Contributions of integrin-linked kinase to breast cancer metastasis and tumourigenesis

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Abstract

Metastasis contributes to more than 90% of mortality in breast cancer. Critical stages in the development of aggressive breast cancer include growth of the primary tumours, and their abilities to spread to distant organs, colonize and establish an independent blood supply. The integrin family of cell adhesion receptors is essential to breast cancer progression. Furthermore, integrin-linked kinase can 'convert' localized breast cancer cells into invasive and metastatic cells. Upon stimulation by growth factors and chemokine ligands, integrin-linked kinase mediates the phosphorylation of Akt Ser^{473}, and glycogen synthase kinase-3. The current notion is that overexpression of integrin-linked kinase resulted in an invasive, metastatic phenotype in several cancer model systems *in vivo* and *in vitro*, thus, implicating a role for integrin-linked kinase in oncogenic transformation, angiogenesis and metastasis. Here, we will review the role of integrin-linked kinase in breast cancer metastasis. Elucidation of signalling events important for breast tumour metastasis should provide insights into successful breast cancer therapies.

Keywords: breast cancer ● metastasis ● integrins ● integrin-linked kinase ● PKB/Akt ● PI3K ● growth factors ● GSK-3 ● loss of heterozygocity

Introduction

Despite its clinical relevance, metastasis is the most poorly understood aspect of carcinogenesis. The potential of malignant cells to spread to distant organs is the leading cause of death from breast cancer. Some breast cancer metastases display tissue-specific patterns to distant organs, such as the brain [1, 2] and bone [3–5]. Although complex, current studies recognize epithelial-to-mesenchymal transitions, cell-to-cell and cell–matrix interactions, activation of specific chemokines/cytokines and proteases, and contributions from signal transduction pathways to the metastastic process [6]. Contrary to normal breast cancer cells, malignant cells must display enhanced migratory behaviour, the ability to breach blood vessel walls and the dense collagenous matrix surrounding tumours. Additionally, metastatic cells must overcome the dynamics of a foreign microenvironment, to colonize and survive at a distant target site.

Once metastasis has occurred, tumour growth is highly dependent on the ability of tumours to induce their own vascularization [7]. Angiogenesis, which is defined as the formation of new blood vessels from the pre-existing vasculature, is regulated by multiple stimulatory and inhibitory factors that are able to modulate the migration and/or proliferation of microvascular cells [8]. Angiogenesis is a normal process in growth and development, as

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well as in wound healing. However, excessive or insufficient blood vessel formation results in critical diseases [8]. Vasculature remodelling is regulated by signals derived from receptors for growth factors and chemokines, as well as extracellular matrix (ECM) molecules [9]. There are key events to which malignant cells must adhere to complete angiogenesis: invasion of the surrounding stromal tissue, intravasation and evasion of programmed cell death, arrests with the vasculature at a distant site, extravasation, as well as establishment and growth within a new microenvironment [6].

This review will discuss the contributions of integrin-mediated signalling, namely the integrin-linked kinase (ILK), to the induction and progression of metastasis in breast cancer.

Role of integrins in tumourigenesis

Integrins and their downstream signalling targets, which regulate tissue integrity and function, are essential in breast cancer cell migration (reviewed in Ref. [10]). The family of 24, heterodimeric adhesion receptors functions as cell-surface glycoproteins, which allows cells to interact with each other and the extracellular environment [11]. Each integrin heterodimer exist as complexes of non-covalently linked α and β subunits, often overlapping with specificity for ECM proteins, such as collagen, fibronectin and laminin (Fig. 4). Thus within breast tissue, this interaction with the basement membrane is required for the structural and functional integrity of the epithelial component of the mammary gland, including proliferation, differentiation and survival of each individual mammary epithelial cell [10, 12–16].

Several studies have implicated integrins in the complex interactions required for tumour cells to expand into normal tissue surroundings, and form the functional vasculature necessary for tumour oxygenation and growth [17–19]. During wound healing, for example, several members of the integrin family of adhesion receptors are expressed on the surface of cultured smooth muscle and endothelial cells [20–23]. Gene ablation for specific integrins, or blockade of their functions, can exert profound effects on the angiogenic response of endothelial cells, supporting that integrins are directly involved, or regulate angiogenic processes [24]. Evidence of their involvement first appeared from studies that introduced small peptides and antibodies against αvβ3 integrin into the chick chorioallantoic membrane (CAM). Brooks *et al.* demonstrated that these antagonists lead to the rapid regression of tumours transplanted onto the CAM, and apoptotic induction of proliferative angiogenic vascular cells [25, 26]. Similar studies soon followed that targeted closely related integrins αvβ5 and αvβ3, and demonstrated effective angiogenesis inhibition [27, 28]. Conversely, studies consistently observed that mice lacking β3- or both β3/β5 integrins increased primary tumour growth and tumour angiogenesis [29–31], suggesting that neither integrin are essential for neovascularization. However, interestingly, human patients suffering from the disease Glanzmann thrombasthenia, many of whom lack a functional β3 subunit, do not show significant defects in vascular development or angiogenesis [32].

Integrins also promote breaching of the ECM and endothelial barrier to pass through to the surrounding blood and lymph vessels for transport [33]. Lung metastasis from an experimental xenograft model of human breast cancer was impaired following administration of an inhibitory anti-β1 integrin antibody [34]. Similarly, a peptide designed against α5β1 and αvβ3 receptors was found to impair the growth and metastasis of invasive human breast cancer cells in a separate xenograft study [10, 35]. Matrix degradation involves membrane-bound or secreted proteases, such as those of the matrix metalloproteinase family (MMP), which is dependent on integrin expression in invasive breast cancer cells [36, 37].

Role of integrins in signalling

Integrin-mediated signalling is significant to normal cellular behaviour, and beneficial to tumourigenesis. Signalling is conveyed in two forms: (*i*) 'inside-out'—the regulation of the affinity and conformation of the receptor from inside the cell; and (*ii*) 'outside-in'—the triggering of intracellular events by ligand occupation of the receptors [11]. Integrin engagement with their ligands transduces various signals through calcium influx or activation of down-stream kinases, such as focal adhesion kinase (FAK), mitogen-activated protein kinase (MAPK) and protein kinase C (PKC), to recruit protein scaffolds to the cell membrane. Moreover, kinase-lacking integrins interact with other kinases and adaptor molecules that have signalling capacities, such as ILK, FAK, talin, paxillin, parvins, p130Cas, Src-family kinases and Rho-GTPases [12]. Integrin expression and ligand binding can be specific to the tissue type. For example, α2β1 is specific for collagen on platelet cells, but can also bind laminin on other cell types [11]. Furthermore, the specificity and affinity of an integrin receptor may not be constant with the same receptor on the same cell [11].

Research substantially documents the contributions of α6β1 and α6β4 to breast cancer cell survival, especially in response to cellular stress [38]. Three proteins that have emerged as important regulators of integrin-mediated signalling in breast metastasis are the ILK, and associated adaptor proteins PINCH and parvin [39–41]. These molecules form a heterotrimeric complex that functions as a signalling platform for integrins by communicating with the actin cytoskeleton and many diverse signalling pathways (Table 1). Further, ILK has been reported to com-immunoprecipitate with β1, and is capable of phosphorylating its cytoplasmic domain *in vitro* [42–44].

Integrin-linked kinase (ILK)

The omnipresent protein, ILK, is a serine/threonine kinase first identified from a yeast 2-hybrid system through its association with the β1 subunit of integrins [42]. Structurally, ILK is composed of three functionally significant domains. The C-terminus

Table 1 Major ILK-binding

Binding protein	ILK binding site	Target on ILK
Transmembrane receptors		
β1 integrins	β1 cytoplasmic domain	Kinase domain
β3 integrins	N/A	N/A
Adaptor proteins		
PINCH	LIM1	ANK domain
CH-ILKBP	CH2	Kinase domain
Affixin	CH2	Kinase domain
Paxillin	LD1	Kinase domain
Catalytic proteins		
ILKAP	N/A	ANK-PH domains
PKB/Akt	N/A	Kinase domains (?)
GSK-3	N/A	Kinase domains (?)
PDK-1	N/A	Not determined
PIP3	PH domain	Not determined

Major ILK-binding proteins were described by Wu *et al.* [122]. CH, calponin homology; CH-ILKBP, calponin homology (CH) domain-containing ILK-binding protein; PDK-1, 3-phosphoinositide-dependent protein kinase 1; PIP3, phosphatidylinositol-3,4,5-trisphosphate; PH, pleckstrin homology domain.

Table 2 Molecules associated with ILK signalling

Action	Molecule
Stimulates ILK activity	Integrins
	PI3K (?)
Activated by ILK	Affixin
	MLC
	ERK
	Akt/PKB (?)
Inhibits ILK	PTEN
	ILKAP
Inhibited by ILK	GSK-3 (?)
	mTOR (?)
Growth factors/Cytokines	ERα
	Osteopontin
	VEGF(?)
	HER2/ErbB2(?)
Tumour suppressors	DOC-2/hDab-2
	MDA-7
Other molecules affected by ILK	CREB
	Cyclin D1
	β-catenin
	AP-1
	MMPs

AKT, protein kinase B (PKB); AP-1, activator protein-1; CREB, cAMP-response-element-binding protein; DOC-2/hDab-2, differentially-expressed in ovarian carcinoma-2/human disabled-2; ErbB2, epidermal growth factor receptor 2; ECM, extracellular matrix; ERα, estrogen receptor α; ERK, extracellular signal-regulated kinases; GSK3β, glycogen-synthase kinase-3β; ILKAP, integrin-linked kinase-associated serine/threonine phosphatase 2C; MDA-7, melanoma differentiation associated gene; MLC, myosin light chain; MMP, matrix metalloprotease; mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositol-3-kinase; PTEN, phosphatase and tensin homolog VEGF, vascular endothelial growth factor. (?)-Denotes that action or pathway is undetermined.

harbours a protein kinase domain that serves as a binding site for the β- integrin subunits. Four ankyrin (ANK) repeat domains exist at the N-terminus, which mediate protein–protein interactions (Table 2) (as reviewed in Ref. [45]). Additionally, the N-terminus regulates the localization of ILK within focal adhesion plaques [46]. Although the identity of a physiological ligand has not been resolved, reports have demonstrated that phosphatidylinositol-3,4,5-trisphosphate binds to a pleckstrin homology (PH) domain, between the C- and N- termini in ILK [47, 48]. Regulators of ILK activity include a PI3K phosphatase, phosphatase and tensin homolog (PTEN) [48–50] and the protein phosphatase 2C, ILKAP [51, 52].

ILK lacks key catalytic domains that are significant to serine/threonine kinases [50, 51]. An alignment of ILK sequences among species, compared to protein kinase B-Raf, showed that ILK lacks both the HRDLXXN domain (catalytic aspartate residue), and the DFG domain (involved in magnesium ion chelation). However, a phe-ser-phe (FSF) motif is present in ILK, within the sequence corresponding to the activation loop [52, 53]. The charged FSF motif is similar to the consensus sequence defined for phosphorylation by PDK-2 (phe-X-X-phe-ser-phe(tyr) [52, 53], which lead many to believe that ILK is the Akt Ser[473] kinase. Delcommenne *et al.* suggested that ILK is directly involved in apoptosis and cell survival through its phosphorylation of Akt, and glycogen synthase kinase-3

(GSK-3), a negative regulator of the Wnt signalling pathway [48]. In normal mammary epithelial cells, stable overexpression of kinase-active ILK directly inhibited GSK-3 activity *in vitro*. Moreover, expression of a kinase-deficient ILK resulted in inhibition of phosphorylation on Ser[473] in Akt. However, ILK failed to directly phosphorylate Ser[9] on GSK-3, which results in its inhibition [48].

Lynch *et al.* suggested that ILK cannot be the immediate kinase for Ser[473] on Akt, since point-mutations to charged residues

restored activity in an ILK mutant [54, 55]. In adipocytes, Hresko and Mueckler observed a 25% increase in insulin-activated Akt Ser[473], despite the presence of an ILK siRNA [56]. Several studies strongly support that the Rictor-mammalian target of rapamycin (mTOR) or DNA-dependent protein kinase (DNA-PK) better meet the requirements as the direct kinase of Ser[473] in Akt, than ILK [57–60]. Instead, ILK may function as a scaffolding protein, allowing catalytic proteins to interact. Regardless of the mystery surrounding its kinase activity, ILK overexpression is a hallmark of several solid tumours [61]. Deregulation of ILK signalling is reported in anchorage-independent growth and cell survival, oncogenic transformation, increased tumourigenicity and increased invasive potential [62, 63]. In spite of this, the catalytic mechanism of ILK remains unclear [64].

ILK and binding complexes

The protein–protein interactions (Table 1) of ILK couple integrins, growth factors and their receptors, and the actin cytoskeleton to the ECM; which maintains matrix integrity. This association is crucial to cellular development and invasion since the actin cytoskeleton is continuously organized through signalling events [63]. The collective ECM provides a structural framework for the formation of tissues and organs. Components of the ECM bind to substrate-adhesion molecules on the surface, which influences various intracellular signalling pathways that regulate survival, proliferation, polarity and differentiation. Among the ECM components, β1 integrins contribute to a large number of integrin heterodimers and are widely expressed. Deletion of β1 integrin in different organisms has been associated with defects in adhesion, proliferation, survival and polarity [65], which indicates that associated binding complexes have key roles in the regulation of cellular behaviour.

Adaptor proteins PINCH (particularly interesting Cys-His-rich protein) and parvin have emerged as important regulators of integrin-mediated signalling. An ILK-PINCH1 interaction was identified in 1994, and PINCH2/LIMS2 was later characterized. PINCH proteins, which contain five ILK-binding LIM domains and tandem nuclear localization sequences [66–68], are expressed abundantly in the stroma of breast carcinomas, compared to normal breast cells [69]. PINCH1 plays a role in mediating epithelial-mesenchymal transition (EMT), as TGFβ-1 induced PINCH1 mRNA and protein expression, while suppressing epithelial markers (E-cadherin and Zo-1) and increasing fibronectin expression an extracellular assembly [70]. Parvin family members, parvin α-, β- and γ-, bind to ILK through one of two calponin homology (CH) domains, and are widely expressed in human mammalian tissues [71]. Binding by parvin α is partially dependent on PIP3, and phosphorylation by cyclin-dependent kinase (CDC2), and MAPK [72]. The biological significance of parvin β is unclear [73], however, Mongroo *et al.* demonstrated that parvin β expression was significantly down-regulated in a number of breast tumours, which correlated with the up-regulation of ILK signalling. In breast cancer cell lines with suboptimal expression of parvin β, its transfection demonstrated

significant suppression of colony formation, increased cell adhesion to collagen and suppressed epidermal growth factor (EGF)-stimulated invasiveness through Matrigel; possibly through inhibition of ILK-mediated phosphorylation [74]. Inverse relationship between parvin β expression and ILK signalling suggests that parvin β suppresses oncogenic ILK signalling. Parvin γ is reported to be more restricted in tissue expression, but possibly interacts with ILK. Parvin γ forms complexes with cytoskeletal proteins, such as αPIX, α-actinin and paxillin. Interestingly, the ILK-parvin γ complex is critically involved in the initial integrin signalling for leucocyte migration [75].

ILK in breast cancer

A role for ILK in breast cancer is controversial. Early reports suggested that ILK overexpression resulted in apoptosis in mammary epithelial cells through Akt activation [76], and reduced the adhesive properties of epithelial cells when plated on integrin ligands [77]. Overexpressed ILK in epithelial cells also disrupted cell–cell contacts, which resulted in anchorage-independent growth and survival [77]. Similarly, overexpression of ILK in the mammary epithelia resulted in mammary gland hyperplasias, which correlated with elevated levels of Akt, GSK-3β and MAPK phosphorylation [78].

Chen *et al.* presented divergent data for the role of ILK in malignant growth and invasion [79]. In their system, ILK expression was lost or down-regulated, and suppressed the growth and invasive properties of tumour *versus* normal breast epithelial cells. One discrepancy, however, was that experiments relied heavily on mRNA expression, which is not indicative of protein expression or kinase activity. Further reports demonstrated that ILK contributed to the survival of breast cancer cells, but not normal mammary epithelial cells, through Akt activation. Troussard *et al.* suggested an 'oncogenic addiction' model: a preferential dependence of breast cancer cells on ILK for Akt activation and cell survival; given that inhibition of ILK resulted in inhibition of Akt Ser[473] and stimulated apoptosis [80]. This report was unique for mammary carcinomas, but other systems reported similar models, such as glioblastomas, pancreatic adenocarcinomas and thyroid cancer [81–83].

Regulation of ILK activity in breast cancer: growth factors, cytokines and tumour suppressors

Cancer progression induced by ILK is a result of the induction of its downstream targets. Cross-talk between growth factor pathways and ILK has been identified in its regulation in breast cancer, which appears to be crucial to the eventual progression and invasion of cancer cells. In MCF-7 breast caner cells, ILK overexpression elevated cyclin D1 induction and expression, which involved

PI3K and Akt, and resulted in CREB (cAMP response element-binding) transactivation through binding at the CRE (cAMP response elements) promoter on cyclin D1. Wnt-1 overexpression also increased ILK kinase activity and cyclin D1 *in vivo* in mammary tissue [84].

ILK has been shown to play a 'survival' role in the progression and invasion of estrogen receptor positive (ER$^+$) breast cancers, compared to normal human breast epithelia [80]. A relationship between estrogen receptors and ILK is likely, since ER$^+$ and ILK regulate overlapping physiological processes and share common interacting proteins (hs90 and caveolin-1). Estrogen (E2) stimulated morphological changes in ER$^+$ ductal carcinoma cells through the formation of pseudopodia and filopodia, as well as increased cellular focal points and significant wound closure and migration, which required estrogen-mediated activation of ILK and PI3K-derived phosphoinositides. Hence, down-regulation of ILK, and pharmacological inhibition of ILK and related kinases, prevented estrogen-dependent migration and wound closure, Akt phosphorylation on Ser^{473} and phenotypic appearances of pseudopodia and focal contacts, and reduced migration [85, 86]. Likewise, the growth factor angiopoietin-2 (Ang-2) promoted breast tumour metastasis through an ILK-mediated pathway, independent of its known receptor, Tie-2. A small-interfering RNA (siRNA) against ILK attenuated the Ang-2-stimulated phosphorylation of Akt. Moreover, inhibition of β1 and β5 integrins and ILK abrogated cell migration and invasion [87].

Cytokines are involved in ILK-mediated mammary metastasis. Osteopontin (OPN), which functions as a cytokine through αvβ3-integrin and CD44, and as a cell attachment protein. Correlating expression of OPN was demonstrated with ILK in a metastatic mouse mammary tumour cell line, but not in a tumourigenic, non-metastatic cell line. Expression also correlated with up-regulated MMP-2, urokinase-type plasminogen activator (uPA) expression and AP-1 activation. OPN and ILK activities also contributed to mouse mammary tumour cell adhesion [88].

Tumour suppressors have been deemed a role in the ILK-mediated regulation of metastasis. The tumour suppressor PTEN, a phosphodiesterase through 3′ phosphatase activity on PIP3, is established as a regulator of ILK [89]. ILK was constitutively activated in prostate cancer cells lacking PTEN expression [47]. Similarly, lung cancer cells expressing the tumour suppressor and cytokine MDA-7 exhibited increased expression of PTEN, but suppressed the functioning of proto-oncogenes, such as ILK [90]. However, strong relationships between PTEN/ILK and MDA-7/ILK have not been fully established in breast cancer. A relationship between a tumour suppressor and ILK has been recognized with DOC-2/hDab-2 (differentially expressed in ovarian carcinoma-2/human disabled-2), a gene whose expression correlates with the presence of a basement membrane in ovarian and breast tumours. In breast cancer cells, overexpression of DOC-2/hDab-2 resulted in a significant down-regulation of ILK activity, which closely correlated with the induction of anoikis [91]. Given the importance of the loss of tumour suppressor function in breast cancers [91], elucidation of these relationships with ILK would be of great interest.

ILK: tumour suppressor?

The genetic alterations that initiate breast cancer, especially sporadic cases, are vast and rarely identical. While genetic mutations are hallmark in familial breast cancers, loss of heterozygocity (LOH), somatic mutations and decreased protein expression (for an infinite number of reasons) are frequently observed in sporadic breast cancers-leading to a loss of control of cellular proliferation, differentiation, apoptosis and genetic integrity [92, 93]. In contrast to the work that identifies ILK in enhancing metastasis, studies have surfaced that implies a role for ILK in tumour suppression.

Breast cancer metastasis has been linked to LOH on chromosomes, such as 3p21, 15q14, 16p22, 11p15. Hannigan *et al.* mapped ILK to the human chromosome 11p15.5-15.4; a chromosome frequently subjected to LOH in breast, and other childhood and adult cancers [94–100]. Karnik *et al.* identified two distinct regions on chromosome 11p15.5 that are subject to LOH during breast tumour progression and metastasis [94], corroborating earlier reports.

Chen *et al.* described that ILK may not be essential to the progression of breast cancer metastasis, but contributes to the suppression of breast carcinoma cell growth and invasion [101]. In breast cancer cell lines previously identified to contain LOH at 11p15.5, there was a complete loss or significant down-regulation of ILK, whereas the expression was inverse in normal and non-malignant breast epithelial cells. When an ILK cDNA was re-introduced into MDA-MB-435 breast cancer cells, cells grew at a low saturation density *in vivo*, compared to untransfected cells. Additionally, ILK-mediated growth suppression through cell cycle arrest at G1 [101]. From these data, one may suggest ILK a new function as a tumour suppressor, which strongly conflicts with previous reports. What's more, ILK overexpression induced cellular senescence, characterized by larger cell shapes, lower proliferation capacity and loss of β-galactosidase activity in rat primary cardiac fibroblasts [102]. This data is an interesting contrast to the well-published role for ILK, and definitely adds to the body of breast carcinoma knowledge especially to sporadic cases where genetic mutations are rare and erratic gene inactivity is prevalent.

VEGF- and HER2-associated cross-talks with ILK

VEGF and HER2 are two key mediators critical to the pathogenesis of breast cancer, and demonstrate relationships with ILK kinase activity in other cell lines [103]. Vascular endothelial growth factor (VEGF) is involved in vasculogenesis, angiogenesis and cell survival. In breast cancer cells, it was observed that transfection with antisense VEGF cDNA, or with siRNA-VEGF, increased apoptosis as compared to control cells. VEGF receptor-1 (VEGFR1) expression was abundant, and a specifically targeted siVEGFR1 significantly decreased the survival of breast cancer

cells through down-regulation of Akt phosphorylation [104]. Tan *et al.* observed, in various cell lines, that overexpression of ILK resulted in the expression of VEGF in a HIF1α and Akt-dependent manner. The effects were reversed by pharmacological inhibitors or siRNA against ILK, which resulted in decreased VEGF expression. Importantly, ILK was required for the stimulation of VEGF-mediated endothelial cell migration, which was retracted when ILK-siRNA was introduced; thus confirming a role for ILK in angiogenesis [105].

Correspondingly, HER2 also demonstrated a potential relationship with ILK. A member of the epidermal growth factor receptor family (ErbB), ErbB-2/HER2 is infamous for its role in the pathogenesis of breast cancer, and as a target of treatment. In mouse embryonic fibroblasts overexpressing HER2, ILK was required for HER2/neu-induced survival signalling, which conferred resistance to TNFα and anoikis. ILK kinase activity was significantly up-regulated and required PI3K. Conversely, the activity was absent in the presence of PI3K inhibitor, wortmannin. Moreover, kinase-deficient clones of ILK were more resistant to TNFα-induced apoptosis and anoikis in mouse fibroblast cells overexpressing HER2 [106, 107]. Further, HER2-overexpressing breast cancer cells treated with the ILK antagonist, QLT-0267, inhibited the phosphorylation of Akt Ser^{473} [80]. Considering that VEGF and HER2 are imperative to breast cancer angiogenesis, metastasis, progression and survival, it would be interesting to explore these growth factor relationships with ILK in human breast cancer cells.

Conclusions

Molecular mechanisms of ILK in other tumours

Consistent with reports in breast cancer, increased ILK expression is noted in other malignant tumours [108–110]. In gastric carcinomas, increased expression of ILK was detected in over 60% of samples studied; as compared to non-neoplastic gastric carcinomas did not overexpress the kinase [108]. Similar results were observed in laryngeal squamous carcinomas [112]. Likewise, tumour samples from non-small cell lung cancer (NSCLC) cells detected ILK at 31% *versus* no detection in non-cancerous pulmonary tissue samples, and minimal detection in healthy cells [112]. These studies, and others of prostate, colon and ovarian carcinomas, have significantly correlated ILK expression with depth of invasion, nodal metastasis and amount of stromal tissue [114–118]. Overall prognosis of patients with strong ILK expression was reported to be significantly poorer than that of patients with weak or no expression of ILK [112, 113]. Furthermore, strong expression of ILK was associated with an increased recurrence, which suggests that patients with strong ILK expression may be prone to metastasis, or may already have occult systemic disease [113]. The observation that there was no difference in the expression of ILK between primary and metastatic colorectal tumours may suggest that the ILK overexpression has an early role in disease development, but does not contribute to the acquisition of more aggressive, highly invasive metastatic tumour phenotype [114, 115].

Dysregulated signalling axes have been shown to increase ILK expression and activity [116] in a variety of cell lines, such as: (*i*) endothelin-1 [117] and vitronectin [118] in metastatic ovarian carcinomas; (*ii*) TGFβ1 in metastatic melanomas [119] and (*iii*) thymosin β4 in metastatic colorectal carcinomas [120]. Corroborating with the notion that ILK may serve as a point of inhibition in metastatic cell diffusion, treating glioma cells with the ILK specific inhibitor QLT-0254 *in vitro*, suppressed phosphorylation of ILK typical targets (Akt, GSK-3β and mTOR), excluding interference with total protein levels [83]. Notably reduced by QLT-0254 was MMP-2 secretion, VEGF and HIF1α, as well as the invasive capability of glioma cells. Correspondingly in NSCLC cells, the ILK inhibitor KP-392 along with cisplatin impacted the incidence of metastasis to the kidney, bone and contralateral lung [121]. Therefore, based on these collective data, it would be imprudent not to consider ILK as a therapeutic target for breast malignancies and other cancers [83, 122], although, the involvement of ILK in tumour suppression and senescence contributes to the complexity of its function.

The involvement of ILK in angiogenesis, anoikis, anchorage-independent cell cycle progression, migration, invasion and tumourigenesis implicates ILK as extremely influential in malignant progression, and as a putative medicinal target. ILK exerts regulation through an ill-described phosphorylation of Akt Ser^{473} and GSK-3 [56]. Transfection of dominant-negative ILK constructs or siRNA-ILK molecules have been shown to modulate ILK expression, as well as the phosphorylation of Akt Ser^{473}, ultimately affecting transformation. An ILK kinase deficiency was shown to suppress the constitutive phosphorylation of Akt in PTEN-mutant cells [41]. Furthermore, ILK kinase inhibition induced anoikis in an anoikis-resistant breast cancer cell line [107], inhibited nuclear β-catenin localization and cyclin D1 expression [108], and reduced the invasive properties of ILK-overexpressing and malignant cells, and *in vitro* cell growth [109]. Small molecule antagonist of ILK activity (QLT-0267 and QLT-0254) have been identified as having therapeutic potential in breast cancer [81, 83].

Acknowledgements

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