Occupy tissue: the movement in cancer metastasis

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Abbreviations

2D 2-dimensional

3D 3-dimensional

ECM extracellular matrix

CDM cell-derived matrix

Abstract

The critical role of migration and invasion in cancer metastasis warrants new therapeutic approaches targeting the machinery regulating cell migration and invasion. While 2-dimensional (2D) models have helped identify a range of adhesion molecules, cytoskeletal components and regulators that are potentially important for cell migration, the use of models that better mimic the 3-dimensional (3D) environment has yielded new insights into the physiology of cell movement. For example, studying cells in 3D models has revealed that invading cancer cells may switch between heterogeneous invasion modes and thus evade pharmacological inhibition of invasion. Here we summarize published data in which the role of cell adhesion molecules in 2D versus 3D migration have been directly compared, and discuss mechanisms that regulate migration speed and persistence in 2D and 3D. Finally we discuss limits of 3D culture models to recapitulate the *in vivo* situation.

Introduction

Nearly all adherent cells are able to crawl and migrate under standard 2D cell culture conditions. Time-lapse recordings of migrating cells never fail to excite a sense of awe about the complexity of the migration process, as lamellipodia and filopodia form, extend and make contact with the matrix, as cells change their shape, lunge forward, contract and detach. Cell migration depends on a variety of temporally and spatially orchestrated parameters, with several hundreds of proteins involved. Although changes in cell migration after knock-out or mutation of a protein may not be the ultimate proof of a causal relationship; watching cells migrate and measuring parameters of cell speed and directional persistence have proven invaluable in elucidating the functional components regulating intrinsic cell migration in 2D. A number of studies have now begun to examine these same parameters using 3D culture systems.

Interstitial dissemination

The ability of cancer cells to invade tissue surrounding the primary tumour site, leading to the development of metastatic tumours, is one of the hallmarks of cancer.[1] Disseminating tumour cells encounter a variety of tissue architectures through which they must transmigrate. While the majority of tumour cells are confronted by collagen-rich connective tissue found around most organs in the body, invasion in the brain represents a specialized form of transmigration due to the unique makeup of the brain interstitium. For more detail we direct readers to an excellent, recent review that provides a comprehensive description of tissue structures through which cancer cells transmigrate *in vivo*.[2] The structure and composition of the interstitial tissues determines the mechanisms that must be employed by the cancer cells for successful

navigation. Gritsenko and colleagues[2] suggest three generic mechanisms employed by invasive cancer cells: contact guidance along stiff matrix elements through integrin mediated mechanisms, directed migration towards chemokines and growth factors, and physical pushing through small spaces.

Cell migration *in vivo*

The process of cell migration and invasion is, of course, not restricted to metastatic cancer. Cell migration and invasion occurs during embryonic development and continues throughout adulthood as it is critical for the correct execution of a variety of biological programs. During embryonic development there are precise migration and invasion events that are necessary for the final correct organization of the adult tissue. A particularly well-studied example of this is during the development of the mammalian brain, where cells undergo precisely timed migration events that are essential for the final tissue organization and wiring of the fully developed brain (for review see ref 3). The migration speed and persistence of distinct cell populations appear to be important parameters for determining their final destination in the maturing brain tissue. Moreover, cell populations change speed as they migrate throughout different zones of the brain.[4] Mutations which alter speed and directional persistence of migrating neuronal cell populations can therefore perturb the organization of the brain tissue into discrete layers.[5, 6]

The consequences for changes in speed and persistence of invasive cancer cells are currently less well known. Approaches that abrogate cell movement in environments that mimic the tissue organization of the *in vivo* tumour are generally considered to be strong candidates for novel therapies to treat metastatic cancer. Similarly, approaches

that do not completely abrogate movement but instead slow down the rate of cell migration are expected to restrict the dissemination of cells from the primary tumour and increase the window of opportunity for localized and targeted delivery of therapies. In the particular case of highly invasive primary brain tumours such as the deadly grade IV gliomas (also known as glioblastoma), there is extensive collateral damage to the brain tissue as a result of the invading tumour cells, leading to significant loss of quality of life.[7] Thus, even a partial reduction of tumour invasion may ameliorate some of the collateral damage that occurs in the brains of these patients. Moreover, targeting the cell's migration machinery may in turn sensitize the cells to different therapies. One example of such sensitization is tumour cells lacking expression of the invasion/metastasis promoter NEDD9. These cells appear to concomitantly become sensitized to the effects of Src kinase inhibition.[8] Similarly, decreasing the persistence of the cancer cell movement will necessarily decrease the numbers of successful escapers from the primary tumour site. But to confirm these speculations and expectations, any anti-invasive therapy must first demonstrate its effectiveness in models that mimic the natural *in vivo* 3D organization of the relevant tissue surrounding a particular tumour.

3D culture models

The interactions between cells and the external environment, either with the ECM or with neighbouring cells, are in a literal sense vitally important. Once thought to solely provide physical and structural support, the ECM is now known to regulate and influence survival,[9] proliferation,[9] migration and adhesion,[10, 11] morphology,[12] internal cellular structures,[13] and signalling pathways.[11] During tumorigenesis, cellular interaction with the ECM can become deregulated not only as a result of altered

integrin and focal adhesion protein expression, but also because tumour cells can rearrange the local native tissue architecture, alter its chemical composition, structure and stiffness, which in turn often promotes cell migration and invasion.[14, 15] For example, in breast carcinomas the formation of the distinctive palpable 'lump', known as demosplasia, is attributed to tumour cell secretion of platelet derived growth factor (PDGF). This induces the deposition of large quantities of collagen and collagen cross-linkers by surrounding fibroblasts, thus increasing local tissue stiffness and rigidity.[16, 17]

In vivo, most invading cancer cells encounter two major ECM groups. The first is the basement membrane that directly interacts with both the epithelium and endothelium layers. The second is the interstitial matrix. It harbours numerous collagen isoforms and fibronectin and is thought to contribute to the overall mechanical strength of the tissue. *In vitro*, the extracellular matrix is most commonly planar, tissue-culture treated plastic, or sometimes glass coated with collagen, fibronectin or other ECM-derived proteins. Even then, comparative analysis of cell migration and morphology has revealed striking inconsistencies between cells grown on 2D surfaces versus those seeded within 3D matrices.[13, 18] Therefore, in attempting to recreate a cell's *in vivo* environment, it is not sufficient to only consider which ECM group the cell type in question is most likely to interact with; it is equally important that the 3D structure and mechanical properties of the matrix closely mirror that of the *in vivo* environment. Three of the most commonly used *in vitro* 3D matrix models - fibrous gels, basement membrane extracts (or Matrigel), and cell-derived matrices - are discussed in the following sections.

Fibrous gels

Fibrous gels, namely reconstituted collagen and fibrin gels, aim to replicate the major architectural and structural components of the extracellular matrix. Collagen and fibrin emerged as ideal candidates for single-protein 3D matrix models as both are major structural components of the extracellular matrix. It is worth noting, however, that interstitial tissue is composed of heterogenous mixtures of ECM components, which these gels do not recapitulate.[19] Nevertheless, due to the abundance of collagen and fibrin *in vivo*, reconstituted fibrous gels are physiologically compatible with numerous cells types. Fibrin gels are particularly suitable to study migratory events involved in wound healing,[20, 21] while collagen gels are more suitable to study interstitial cell migration and metastatic invasion.[10, 22, 23]

Not all fibrous gels are created alike however. Seemingly minor protocol variations can have major effects on the gel properties. For instance, an increase in fibre concentration enhances fibre density,[24, 25] resulting in an overall increase in the mechanical strength of the gel.[26] Additionally, the effective pore size of the fibrous meshwork decreases at higher fibre concentrations,[27] thus increasing steric hindrance for migrating cells.[28, 29] The gelation temperature will also affect the mechanical and structural details of the gel; for instance, the pore size and fibril diameter decreases and the number of fibrils formed increases at higher polymerization temperatures.[27, 30]

The production details of harvesting, dissolving and purifying collagen or fibrin from different animal tissues are also a source of substantial variability. This is especially true in the case of collagen, where fibres harvested e.g. from bovine Achilles tendon demonstrate a higher denaturation temperature and a thicker fibre diameter when compared to collagen harvested from rat tail tendon.[31,27, 32] The method for dissolving

the collagen, whether with acid or by proteolytic digestion (i.e. pepsin or trypsin) similarly influences the structure and polymerization kinetics of the reconstituted gels. Acid-dissolved collagen will begin the fibrillogenesis process more rapidly,[31, 33, 34] showing characteristic fibre lengths and diameters that closely resemble those observed *in vivo*.[35] In comparison, collagen extracted by proteolytic enzyme digestion shows significantly smaller fibre diameters and reduced fibril formation, thus reducing the gels' strength and rigidity.[30, 31, 36]

Basement membrane extracts

Matrigel is a reconstituted basement membrane matrix product extracted from Engelbreth-Holm-Swarm (EHS) mouse sarcoma cells. When polymerized, Matrigel mimics the microenvironment of *in vivo* extra-cellular basement membrane.[37-39] Composed chiefly of laminin, collagen IV isoforms and heparan sulphate proteoglycans, basement membranes provide a unique signalling platform for cells that adhere to it. [40, 41] Matrigel 3D substrates can restore the normal morphological characteristics and specific cell functions that would otherwise be lost under standard cell culture conditions, especially in epithelial-,[42, 43] endothelial-,[44] and Schwan-derived cells.[45] But this is not generally true for all cells; for instance fibroblasts that are not normally in contact with a basement membrane *in vivo* adopt a noticeably uncharacteristic rounded morphology and exhibit a non-migratory phenotype when cultured in Matrigel.[12, 39]

Cell-derived matrix

When cultured at high density, fibroblasts "bioengineer" their own extracellular matrix.[46] This cell-derived matrix (CDM) is composed of fibronectin,[47] collagen types I and II,[48] hyaluronic acid[49] and heparin sulphate proteoglycans.[49, 50] CDMs mirror the flexibility and malleability of an *in vivo* ECM, as cells seeded onto a CDM can

reorganise and modulate the matrix, recapitulating the migration and invasion events of both physiological and metastatic processes observed *in vivo*.[11, 12, 51] However, as with all protocols, there are limitations to this method also. Somewhat problematic is the poorly defined and highly variable composition of fibroblastic CDMs. Moreover, although CDMs display a 3D matrix organization, these matrices are typically thin and therefore subject to the rigidity of the underlying 2D surface. Regardless, the use of CDM has permitted insights into the signalling pathways that govern proliferation, morphology, cell-matrix attachments and individual cell migration, all of which impact and promote tumorigenesis.[11, 12, 51]

Cell adhesion

The role of focal adhesions has been thoroughly studied in 2D cultures and has provided important insight into the mechanisms of cell movement in 2D. However, as investigators have turned to the analysis of focal adhesions in 3D, there has been conflicting reports regarding their presence and detection in 3D.[12, 52, 53] We direct readers to the excellent review by Harunaga[54] for a comprehensive analysis of the issue of cell-ECM adhesions in 3D culture models. The upshot of which is that focal adhesions do exist in 3D, but the composition, mechanical properties and structure of the 3D matrix profoundly affects the appearance and distribution of focal adhesions.[12]

In Table 1 we summarize data from studies in which migration parameters of speed and persistence have been directly compared between 2D and 3D culture conditions in the same cell background. Treatments that are expected to promote invasion and metastasis *in vivo* such as stimulation with EGF, complete oncogenic transformation following combined expression of ErbB2 and 14-3-3ζ and high α5β1 expression all promoted faster migration speed in 3D cultures, yet had variable effects in 2D (Table

1). For example, breast epithelial cells induced to undergo oncogenic transformation displayed reduced migration speed in 2D,[55] while EGF treatment of glioblastoma cells increased 2D speed.[56] Similarly, cells from a metastatic progression series showed increasing speeds in 3D collagen gels, but decreasing speeds in 2D.[57] Without exception, the knock-down or loss of cytoplasmic members of focal adhesion sites (p130Cas, NEDD9, vinculin, talin, FAK, VASP, paxillin and Hic-5) inhibited cell migration speed in 3D cultures, yet again varied results are seen in 2D cultures. Depletion of these molecules variously increased 2D speed (p130Cas, NEDD9, vinculin knockout fibroblasts), decreased 2D speed (talin, FAK) or did not alter speed relative to controls (vinculin shRNA in HT1080 fibroblasts, VASP). With the exception of EGF stimulation and high $\alpha5\beta1$ expression, all treatments resulted in reduced intrinsic persistence of migration in 3D. As was seen for the speed data, there is no clear correlation between effects on migration persistence in 2D and effects seen in 3D.

Forces in cell migration

The disparate results for 2D and 3D speed and persistence in exactly the same cell background highlight critical differences between these different model systems. Cell migration in 2D differs in one fundamental aspect from 3D migration in that steric hindrance is absent. In 2D, the cell needs to overcome only the frictional (drag) forces form the surrounding liquid and the 2D surface. At a speed of only a few microns per minute, liquid drag can be neglected, as the force of a single myosin motor would be sufficient to propel the cell forward. Thus, the better a cell adheres to its substrate, the greater the contractile force needed to overcome the adhesive friction from cell-matrix adhesions; as a general rule, a cell with poorer adhesion can migrate faster on a 2D surface. Poorer adhesion can either be a consequence of a lower matrix protein

density on the surface,[58] or a consequence of a reduced expression level of adhesion proteins on the cell (for examples, see Table 1). Of course, a minimum amount of adhesion is essential for cell movement as otherwise the cell cannot polarize or activate its contractile machinery.[58] Thus, in a 2D context, changes in focal adhesion dynamics[59, 60] dominantly effect cell migration speed and likely explain the disparate effects on 2D cell migration seen following depletion of focal adhesion-associated molecules (Table 1).

In 3D, in addition to frictional forces, the cell also has to overcome forces that arise from the steric hindrance of the matrix network, provided that the pores and crevices through which the cell migrates are smaller than the cell itself. Here, the cell has two options. It can either deform itself until it can fit through the pores; or it can deform the network until the pores are large enough for passage. Switching between cell body deforming versus matrix deforming migration strategies is evidenced by changes in cell morphology from rounded cell shapes to elongated cell shapes.[61] Deforming the cell body sufficiently to squeeze through small pores requires forces for overcoming the elastic and frictional forces of the cytoskeleton. The cell can decrease cytoskeletal elasticity (stiffness) and friction by depolymerizing cytoskeletal filaments, but although this would reduce the forces necessary to deform the cell until it fits through a pore, it also reduces the force-generating capacity of the acto-myosin contractile apparatus.

Analogously, deforming the matrix network requires forces for overcoming the elastic and frictional forces of the matrix. By secreting matrix-digesting enzymes, the cell can soften the network structure or increase its porosity, thereby reducing the

necessary forces for deforming the matrix network until the cell can fit through. But at the same time this also limits the ability of the cell to adhere to the matrix, which is a prerequisite for contractile force generation. Therefore, does the speed of intrinsic cell migration in 3D give clues as to whether cells are using the rounded (cell body deforming) versus elongated (matrix deforming) cell migration strategies? Initial descriptions suggested that there is no loss of cell speed between the two modes.[10] However, absolute cell speed depends on the make-up of the surrounding matrix. While partial transformation of breast epithelial cells (Erb2 or 14-3-3ζ overexpression) did not affect cell speed in "compliant" collagen gels (103Pa elastic modulus), cells had reduced migration speed in "stiff" collagen gels (391Pa elastic modulus).[55] Similarly, increasing steric hindrance at high collagen concentrations can inhibit cell invasion.[62] Thus the concentration of the matrix, extent of cross-linking and matrix pore size all critically determine cell speed and moreover, whether a matrix deforming or cell body deforming mode of invasion is possible. Given the ultimate goal of determining the molecular mechanisms that promote cancer cell invasion and metastasis, it will be increasingly important to adopt a range of 3D culture models that can mimic the various different extra-cellular environments encountered by invading cancer cells *in vivo*. Two examples from recent studies with opposing findings highlight the problem in interpreting data that are measured under only a single condition: Several studies have reported that invasive, metastatic cancer cells are softer than their nonmetastatic counterparts derived from the same tissue[63, 64] when measured under non-adherent conditions. By contrast, in adherent cancer cells, the opposite behaviour is seen, with more invasive cells tending to be more contractile and consequently stiffer.[65, 66]

Predictive power of 3D invasion models for cancer metastasis.

The goal of the 3D cell migration models is to better mimic the physiological environment. An important question in cancer research therefore is how successfully data from the 3D models predicts *in vivo* metastatic behaviour. To address this question we have searched the literature for examples where adhesion molecules that have been directly compared in 2D versus 3D models (Table 1) have been analysed using *in vivo* models of metastasis and invasion (Table 2). There is an extensive literature on mouse models of cancer cell invasion, but for our purposes we have limited our analysis to studies where adhesion molecules from Table 1 have been manipulated by overexpression or knockdown. It is noticeable that surprisingly few studies have been carried out to directly test the effects of these molecules on *in vivo* tumour invasion and metastasis – the bulk of studies have focused on tumour initiation. Our search yielded examples for FAK, p130Cas, NEDD9, vinculin, talin, β1 integrin and α5β1 and αvβ3 integrin receptors (Table 2).

The *in vivo* models used in the summarized studies can be divided into three categories: (1) tissue specific neo-oncogenesis that recapitulates carcinoma progression seen in human disease[67-72] and orthotopic injection: both have the advantage of mimicking the correct tissue context of the human disease;[73-77] (2) sub-cutaneous injection at non-orthotopic sites;[78-80] and (3) intravenous injection.[8, 81-85] In the case of the first two categories successful metastasis requires the tumour cells to invade locally, intravasate into blood or lymph vessels, extravasate at secondary sites and form new tumours. Conversely, intravenous injection does not depend on the formation of a primary tumour, thus allowing separation of the effects of primary tumour formation from dissemination into secondary tissues. This approach is

frequently used when the molecule or pathway of interest inhibits initial tumour formation. A limitation is that this does not address initial migration and invasion away from primary tumour sites. A further important caveat to all of these models is that none measure migration and invasion alone. The ability of the tumour cells to disseminate and form secondary tumours is a multifactorial process, which additionally requires survival in the circulation, adhesion to vessel endothelial layers, and transmigration through the endothelium. Below we compare data for 3D migration speed and persistence with *in vivo* metastasis results in response to altered adhesion molecule expression and activities.

FAK, p130Cas, talin and vinculin

To date, a large number of studies have focused on the role of FAK for *in vivo* metastasis. FAK has long been known to be up-regulated in a wide variety of invasive cancer types (reviewed in ref 86). Cancer cells in which FAK is either homozygously deleted or down-regulated via expression of a dominant negative form of FAK universally display reduced metastatic tumour formation (Table 2), matching the findings that FAK depletion reduces 3D cell migration speed (Table 1). FAK depletion inhibited metastatic tumour formation in all three categories of *in vivo* invasion models (see above). Congruent results were also seen between 3D culture migration speed and *in vivo* metastases for p130Cas and talin (Tables 1 and 2). However, the data are not so clear for vinculin. The depletion or absence of vinculin had no effect[52] or led to reduced migration speed[87] in 3D models. In contrast, a high level of exogenous vinculin expression reduced lung tumour formation *in vivo*.[80]

NEDD9

NEDD9 overexpression promotes invasion and metastasis in a range of cancer cell types[88]. In apparent concordance with this, NEDD9 depletion reduces fibroblast migration in 3D collagen gels (Table 1). However, while NEDD9-/- tumour cells in a model of oncogenic transformation of mammary epithelia had a tendency to form fewer metastatic tumours,[67] tail vein injection of these isolated cells revealed aggressive lung tumour formation.[8] Potentially, this may represent a switch to the amoeboid/cell deforming invasion mode that occurs following NEDD9 depletion[22] which may promote lung colonization. By contrast, the dense, rigid matrix that is reported to characteristically surround breast tumours,[89] which is presumably present in the transformed breast epithelia model, may obviate the rounded/cell deforming mode of invasion. Alternatively, increased metastatic lesions following tail vein injection may reflect changes in survival in circulation, adhesion and transmigration of the endothelial cell layer of the vessel.

β1 integrin

The effects of β1 integrin depletion on migration in Matrigel are dependent on the Matrigel concentration; at high concentrations when the gel is less deformable, the loss of β1 impaired the ability of the cells to invade.[90] These results are in agreement with *in vivo* data where β1 depletion also caused reduced metastatic lesions (Table 2). In one of the studies, increased numbers of tumour emboli in the lymphatic vasculature were noted[68]. This suggests that the cells in this model were competent to locally invade and intravasate, but were not able to execute the final stages required for secondary tumour formation.

α5β1 and αvβ3 integrin receptors

The finding that high level α5β1 is associated with enhanced migration speed and persistence of breast cancer cells in 3D collagen gels[62] appears to agree with reports that α5 depletion reduced lung colonization in a tail vein injection model.[83] However, when α5 and β1 are analysed separately, the findings are conflicting. Exogenous α5 expression was shown to significantly reduce lung and extrapulmonary metastases arising from injected colon cancer cells[85]. Upregulation of β3 expression increased bone and lung metastases,[76, 81] yet decreased lung tumour formation in an invasive melanoma model.[91] Fibroblasts lacking β3 integrin expression have significantly faster migration speeds and reduced migration persistence in a 3D CDM assay [59]. It is difficult to estimate to what degree the usage of different cell types has contributed to the disparate findings. For instance, the differential expression of other integrin receptors in different cell types may have influenced the invasion and metastasis behaviour. In particular, it has been suggested that cross-talk between recycling α5β1 and αVβ3 receptors may be an important mechanism of regulating migration persistence.[92] These data highlight the need for direct comparison between 3D culture models and *in vivo* metastasis models for validation.

CONCLUSION

Our comparison of the effects of a handful of studies that have investigated adhesion proteins in 2D versus 3D culture models with *in vivo* measurements of metastasis has revealed a complex picture. For instance, it is notable that without exception, the depletion of the adhesion molecules summarized in Table 1 resulted in decreased migration persistence in 3D models but not always in reduced metastasis formation *in vivo* (Table 2). To accurately predict *in vivo* invasion behaviour, we need 3D model systems are required that faithfully replicate the physical characteristics of the

environments encountered by transmigrating cancer cells. In particular it will be critical to develop model systems mimicking the interstitial tissue organization both at the primary tumour site and at the metastatic niches that characterize different tumours. Such tailored 3D models to map the journey taken by metastasizing cancer cells are still lacking for interactions with the vasculature, host immune responses and other cell-cell interactions, and can generally mimic only short-term (< 1 week) conditions (Yamada & Cukierman Cell 2007). But even with these limitations, 3D models are essential for gaining a basic understanding of cell migration and invasion through tissue and will ultimately lead to novel cancer therapies. Invasion away from the primary tumour site fundamentally underpins metastatic progression, and treatments that target invasion therefore hold the promise of significantly improving survival rates for most cancers.

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Adhesion Molecule	Cell Line	2D		3D	
		Speed	Persistence	Speed	Persistence
p130Cas shRNA[52]	HT1080 fibrosarcoma	↑	↑	3D collagen ↓	↓
NEDD9 knockout[(b)60]	Fibroblast	↑	ND	3D collagen ↓	↓
Vinculin knockout[87]	Fibroblast	↑	↑	3D collagen ↓	ND
Vinculin shRNA[52]	HT1080 fibrosarcoma	=	=	3D collagen No change	↓
β3 integrin knockout[(a)59]	Fibroblast	↑	↓	CDM[#] ↑	↓
EGF stimulation[56]	U87MG glioblastoma	↑	↓	3D collagen[e] ↑	3D collagen ↑
β1 integrin inhibition[52]	DU-145 prostate cancer	Biphasic	ND	Low Matrigel %: ↑ High Matrigel %: ↓	ND
Talin shRNA[52]	HT1080 fibrosarcoma	↓	↑	3D collagen ↓	↓
FAK shRNA[52]	HT1080 fibrosarcoma	↓	↑	3D collagen ↓	↓
VASP shRNA[52]	HT1080 fibrosarcoma	=	=	3D collagen ↓	↓
Transformation progression series:[55] (1) + ErbB2 (2) + 14-3-3ζ (3) +ErbB2+14-3-3ζ	MCF10a non-transformed breast epithelia	(1) ↑ (2) ↓ (3) ↓	(1) ↓ (2) ↓ (3) ↓	"Compliant" 3D collagen[c]: (1) = (2) = (3) ↑ "Stiff" 3D collagen[d]: (1) ↓ (2) ↓ (3) ↑	"Compliant" 3D collagen[c]: (1) = (2) = (3) ↓ "Stiff" 3D collagen[d]: (1) ↓ (2) ↓ (3) ↓
Metastasis progression series:[57] (1) 67NR - tumorigenic (2) 168FARN – micrometastases (3) 4T07 – secondary micrometastases (4) 66c14 –	NmuMg mouse breast epithelial line	↓ (relative to 67NR)	↓ (relative to NmuMG)	Collagen gel: ↑ (relative to 67NR)	Collagen gel: 66c14 ↓

secondary tumours					
Paxillin siRNA[f][94]	MDA-MB-231 breast cancer	ND	ND	CDM[#] ↓	↓
Hic-5 siRNA[94]	MDA-MB-231 breast cancer	ND	ND	CDM[#] ↓	↓
High α5β1[62]	MDA-MB-231 breast cancer	ND	ND	3D collagen ↑	↑

Table 1: Studies reporting cell speed and persistence in 3D cultures compared with 2D migration parameters in the same cell background.

[a]reduced focal adhesion length and increased rates of focal adhesion turnover, [b]increased rates of focal adhesion disassembly, [c]elastic modulus = 103Pa, [d]elastic modulus = 391Pa, [e]effects were compared across a range of collagen concentrations (2, 3 and 4 mg/mL), [f]measured dynamics of GFP-tagged talin adhesions in CDM and showed multiple roles – paxillin depletion caused increased numbers of highly dynamic, peripheral, short-lived adhesions, increased stability of more centrally located adhesions accompanied by reduced rates of adhesion assembly and disassembly. [#]Cell derived matrix, ND not determined, = no change. Red arrows indicate examples in increased speed or persistence in 3D.

Adhesion molecule	*in vivo* Model	Metastasis Phenotype
FAK	Intravenous injection of FAK-/- tumour cells.[82]	Failed to form lung tumours; cells retained in the lung capillary bed were rounded and lacked membrane extensions into the vessel.
	Activated ErbB2 mammary tumour model with epithelial-specific FAK deletion.[69]	Metastatic lung tumours all negative for cells with homozygous deletion of FAK.
	Mammary epithelium-specific deletion of FAK in MMTV-PyVmT Mouse Tumor Model.[70]	Metastatic lung tumours all negative for cells with homozygous deletion of FAK.
	Mammary epithelium-specific deletion of FAK in MMTV-PyVmT Mouse Tumor Model.[71]	Reduced lung tumour metastases.
	Orthotopic injection of pancreatic cancer cells treated with FAK siRNA.[73]	Prevented formation of liver metastases.
p130Cas	Mammary tumour model – injection of cells expressing inducible p130Cas shRNA.[77]	Inhibits lung colonisation.
	Athymic nude mice injected sub-cutaneously with p130Cas-/- fibroblasts transformed with oncogenic Src and expressing p130Cas.[78]	Exogenous p130Cas expression increased formation of metastatic lung tumours after surgical removal of primary tumours; authors comment that "the capacity of the cells to invade through Matrigel was strongly correlated with their capacity to invade and metastasize *in vivo*."
Vinculin	Exogenous vinculin expression in highly metastatic rat adenocarcinoma injected into foot pad.[80]	Highest levels of vinculin expression suppressed formation of lung metastases, low to moderate expressors formed tumours in lymph nodes close to injection site but failed to form lung metastases.
NEDD9	Mammary epithelium-specific deletion of NEDD9 in MMTV-PyVmT Mouse Tumor Model.[67]	Trend to fewer lung metastases.
	Tail vein injection of NEDD9-null primary tumours.[8]	Tumours formed of null-cell lines exhibited increased aggressiveness, with all injected mice generating secondary tumours.

Talin	Tail vein injections of prostate cancer cell lines treated with talin shRNA.[84]	Reduced numbers of metastatic lung lesions.
αvβ3	Orthotopic injection into mammary fat pad with mammary carcinoma cell line expressing exogenous β3.[76]	Drove unique formation of bone metastases; authors show increased haptotactic and chemotactic response to bone-matrix proteins and soluble factors.
	MDA-MB-435 breast cancer cells expressing constitutively active αvβ3 injected into mouse tail vein.[81]	Enhanced lung colonization.
	Intravenous injection of metastatic αvβ3 negative melanoma cells expressing exogenous β3.[91]	Re-expression of β3 in metastatic, β3 negative lines reduced lung colonization.
β1 integrin	Conditional deletion of β1 integrin from mammary epithelia, crossed with MMTV/activated *erbB2*.[72]	Significantly reduced formation of lung metastases.
	Orthotopic injection of pancreatic cancer cells treated with β1 integrin siRNA.[74]	Absence of any metastatic tumours; controls treated with α2 or α3 integrin subunit siRNA displayed metastatic tumours.
	Conditional deletion of β1 integrin from pancreatic β cells crossed with Rip1Tag2 mice.[68]	Loss of β1 expression induced increased tumour cell emboli in the lymphatic vasculature but no metastasis formation; similarly, tail vein injections of β tumour cells lacking β1 integrin expression did not form metastases.
	Ras-myc transformed β1 null ES cells injected sub-cutaneously.[79]	Reduced numbers and size of metastatic foci in the lung.
α5β1 integrin	HT-29 colon cancer cells expressing exogenous α5 integrin injected intravenously.[85]	Significantly reduced lung and extrapulmonary metastases.
	Lewis Lung Carcinoma cells expressing α5 shRNA injected into tail vein.[83]	Fewer lung tumours.

Table 2. Summary of *in vivo* metastasis data, targeting the adhesion molecules described in Table 1.