

Chapter 23

The Role of Drebrin in Cancer Cell Invasion

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Abstract Cancer progression is characterized by the capacity of malignant cells to exploit an innate migratory ability in order to invade adjacent tissues, enter the vasculature and eventually metastasize to secondary organs. It is this spread of cancer cells that is the major cause of death in cancer patients. Understanding the basic biology of how cancer cells generate an invasive phenotype will be crucial to the identification of drug targets with the aim of impeding tumour dissemination. Ten years on from its initial description in neuronal cells, drebrin expression was found in a wide variety of non-neuronal cells that importantly included cancer cell lines. Since then mounting evidence suggests that drebrin may be a key player in the advancement of several diverse cancer types where its expression is frequently upregulated. Cancer cell motility and invasion are crucial elements in the metastatic cascade and involve dramatic changes in cellular morphology that are associated with dynamic remodelling of the cytoskeleton. Interestingly, it now appears that drebrin could deliver this role during cancer development.

Keywords Drebrin • Cancer • EMT • Migration • Invasion • Metastasis

23.1 Introduction

Cancer metastasis is a disease arising from deregulated cellular behaviour whereby malignant cells are able to use their intrinsic motility machinery to escape their tissue of origin, invade other tissues and vasculature and ultimately disseminate to distant sites (Fig. 23.1). Minimally, for malignant cells to metastasize, these cells must first detach from the primary tumour, gain a migratory phenotype and breach the extracellular matrix (ECM) of basement membranes and stroma, which physically divide the tumour from the blood and lymph circulation. From here, the cancer cells must transmigrate across the endothelium to enter the bloodstream or lymph in

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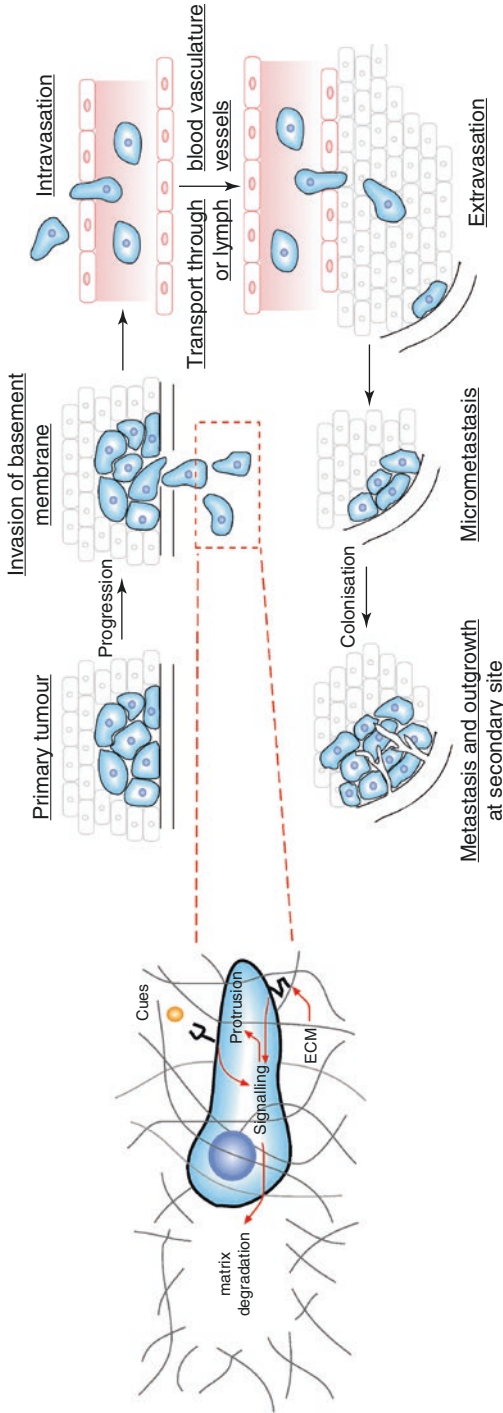


Fig. 23.1 The metastatic cascade. Schematic representation of the multistep process of cancer metastasis, which is characterized by the physical translocation of malignant cells from the primary tumour to a distal organ. Cancer cells within the initial tumour must progressively acquire enhanced motile and invasive traits allowing them to degrade underlying basement membranes, infiltrate the extracellular matrix (ECM) of the surrounding tissue and migrate towards blood and lymphatic vessels. From here, tumour cells intravasate to enter the circulation and be disseminated to secondary sites. Upon reaching microvessels of distant tissues, cancer cells exit the circulation via extravasation and begin to colonize these foreign niches by adaptation to the microenvironment. Active proliferation through recruitment of a blood supply ensures a small cluster of malignant cells can develop from a micrometastasis to a macrometastasis. In order to seed metastases, primary tumour cells must have the capacity to invade ECM, and this crucially requires a change in cell morphology driven by cytoskeletal reorganization. Cues emanating extracellularly trigger a diverse array of intracellular signalling pathways that generate protrusive structures, dynamic cycling of focal adhesions and proteolysis of the matrix for efficient, directed invasion

a process known as intravasation. Only those cells that survive transport through the circulation can undertake transmigration or extravasation through the endothelial cell lining into the stroma of the targeted tissue. Once in this microenvironment, malignant cells can switch to a proliferative phenotype and colonize the secondary niche (Chaffer and Weinberg 2011). Inherent in the progression of cancer from tumour initiation to invasion are dramatic transformations of cell shape primarily resultant from dynamic changes in the cell cytoskeleton (Fig. 23.1).

To facilitate invasion, the cancer cell undergoes a gradual acquisition of traits through an epithelial to mesenchymal transition (EMT). Its diverse cellular responses are tightly controlled by intracellular signalling cascades evoked by extracellular stimuli, such as growth factors, chemokines and cytokines, in the ECM. Indeed it is becoming clear that cancer cells frequently divert extrinsic signalling pathways associated with physiological processes to support tumour cell dissemination (Roussos et al. 2011). In turn, the signalling pathways triggered by receptor activation can generate an array of invasive properties, namely, (1) cytoskeletal remodelling and actomyosin contractility to promote the formation of protrusive structures for polarized migration, (2) initiation of proteolysis by matrix-degrading enzymes to degrade ECM components for efficient invasion, (3) matrix remodelling by secretion of ECM proteins and (4) dynamic turnover of focal adhesions between the integrins of the cell and the ECM components (Fig. 23.1; enlarged schematic of a cancer cell).

As such, cancer pathogenesis can be seen as a multistage process culminating in metastasis, the foremost cause of cancer-related mortality (Chaffer and Weinberg 2011). Identifying the broad spectrum of fundamental motility mechanisms and their associated molecular triggers which ultimately underlie the critical steps of invasion and metastasis will allow us to gain an understanding of both the basic biology and the pathology of cancer. Moreover, this knowledge will be crucial in the discovery of new diagnostic methods and targets for cancer therapy.

For just over 10 years following its original identification, drebrin was generally believed to be a neuronal-specific protein. However by 1999, drebrin E was described as being expressed in a range of non-neuronal cell types and tissues of diverse origin that importantly included several cancer-derived cell lines (Peitsch et al. 1999; Shirao et al. 1994). Since then drebrin has been characterized in non-neuronal cell types from multiple organs and recognized as being integral to the developmental stages of these organs. It is perhaps then not surprising that drebrin is emerging as an important player in cancer cell biology.

23.2 Drebrin Expression in Cancer Types

Drebrin E was initially observed, by immunoblotting, to be present in the human cell lines: metastatic-derived breast carcinoma MCF-7, primary liver carcinoma PLC and primary cervix adenocarcinoma HeLa (Peitsch et al. 1999). Subsequently, drebrin expression has been identified in a range of cancer cell lines encompassing cancer

types derived from both simple epithelia and astrocytes. These include human colon adenocarcinoma Caco-2 and glioma U333 CG/343 (Keon et al. 2000; Peitsch et al. 2001). Early profiling of cancer cell lines revealed that drebrin was undetectable in a cell line derived from an epidermoid carcinoma A-431. This raised the intriguing possibility that drebrin may not be universally expressed in all cancer types with an absence in tumours derived from stratified epithelia (Keon et al. 2000). However, this could have been an oversimplification of the expression profile, as drebrin was later detected in many cancers originating in the skin, a stratified epithelium. These include basal cell carcinoma (BCC), squamous cell carcinoma (SCC) and a subcutaneous metastasis of a melanoma (Mizutani et al. 2014; Peitsch et al. 2005). In normal human skin, drebrin is albeit absent from the epidermis and only heavily enriched in eccrine sweat glands and hair follicles, regions where BCC is thought to derive from. Moreover, it has been noted that the drebrin distribution in SCC is often variable with a mosaic patterning (Peitsch et al. 2005). Taken altogether, these observations suggest that the discrepancy in drebrin expression in cancers of stratified epithelia may be dependent on the specific cancer stem cell of origin. It would be of great interest for future studies to systematically investigate the drebrin expression pattern in an expansive panel of simple versus stratified epithelial cancer cell types.

The number of human cancer types now known to express drebrin is extensive and wide-ranging. To date, drebrin has been detected in gliomas (Terakawa et al. 2013), skin cancers (Mizutani et al. 2014; Peitsch et al. 2005), breast cancer (Yagoub et al. 2014), bladder cancer (Xu et al. 2015), leiomyosarcoma smooth muscle tumours (Peitsch et al. 2005), recurrent non-small cell lung cancer (Mittra et al. 2011), lymphoblastic leukaemia (Vaskova et al. 2011), mantle cell lymphoma (Wang et al. 2010), prostate adenocarcinoma (Dart et al. 2017), colorectal cancer lymph node-positive (Kwon et al. 2004) and liver-positive (Lin et al. 2014) metastasis and pancreatic cancer (Iacobuzio-Donahue et al. 2002). Nonetheless, drebrin expression is unlikely to be limited to these cancer types as it is yet to be fully characterized, and thus, the full extent of drebrin expression in human cancers is undoubtedly underestimated.

Regardless of the cancer type, a unifying theme is the upregulation of drebrin, conceivably indicating that this event may be a common factor in malignancy. Illustrating this is the greater than fivefold change in drebrin expression in pancreatic cancer cell lines and tissues compared to normal pancreas tissue (Iacobuzio-Donahue et al. 2002), and the marked increase in different skin cancers contrasted with the virtual absence in non-tumourigenic skin disorders and normal skin (Peitsch et al. 2005). As reports of drebrin in cancer have accrued, insights into potential mechanisms of aberrant upregulation are also emerging. Recently, drebrin has been identified as a direct transcriptional target of the protein SOX11 (Wang et al. 2010). This is particularly pertinent to tumourigenesis since the transcription factor SOX11 is itself upregulated in several malignancies including ovarian cancer (Brennan et al. 2009), gliomas (Weigle et al. 2005) and mantle cell lymphoma (Wang et al. 2010). Importantly in validation of drebrin being modulated by SOX11 in cancer is the finding that SOX11 expression is highly correlated with that of drebrin in both mantle cell lymphoma primary cells and cell lines (Wang

et al. 2010). Post-transcriptional modifications can also control gene expression, and one such modulation is through the activity of microRNAs. There is growing evidence that microRNAs are abnormally expressed or mutated in various types of human cancer, and in turn these changes can impact on the targets they regulate (Jansson and Lund 2012). Specifically, some microRNAs that act as tumour suppressors undergo epigenetic silencing during carcinogenesis, an example of which is the downregulation of miR-517a in bladder cancer and hepatocellular carcinoma (Liu et al. 2013; Yoshitomi et al. 2011). Significantly, ectopic restoration of miR-517a in bladder cancer cell lines results in a downregulation of drebrin (Yoshitomi et al. 2011) suggesting that for some cancers at least, the dysregulation of miR-517a could be instrumental in inducing drebrin gene expression.

Upregulation of a protein in cancer can often be viewed as a predictor of malignancy. This may also be the case for drebrin as its expression has now been correlated with malignancy in recurrent non-small cell lung cancer and colorectal and bladder cancer tissues and prostate adenocarcinoma tissues (Dart et al. 2017; Lin et al. 2014; Mitra et al. 2011; Terakawa et al. 2013; Xu et al. 2015). A tissue microarray (TMA) of human bladder cancer has shown that drebrin expression is prominently upregulated in a large number of malignant tissues ranging from highly to poorly differentiated bladder cancer in comparison to normal bladder tissue (Xu et al. 2015). Of particular note in this study was the finding that the drebrin staining intensity was substantially elevated in higher-grade (T2, T3 and T4) bladder carcinoma tissues over low-grade (Ta, T1) and normal bladder tissues (Xu et al. 2015). Colorectal cancer exhibits a strong propensity to metastasize to the liver, and drebrin has been identified as a differentially expressed protein between the proteomic profiles of the poorly metastatic human colon adenocarcinoma cell line HCT-116 and its liver metastatic derivative, E1 (Lin et al. 2014). Significantly, these two cell lines are derived from the same genetic background suggesting that drebrin upregulation could be both causal and indicative of a metastatic phenotype. Furthermore, drebrin overexpression has additionally been validated in a clinical context for colorectal cancer as immunohistochemical analysis of patient sections has revealed drebrin is absent from primary adenocarcinoma tumours whilst present in matched lymph node and liver metastases (Lin et al. 2014).

However, a negative relationship between drebrin expression and disease is not always absolute for all cancers. In clinical samples of human glioblastoma, the link between drebrin levels and malignancy is less well defined with only 40.3% of examined specimens displaying drebrin expression and, moreover, no association with patient outcome (Terakawa et al. 2013). As alluded to by the authors of this work, analyses which consider overall protein levels in a randomly selected tissue sample may not accurately reflect the impact of a protein on tumour progression and/or invasiveness, and hence, more detailed examinations of the heterogeneity of protein expression within a tumour may be more informative. Reinforcing this idea is the observation that in drebrin-positive glioblastoma samples, drebrin expression was substantially higher in the periphery of the tumours compared to central regions, perhaps denoting the more invasive nature of these outermost cells (Terakawa et al. 2013). Collectively, these reports advocate the potential of drebrin as a novel bio-

marker and highlight how its expression could carry prognostic information (Iacobuzio-Donahue et al. 2002; Mitra et al. 2011; Mizutani et al. 2014; Vaskova et al. 2011; Xu et al. 2015). In a similar vein, there is accumulating evidence to suggest that the presence of autoantibodies is symptomatic of cancer progression, and hence, their identification in sera from cancer patients could be used as a diagnostic tool. In light of this, it is interesting to note that drebrin has been recognized as an immunogenic tumour-associated autoantigen in prostate cancer patients (Fossa et al. 2000).

Mutations in proteins are often causal in the development of many types of cancer, and therefore perhaps not unexpectedly, such mutations have been identified in drebrin. Specifically, two point mutations have been found in cases of breast cancer: glutamate to lysine at position 278 (E278K) and glutamate to glutamine at position 640 (E640Q) (Sjoblom et al. 2006). These correspond to single nucleotide changes that fall in the helical (Hel) and blue box (BB) domains of drebrin, respectively (Fig. 23.2) (Worth et al. 2013). However, the implications for the functional and structural properties of the protein and how these would ultimately impact on breast cancer progression are presently unknown. If one were to speculate on the nature of the effect of these mutations, it would be important to highlight that the E278K

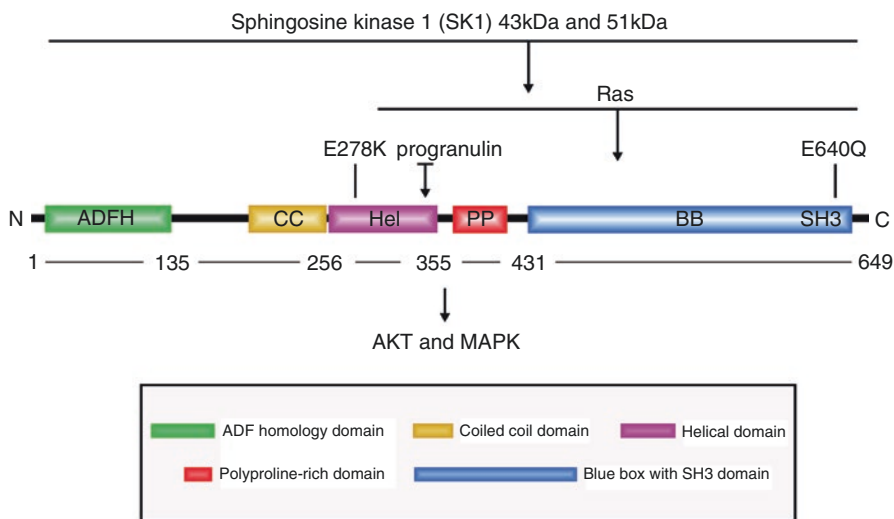


Fig. 23.2 Drebrin signalling in cancer. Schematic representation of the domain structure of drebrin highlighting key drebrin-mediated signalling elements associated with cancer. Two point mutations in drebrin have been identified in breast cancer patients at positions E278K and E640Q. The growth factor progranulin interacts with drebrin in a region encompassing the end of the Hel domain and the start of the PP domain. This interaction triggers downstream activation, by phosphorylation, of Akt and MAPK in bladder cancer cells (Xu et al. 2015). Both isoforms of sphingosine kinase 1 (SK1) have been reported to interact with drebrin in breast cancer cells, although the binding site is yet unknown (Yagoub et al. 2014). Lastly, Ras, the most common oncogene in human cancer, has been shown to drive drebrin-directed signalling in neuronal cells, but an interaction has so far not been demonstrated in cancer cells (Biou et al. 2008). Amino acid numbering is for human drebrin E

mutation lies within a region of drebrin sufficient for cell surface localization and filopodia formation (Xu and Stamnes 2006), whilst the E640Q is positioned in an assumed SH3 domain (Hayashi and Shirao 1999; Lappalainen et al. 1998). At present, it seems likely that such mutations could influence the downstream signalling cascades of drebrin, resulting in changes to cytoskeletal dynamics that culminate in a pro-migratory phenotype. Future studies will need to address if similar mutations can be identified in other cancer types and whether they can potentially confer a gain of function upon drebrin.

23.3 Drebrin Localization in Cancer Cells

Aberrant cell migration is essential for cancer cells to invade and metastasize, and importantly this involves the precise control of changes in cell shape. Typically, the malignant cell will become polarized in the direction of migration by generating protrusive structures in the leading edge cell membrane. Current thinking is that these structures can detect extracellular cue gradients in the surrounding matrix to orchestrate a cellular response whereby the structures in a favourable position can then guide cell movement (Lidke et al. 2005). These protrusive structures can take the form of filopodia, lamellipodia and invadopodia which are all morphologically and structurally different. Nonetheless, these structures have in common a requirement for spatially and temporally regulated dynamic cytoskeletal remodelling that is indicative of an EMT transition. Consistent with a role for drebrin in promoting a motile phenotype, it has been observed accumulating discretely in cellular protrusions or membrane ruffles at the leading edge of lamellipodia and also at the base of filopodia in the human glioma cell line U333 (Peitsch et al. 2001). Notably, drebrin was absent from stress fibres (Peitsch et al. 2001), the contractile actomyosin bundles that are often a prominent cytoskeletal feature of cancer cells on two-dimensional (2D) substrates (Tojkander et al. 2012). The disparity between drebrin localization in filopodia and not in stress fibres is likely explained by the difference in F-actin organization within these structures. In filopodia, F-actin is arranged in parallel bundles, whereas in stress fibres, the F-actin is antiparallel proposing that drebrin can exclusively recognize parallel F-actin bundles (Worth et al. 2013).

More recently, drebrin expression levels have been shown to induce morphological changes in a range of human glioma cell lines (Terakawa et al. 2013). Overexpression of drebrin in the Glioma cell line U87 triggered a spindle/stellate cell shape accompanied by a larger number of cellular projections. In contrast, depletion of drebrin from another glioma cell line A172 (which has significantly high drebrin expression in comparison to other glioma cell lines) produced a more rounded, spread phenotype that had substantially less cellular protrusions concomitant with an increase in stress fibre formation (Terakawa et al. 2013). This result is consistent with current thinking that stress fibres are more conspicuous in stationary cells on 2D surfaces suggesting that they may be inhibitory to cell migration in three-dimensional (3D) environments (Tojkander et al. 2012). Furthermore,

in line with earlier studies, drebrin was also focally localized at the projecting tips and membrane ruffles of lamellipodia where it co-localized with F-actin in these glioma cell lines (Terakawa et al. 2013). Similarly, drebrin is detected in F-actin-enriched spikes at the membrane edge of growth factor-stimulated 5637 bladder cancer cells (Xu et al. 2015).

Besides the leading edge protrusions of motile cells, drebrin has often been observed in a juxta-nuclear cytoplasmic position within non-neuronal cells, including cancer cells, such as glioma U333 cells (Peitsch et al. 2001). Whilst the exact nature of this cytoplasmic pool of drebrin has not been fully characterized, some reports indicate drebrin can be found in complex with actin on the cisternae of Golgi and associated budding vesicles in rat liver fractions (Fucini et al. 2000). Although there is no direct data to point towards this potential drebrin accumulation at Golgi impacting on the malignancy of cells, it is significant to note that a proteomics approach looking at the protein content of breast tumour cell membranes revealed that drebrin was heavily enriched in integral membranes of four breast cancer cell lines MDA-MB-468, T-47D, BT-474 and MCF-7, three of which derive from metastatic sites (Adam et al. 2003).

Another unique distribution pattern of drebrin in non-neuronal cells can be seen in polarized epithelial cancers. In these cancer types, which include human primary liver carcinoma and epithelial skin tumours, drebrin is strongly enriched at cell-cell boundaries and, more specifically, at intercellular adherens junctions (Keon et al. 2000; Peitsch et al. 1999, 2005). In both basal and squamous cell carcinomas, drebrin decorates the submembranous actin cortex of cell-cell borders where it co-localizes with F-actin. Similarly, in the PLC cell line, drebrin intensely labels apical plasma membranes between neighbouring cells but is notably absent from cell free borders (Keon et al. 2000). Furthermore, overexpression of drebrin in a cell line derived from a human epidermoid carcinoma A-431 that ordinarily does not express drebrin produces the corresponding localization of drebrin at intercellular borders, where it partially co-localizes with adherens junction constituents, such as β -catenin and E-cadherin. Drebrin localization did not however overlap with vinculin, a component of focal adhesions. Additionally, these drebrin-GFP-A-431 cells frequently formed small cellular processes that became more pronounced and filopodial-like upon EGF stimulation, features indicative of an EMT transition (Peitsch et al. 2005). In normal epithelium, drebrin may be required to control actin filament attachment to adherens junctions, but in terms of tumourigenesis, the relevance of junctional drebrin is unclear. Yet, it is tempting to speculate that this localization could become misregulated to provide a protrusive force at cell-free borders and perhaps a contractile/retractive force at cell-cell boundaries enabling extrusion of a single cell from its surrounding neighbours within a tumour mass (Slattum and Rosenblatt 2014).

All studies so far of drebrin involvement in cancer have exclusively looked at malignant cells on 2D substrates, and as such, it remains to be seen whether these specific localizations of drebrin translate to invading cancer cells in 3D microenvironments. One such protrusive structure widely observed in *in vitro* 3D ECM mod-

els are F-actin-rich projections known as invadopodia, which contain a high density of proteases capable of degrading the matrix. Although drebrin has not been definitively localized within this structure, it has been shown by mass spectrometry to bind Tks5, a component of invadopodia important in linking actin assembly to matrix proteolysis (Stylli et al. 2009). Furthermore, TIRF microscopy has highlighted that in a metastatic breast cancer cell line, the formation of the initial primary invadopodial process is accompanied by transient filament-like extensions reminiscent of filopodia (Artym et al. 2011). This finding intimates it is plausible drebrin could be functioning in the actin dynamics associated with invadopodia. At present however, this remains to be investigated.

23.4 Drebrin Signalling in Cancer Cells

Delineating the signalling cascades that are subverted to afford cancer cells an invasive advantage is an active field of cancer research that will ultimately provide drug targets. This is also true of drebrin-mediated signalling pathways during cancer progression as many large proteomic and mass spectrometry-based screens are frequently revealing drebrin as an interaction partner. One such example is the novel identification of drebrin as a sphingosine kinase 1-binding protein in MCF-7 epithelial breast cancer cells (Yagoub et al. 2014). Sphingosine kinase 1 (SK1) is a signalling enzyme that catalyzes the formation of sphingosine-1-phosphate (S1P) through the phosphorylation of the lipid sphingosine. The dysregulation of the intricate balance between these two lipid forms is expected to influence the tumourigenic potential of SK1. Furthermore, the tumourigenic effects of SK1 are well recognized in breast cancer, specifically stimulating the growth and oestrogen receptor regulation and responsiveness of breast cancer cells (Sukocheva et al. 2003). Importantly, drebrin has been shown to interact with both major transcriptional isoforms of SK1, SK1 43kDa and SK1 51 kDa, with the 51 kDa variant being more abundant in breast cancer cells. Although the function of drebrin in SK1 signalling is yet to be defined, it seems likely it could be central in the acquisition of cancer cell migratory phenotypes by providing a bridge between the membrane of the cell and the F-actin network. Strengthening this concept is the finding that SK1 has been implicated in EGF-mediated MCF-7 cell motility (Sarkar et al. 2005).

In another signalling pathway that impacts on cancer cell motility and invasiveness of bladder cancer, a secreted growth factor glycoprotein known as progranulin associates with drebrin to regulate F-actin reorganization (Xu et al. 2015). Mass spectrometry mapped the progranulin-interactive drebrin fragment to a sequence that ranges from the end of the Hel region to the beginning of the polyproline-rich region (PP) (Fig. 23.2) (Xu et al. 2015). Under steady-state conditions, drebrin was demonstrated to interact weakly with progranulin in two malignant bladder cell lines, 5637 and T24. The kinetics following progranulin stimulation showed that the drebrin complex with progranulin was lost after 5 min but then markedly increased

after 30 min, implying that the interaction between drebrin and progranulin occurs subsequent to progranulin internalization from the cell membrane (Xu et al. 2015). Progranulin-dependent effects on bladder and prostate cancer cell migration and invasion involve the downstream activation of Akt and MAPK pathways. It now appears that progranulin induces the phosphorylation of targets Akt and ERK1/2 via drebrin since depletion of drebrin from bladder cancer cell lines effectively prevents the activation of these two signalling cascades (Xu et al. 2015).

Although very little evidence has directly placed drebrin into signalling networks that give rise to oncogenic attributes, we may be able to infer drebrin-mediated signalling patterns from studies of drebrin function in neuronal systems. An example of this is the regulation of the drebrin-driven effects on dendritic spine actin cytoskeleton and morphology by Ras (Biou et al. 2008). This modulation requires the C-terminal domain of drebrin, specifically a region encompassing amino acids 233–317 (personal communication RC Malenka), and implies that a direct interaction between drebrin and Ras may occur. Importantly, Ras is a common oncogene in a wide variety of cancer subtypes (Pylayeva-Gupta et al. 2011). Thus, it is entirely possible that oncogenic and hence constitutively activated Ras could be causal in the acquisition of an invasive phenotype by promoting the F-actin-binding activity of drebrin. Further studies will need to determine the relationship between drebrin and Ras, initially determining if there is a direct interaction, but it presently stands as an exciting prospect.

23.5 Drebrin Involvement in Tumourigenesis

For a tumour to develop, it requires a transformation in cellular adhesivity that disrupts the normal tissue architecture and creates a microenvironment conducive to proliferation and angiogenesis to deliver a blood supply for continued tumour growth. Therefore, the ability of cancer cells to proliferate without adhesion to ECM proteins is considered a hallmark of tumourigenicity and can be modelled *in vitro* using anchorage-independent cell growth assays. Interestingly, drebrin depletion in a malignant bladder carcinoma cell line UMUC-3 has been shown to impair the colony-forming capacity of these cells in soft agar (Xu et al. 2015) and is suggestive of drebrin expression being an important determinant of tumourigenic and metastatic potential, at least for bladder cancer.

So far only one study has provided evidence that drebrin could underlie tumour development and aggressiveness *in vivo*. In a mouse xenograft model of bladder cancer, malignant UMUC-3 cells with shRNA-driven reduction of drebrin expression substantially decreased both the occurrence and size of tumours (Xu et al. 2015). However, no definitive relationship between drebrin expression and a proliferative or survival advantage for the cancer cell has been demonstrated *in vitro*, and by itself the mechanistic insight as to how drebrin could drive tumour formation and maintenance *in vivo* is unclear.

23.6 Drebrin Involvement in Cancer Cell Migration and Invasion

The capacity of cancer cells to colonize a metastatic tumour site is predicated on their ability to escape the confines of the primary tumour through acquisition of migratory and invasive programmes. It is now emerging that drebrin could be crucial to both these elements in several cancer types (Terakawa et al. 2013; Xu et al. 2015). Progranulin-mediated migration of malignant bladder cells appears to be regulated by drebrin expression, as suppression of drebrin protein levels in two bladder cancer cell lines, 5637 and T24, produces an inhibition of motility upon progranulin stimulation (Xu et al. 2015). A role for drebrin in glioma cell migration has also been demonstrated using an *in vitro* radial cell migration assay whereby depletion of drebrin attenuated migration rates in several glioma cell lines. Interestingly, these experiments were performed on a range of different ECM proteins (laminin, collagen type IV, vitronectin and fibronectin), all of which displayed a dependency on drebrin expression (Terakawa et al. 2013). This is particularly striking given that diverse modes of cell migration are often determined by the ECM composition, ostensibly as a result of the varying nature of the cell: substratum adhesions formed. Hence, this finding perhaps proposes a unifying role for drebrin in cancer cell motility.

Cell migration is intrinsically linked to invasion, and as such it would be expected that any effect of protein misregulation on the migratory capacity of a cell would be directly correlative with that of its invasive capacity. This appears to be true of drebrin in cancer. With the application of 3D *in vitro* Transwell invasion assays utilizing Matrigel as the matrix constituent, it has been shown that drebrin depletion can decrease invasive rates of U87 and A172 glioma cell lines (Terakawa et al. 2013) and prostate adenocarcinoma cell lines (Dart et al. 2017) as well as progranulin-induced invasion of 5637 bladder cancer cells (Xu et al. 2015). Further evidence demonstrating that the balance of drebrin expression is crucial to the generation of a malignant phenotype comes from overexpression of drebrin in the glioma cell line U87 which results in a greater than ninefold increase in the invasiveness through a Matrigel matrix (Terakawa et al. 2013).

Whilst these studies have provided strong support for a role for drebrin in migration and invasion of several cancer cell types, they do not directly elucidate the drebrin-driven signalling pathway(s) exploited to provide the cancer cell its invasive attributes. One possible hypothesis proposed by Terakawa et al. (2013) is that in some way drebrin is able to modulate the interaction of the cancer cell with the ECM substratum, which principally derives from experiments showing that the glioma cell line U87 exhibits increased adhesion to ECM macromolecules upon drebrin depletion (Terakawa et al. 2013). However, this theory is inconsistent with reports that drebrin displays a strikingly dissimilar localization pattern to that of vinculin, an archetypal protein of focal adhesions. Nonetheless, despite a lack of current evidence, it may be that drebrin could regulate the focal adhesion turnover of migrating cancer cells indirectly through an effect on cytoskeletal dynamics. This

should be fully explored by seeking functional interactions of drebrin with fundamental focal adhesion proteins, such as FAK and paxillin (Xu et al. 2015) (Ikeda et al. 1995, 1996).

23.7 Future Perspectives

Given the presence of drebrin in human cells with a distinctive and specialized need for actin plasticity, such as neurons, parietal cells of the stomach and podocytes of the kidneys (Aoki et al. 2005; Keon et al. 2000; Peitsch et al. 2003), it was perhaps not unexpected to find cancer cells might too be modulating the expression of this actin-binding protein as part of an adaptive strategy to facilitate increased motility and invasion. Evidence is now rapidly accruing to suggest this is the case. Taken together recent data would propose that drebrin becomes upregulated in cancerous cells undergoing EMT and that this misregulation of drebrin expression is associated with a greater invasive potential. However, reports pertaining to a role for drebrin overexpression in cancer progression are still limited, leaving many unanswered questions. Whilst dysfunctional levels of drebrin may be a common consequence of tumourigenesis, we cannot yet say whether it is the primary driver of tumour formation or simply a secondary effect of a sequence of other genetic or epigenetic alterations. An attractive area of research for the future would be to investigate the concept that drebrin expression may be fundamental to the cellular origins of at least some cancer types.

Another path of potential exploration will be to find and characterize binding partners of drebrin as well as signalling cascades activated downstream of drebrin that could help elucidate its potential functionality in cancer. It will also be necessary to determine how drebrin precisely coordinates the cytoskeleton in the varied modes of directed migration and invasion observed during cancer metastasis. Much research of late has focused on the involvement of the actin cytoskeleton and its regulation in cancer cell invasion and metastasis, but it is important to emphasize that the actin cytoskeleton does not work in isolation and its interplay with the microtubule cytoskeleton will ultimately influence the migratory outcome of a cancer cell. This perhaps explains the recent surge in literature highlighting the microtubule system as a crucial factor in cancer cell invasion and metastasis (reviewed in Fife et al. (2014)). In addition, recent reports have underlined the necessity of the mutual regulation of actin and microtubule cytoskeletons indicating that targeting this crosstalk could be an attractive approach for developing novel combinatorial therapeutics (reviewed in Fife et al. (2014)). Therefore, is the ability of drebrin to bind actin and the microtubule +TIP-binding protein EB3, thereby coupling actin filaments to microtubules (Geraldo et al. 2008), necessary for cancer cell invasion and metastasis? Furthermore, the key to metastasis is directional, polarized motility which is acquired in response to extracellular cues. Drebrin has already been shown to be involved in regulating progranulin-dependent signalling in bladder cancer, but importantly, we need to identify if other extracellular chemical gradients mobilize drebrin to influence cytoskeletal dynamics.

The capacity of cancer cells to disseminate is intimately linked to the microenvironment in which they find themselves, and moreover, the interaction of cancer cells with stromal cells and other constituents surrounding the tumour is important for disease development. Taking this into consideration, drebrin expression has also been detected in fibroblasts and endothelial cells (Keon et al. 2000; Peitsch et al. 1999; Xu et al. 2015) indicating it could also facilitate invasion indirectly via other components of the tumour microenvironment. This aspect of drebrin functionality influencing cancer progression certainly warrants further study.

Collectively the current research presents drebrin as not only a necessary but more importantly a feasible drug target. It will be crucial in the future to determine the exact mechanism of action of drebrin in order to generate appropriate target selectivity. As it stands, efforts directed towards the actin-binding capacity of drebrin could prove to be an effective therapeutic.

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