progression.

A second approach is to target key intracellular signaling nodes for motility, such as those that link transcellular contractility to cell adhesion [23] or forward protrusion [25]. In this manner the redundancy of input signals is not an issue as convergent points are inhibited. Although this might risk a high level of toxicity, the fact that the motility that drives tumor progression is distinct from the routine homeostatic haptotaxis [1] suggests that there would be a therapeutic index as long as there are no ongoing wound healing processes that also use this induced cell migration. The balance of physical considerations and active biochemical signaling governed concomitantly by extracellular matrix and growth factors can be quantitatively analyzed to determine how robust the effectiveness of a signaling pathway inhibitory drug could be [61]. Thus, as it is usual for therapies to await the post-operative healing period, this should not be an issue; however, long-term maintenance therapy must account for these comorbidities.

An alternate approach would be to provide anti-motility signals rather targeting pro-motility pathways. Recent investigations have highlighted physiological 'stop' signals such as CXCR3 and decorin, holding the promise that upregulation of these signals could be triggered in a therapeutic manner to limit invasion. We have recently found that presentation of decorin in the matrix can limit melanoma invasion [14]. The unwanted side effects of such forced expression again would be during times of active wound healing. A word of caution must come to the fore in light of the findings of splice isoform switching by carcinoma cells in that the tumor cells turn this 'stop' signal into a 'go' signal by changing the expressed receptor splice variant [52]. Thus one cannot readily extrapolate the efficacy of these 'stop' signals from non-tumor counterparts, but need to validate these signals in the relevant tumor cells and models.

A general cautionary note also must be sounded when targeting an integrated cellular behavior in a long-term manner. This relates the issue that near complete inhibition of a pathway may achieve the desired effect, but partial inhibition may be counterproductive. For instance, targeting the individual components of motility in a model of prostate cancer invasion and metastasis abrogated dissemination; however, when we challenged these same cells with a gradation of inhibition, the invasiveness was increased at partially diminished signaling [62]. This was predictable because the actuation of locomotion depends on a balance of signaling elements and changes to any aspect short of complete inhibition can be compensated by similar directional changes in other signaling cascades. Compensation such as this can be a major limiting factor during long-term maintenance therapies rather than the usual short-term ablative therapies currently used to kill cancer cells. A second caveat relates to the possibility that tumor cells have disseminated quite early prior to detection and initial treatments [63]. In such a circumstance the opportunity to prevent metastasis may be missed, with many of these disseminated cells potentially entering a long period of quiescent dormancy [7]. However, as metastatic deposits can give rise to subsequent disseminations [64], even secondary prevention may be beneficial.

This leads to the major challenge facing progression-targeted therapies – how to design clinical trials to determine efficacy. There are several current trials examining tumor cell migration but these are mainly in the correlative stage and have not progressed to interventional status (clinicaltrials.gov). In addition to a few growth factor inhibition strategies already in clinical use (such as inhibitors of EGFR family signaling, including Herceptin and erlotinib), inhibitors of intracellular signaling nodes are beginning early stage clinical trials. However, as the target molecules also are involved in signaling proliferartion and/or survival, the key mechanism of efficacy remains to be determined. Further limiting clinical testing is the open question of how to measure success of the intervention. By their nature, agents that target motility will not be useable as single agents for extant tumors and thus must be part of a regimen. Because the standard measurements for efficacy involve tumor nodule size (shrinkage or stable size being measured as response), these agents would require a different trial design. Thus, trials would need to provide for progression-free or overall survival measurements, even at the earliest stages of Phase II trials. This would be costly in terms of time and number of patients to be enrolled. The avenue of using rapidly progressive and invasive disease, such as glioblastoma multiforme is complicated by the concomitant proliferative aspects of the tumor that would be under treatment. The true target of these agents would be for limiting the spread of more indolent tumors, such as dormant breast cancer or the field effect bladder and oral carcinomas. In these cases, trials would only be manageable by allowing for surrogate endpoints such as size of field effect and time to limited localized recurrence, as determined by multiple and frequent biopsying of the bladder or oral cavity. Although these adaptations are possibly intuitive from a medical and biological perspective, the regulatory agencies would need to develop new avenues for such testing. The advantage of these trial designs would be that recruitment would potentially be

easier because these treatments will likely have low toxicity and be an add-on to the standard therapies.

Concluding Remarks

Tumor cell migration as induced by various soluble and matrix signals represents a novel avenue for limiting both invasion and metastasis, thus attacking the most morbid and daunting aspects of cancer. Deciphering the basic mechanisms of cell motility in 2D and 3D have highlighted intracellular cascades critical for this motility, and studies of human cancers have shown the signals in the microenvironment that drive this cellular behavior. Thus, there is no shortage of candidate targets, and importantly, there are at least lead compound inhibitors for many of these. However, rapid movement into the clinic is challenged by numerous issues. Signaling and network redundancy will require careful selection of key nodes, or even upregulation of ‘stop’ signals. Even if this is accomplished, the desired effect of preventing extension while not killing or shrinking the tumor per se, and doing this over years, will require new types of clinical trials. Still, the promise of ‘stopping cancer in its tracks’, literally, impels the quest to develop such treatments that limit tumor migration.

Acknowledgments

We thank the Wells, Lauffenburger, Griffith and Camacho laboratories for helpful discussions. These studies were supported by grants from NIH (NIGMS and NCI) (AW and DAL) and VA (Merit Program) (AW).

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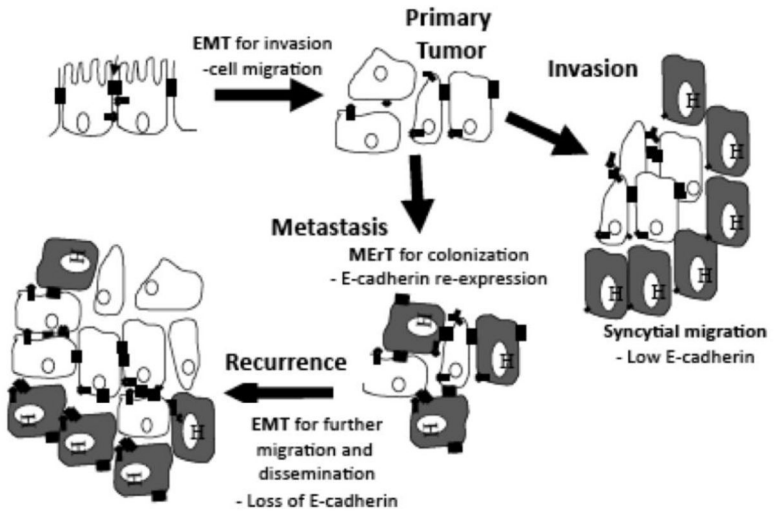

Figure 1.

Schematic of phenotypic changes from a normal, cell-cell connected epithelium to a disseminated carcinoma. The cells downregulate their E-cadherin (solid bars) to allow for motility in a process denoted as ‘epithelial-to-mesenchymal transition’ (EMT) which allows for migration as a syncytial mass for invasion that displaces the normal parenchyma (gray cells) or as singular cells for metastasis. The survival in the distant site likely requires a reversion of the phenotype, a ‘mesenchymal-to-epithelial reverting transtion’ (MErT) to reside among ectopic tissue epithelial cells (gray cells).

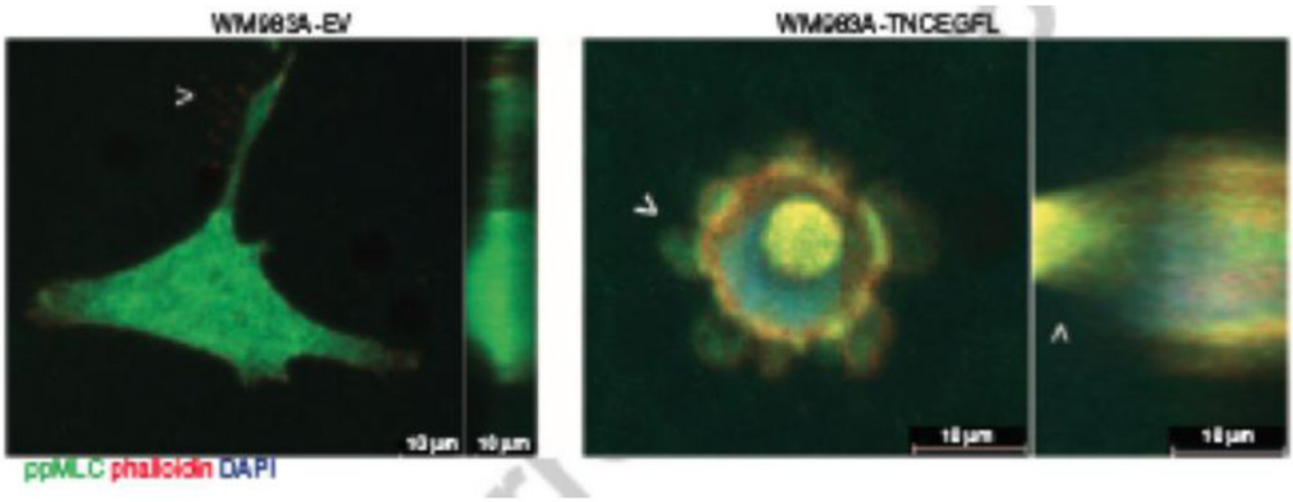

Figure 2.
Invading melanoma cells can passage through tight matrices either in the mesenchymal state requiring extensive matrix remodeling (left) or the amoeboid state in which the cells ‘bleb’ through tight spaces (right). The right panels in each pair are vertical views of cells extending through a pore in the membrane. From [13].

Table 1

Properties that distinguish invasive from metastatic dissemination.

Invasion	Metastasis
Local extension thru tissue and into adnexia	Distant travel through conduits
Contiguous tumor	Distinct tumor
Orthopic microenvironment	Ectopic microenvironment