Emerging roles for LPP in metastatic cancer progression

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Abstract LIM domain containing proteins are important regulators of diverse cellular processes, and play pivotal roles in regulating the actin cytoskeleton. Lipoma Preferred Partner (LPP) is a member of the zyxin family of LIM proteins that has long been characterized as a promoter of mesenchymal/fibroblast cell migration. More recently, LPP has emerged as a critical inducer of tumor cell migration, invasion and metastasis. LPP is thought to contribute to these malignant phenotypes by virtue of its ability to shuttle into the nucleus, localize to adhesions and, most recently, to promote invadopodia formation. In this review, we will examine the mechanisms through which LPP regulates the functions of adhesions and invadopodia, and discuss potential roles of LPP in mediating cellular responses to mechanical cues within these mechanosensory structures.

Keywords LPP · Focal adhesions · Migration · Invadopodia · Invasion · Metastasis

Introduction

Directed cell migration is critical for the proper development and function of multi-cellular organisms. Migratory programs are also engaged in pathological conditions, such as cancer progression, where they contribute to tumor cell dissemination. Interactions between cancer cells and surrounding extracellular matrix (ECM) impact the mechanisms that are engaged to facilitate cellular migration and invasion. In addition to biological cues, such as growth factors, the migratory capacity of cancer cells is influenced by the physical properties of underlying substrates on which they move. These biochemical and biophysical stimuli modulate signalling cascades that manifest in diverse cellular responses, including cell migration.

Cell migration is characterized by four key events: (1) protrusion of the leading edge, (2) adhesion to the ECM, (3) cell body translocation, and (4) retraction of the trailing edge (Friedl and Wolf 2010; Lauffenburger and Horwitz 1996; Welf and Haugh 2011). Adhesion assembly, stability and disassembly allow migrating cells to anchor their cytoskeleton to the ECM and move in a directed fashion. A complex interplay between over 200 different proteins localized to adhesions ultimately results in the generation of traction forces that propel the cell body forward (Geiger et al. 2009).

In the context of cancer, increased cell migration is central to the metastatic process. Cells migrate through the interstitial ECM surrounding the primary tumor, enter the vasculature, and eventually colonize distant organs/tissues. Upregulation of proteins that enhance actin cytoskeleton and adhesion dynamics is often observed in invasive and metastatic cancer cells, while inhibition of these mediators has proven beneficial in blocking cell migration and invasion (Jiang et al. 2015; Wendt and Schiemann 2009; Yamaguchi and Condeelis 2007).

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Breast cancer continues to be the most commonly diagnosed cancer in women (DeSantis et al. 2016). Despite significant progress in detection and early treatment, metastatic disease remains largely incurable. The tumor microenvironment is increasingly implicated in cancer progression (Quail and Joyce 2013). The cellular and biochemical components of the primary tumor and metastatic microenvironments have long been areas of interest; however, the biophysical microenvironment is emerging as a key modulator of cancer cell behaviour (Kai et al. 2016; Parekh and Weaver 2016). A better understanding of how mechanical properties within the surrounding stromal tissue regulate molecular mechanisms engaged in cancer cells may lead to the identification of new targets for intervention that can suppress malignant cancer phenotypes. Lipoma Preferred Partner (LPP) has recently emerged as an important regulator of breast cancer cell migration and invasion (Ngan et al. 2013, 2017). Here, we describe the initial discovery of LPP, its unique molecular interactions that control cell migration, and novel roles for LPP in invadopodia formation and breast cancer metastasis. Potential roles for LPP as a mechanosensory protein that controls cell migration and invasion are also discussed.

Discovery of LPP

LPP was first characterized as a fusion transcript and the preferred translocation partner of High Mobility Group AT-Hook2 (*HMGA2*) in lipoma cell lines and primary lipomas (Petit et al. 1996). *HMGA2* is a transcriptional regulator encoded by chromosome 12 that binds the minor groove of DNA and acts as an architectural factor (Goodwin 1998). Approximately two-thirds of lipomas display chromosome translocations (Mandahl et al. 1994; Sreekantaiah et al. 1991) that likely result in aberrant growth control (Ashar et al. 1995; Schoenmakers et al. 1995; Van de Ven 1998). The most frequent rearrangement, t(3;12)(q27;q28; q13-q15), involves a protein on 3q27–28 that was named LPP (Lipoma Preferred Partner/LIM Domain Containing Preferred Translocation Partner In Lipoma). The rearrangement of exons 1–3 from *HMGA2* and exons 9–11 from *LPP* encodes an *HMGA2*-LPP fusion protein containing the first 3 AT-hooks of *HMGA2* and the last 2 LIM domains (LIM 2 and 3) of *LPP* (Crombez et al. 2005).

Several other rearrangement transcripts of *HMGA* and *LPP* have since been described including: (1) *HMGA1*-LPP t(3;6)(q27;q21) (Wang et al. 2010), (2) *LPP*-*HMGA2* fusion (exons 8–11 of *LPP* and exon 1–4 of *HMGA2*) (Taube et al. 2010), and (3) *LPP*-*HMGA2* (exon 7–11 of *LPP* and exon 1–4 of *HMGA2*) (Kubo et al. 2009). An *MLL*-LPP fusion transcript has also been identified in secondary acute leukemia. This transcript t(3;11)(q28;q23) encodes the AT-hook motifs and methyltransferase domain of mixed lineage leukemia (*MLL*) and the LIM 2–3 domains of *LPP* (Daheron et al. 2001).

LPP protein domain organization

LPP spans a genomic region of over 400 kb and produces a mRNA transcript of approximately 10 kb (Petit et al. 1996). The main promoter element within intron 2 has an open reading frame of 1836 nucleotides, which encodes an 80 kDa protein. Although *LPP* mRNA levels are found in all human tissues (except the brain and peripheral blood), *LPP* protein is highly expressed in smooth muscle cells (SMCs) particularly in the ileum, stomach, corpus cavernosum, uterus, portal vein, aorta and bladder (Gorenne et al. 2003). It is important to note that a smaller transcript has been detected in the testes (Petit et al. 1996), which in mice, is generated through an alternative promoter located in intron 7 (Vervenne et al. 2009).

LPP was initially described as a novel group 3 LIM-domain containing protein based on motif composition and its highly modular organization (Petit et al. 1996). Further characterization, however, led to the re-classification of *LPP* as a zyxin family member due to high sequence similarity within the LIM domains and a similar overall domain structure to zyxin (Macalma et al. 1996). The zyxin family currently consists of Zyxin, *LPP*, *TRIP6/ZRP-1*, *Ajuba*, *LIMD1*, *WT1P* and *FBLP-1/migifilin/Cal*, all of which regulate cellular proliferation, cytoskeletal rearrangements, cellular motility, cell migration/invasion and tumorigenesis (Beckerle 1997; Grunewald et al. 2009, 2013; Hirata et al. 2008b; Willier et al. 2011; Wu 2005). Although *LPP* shares the greatest amino acid sequence similarity to *TRIP6/ZRP-1* (53%), its domain organization most closely resembles zyxin (with 41% amino acid similarity) (Li et al. 2003; Yi and Beckerle 1998).

LPP contains three carboxyl-terminal LIM domains that are preceded by a Proline-Rich amino-terminal region (PRR) (Fig. 1). The LIM domains span amino acid residues 413 to 612 in human *LPP* and are formed by cysteine and histidine-rich double zinc finger protein motifs (Petit et al. 1996). These motifs are comprised of approximately 55 residues with the following consensus sequence: CX₂CX_{16–23}HX₂CX₂CX_{16–23}CX₂C (where X is any amino acid) (Majesky 2006; Petit et al. 2003). The remaining domain sequence is highly variable and thought to confer protein-protein binding specificity (Kadmas and Beckerle 2004). It is unclear whether a single LIM domain binds several proteins simultaneously, or whether tandem LIM domains co-operate to bind a single interaction partner. Discrete consensus binding sequences or structural features that define LIM domain interactions with specific partners have not yet emerged.

LPP localization to the nucleus

LPP possesses a classic leucine-rich nuclear export signal (NES) within its PRR (residues 117–128, sequence LDAEIDSLTSIL). Only a very small amount of *LPP* can be

Lipoma Preferred Partner (LPP)

Fig. 1 Schematic depicting the domain structure of LPP. LPP can be divided into the N-terminal Proline Rich Region (PRR) and the C-terminal LIM domains. Distinct domains within LPP are indicated: ABD: α-actinin binding domain; V: VASP binding domain; NES:

nuclear export signal; 1/2/3: LIM domains. LPP interacting proteins are listed above the LPP schematic. Consensus sequences for the LPP α-actinin binding domain (ABD) and LIM domains are depicted below the LPP schematic. Numbers refer to the amino acid position within LPP

detected in the nucleus (1–6% of cells) under steady state conditions (Petit et al. 2000). Nevertheless, nuclear accumulation of LPP can be increased with Leptomycin B, a drug that blocks nuclear export by preventing the formation of a NES/CRM1/RAN-GTP complex (Petit et al. 2000, 2003). Interestingly, expression of exogenous LIM 1–3 alone can displace LPP and vinculin from adhesions and increase nuclear accumulation (Petit et al. 2003). It is unclear how LPP translocates into the nucleus as it does not possess a predicted Nuclear Localization Signal (NLS) and its molecular weight is too large to permit passive diffusion. Even so, data has emerged to suggest that LPP can shuttle to the nucleus and perform distinct functions (Colas et al. 2012; Grunewald et al. 2009).

Functional roles for LPP within the nucleus

In a yeast two-hybrid screen, LPP was identified as an interacting partner with POT1 Δ-Cterm, a mutant telomere capping protein that retains its DNA binding domain. In fibrosarcoma cells, myc-tagged LPP co-precipitated with POT1, TRF2, TRF1 and TIN2, which are all components of the shelterin complex necessary for telomere function (Sheppard and Loayza 2010). Further characterization revealed that LPP

and TRIP6 interact with shelterin through POT1 within a small subset of telomeres (Sheppard and Loayza 2010). These findings were confirmed by chromatin immunoprecipitation (CHIP) analyses and it has been proposed that LPP localizes to critically short telomeres to repress DNA damage responses (Sheppard and Loayza 2010; Sheppard et al. 2011).

Wild-type LPP has also been found to interact with Polyomavirus Enhancer Activator protein (PEA3) through amino acid residues 284–418 and 412–612 (Guo et al. 2006). PEA3 belongs to a subfamily of ETS domain transcription factors that regulate the transcription of matrix metalloproteinase-1 (MMP-1) and cyclooxygenase-2 (COX-2) (Baker et al. 2010; Guo and Sharrocks 2009; Kapila et al. 2009). As a co-regulatory binding partner of PEA3, LPP can associate with known PEA3-regulated promoters (Guo et al. 2006). Interestingly, overexpression of PEA3, MEK or LPP alone has little effect on *MMP-1* gene activity in 293 cells (Guo et al. 2006). In contrast, co-expression of PEA3 and MEK enhances *MMP-1* expression. This increase is further amplified by LPP overexpression. Co-transfection of PEA3 and LPP also increases reporter gene activity of *COX-2* in a MEK/Erk dependent manner (Guo et al. 2006).

LPP fusion proteins created by aberrant chromosomal translocations may function as transcriptional regulators as

well. GAL4 reporter assays show that the LIM2/3 domains of HMGA2-LPP function as a transactivation domain (Crombez et al. 2005). While HMGA proteins do not have direct transcriptional activation capacity, HMGA2-LPP binding to a PRDII element (an enhancer sequence within the IFN- β gene) can potentiate transcriptional activation by the Nuclear Factor κ B (NF- κ B) protein (Crombez et al. 2005). When expressed alone, residues 248–413 of LPP increase luciferase activity by 6000-fold (Petit et al. 2000). In chondromas, HMGA2-LPP contributes to chondrogenesis and adipogenesis via the transactivation of COL1A2 promoter and upregulation of COL1A2, a cartilage specific collagen gene (Kubo et al. 2006).

LPP localization to adhesions

LIM domain-containing proteins typically function as scaffold and/or adaptor proteins to mediate protein-protein interactions and signal transduction. They have been implicated in the regulation of actin structure and dynamics, integrin-dependent adhesion/signaling, neuronal pathfinding, cell-fate determination and tissue-specific gene expression (Kadmas and Beckerle 2004).

The ability of GFP-tagged LPP to localize at cell-cell contacts and adhesions was first observed in fibroblasts (Petit et al. 2000). The second LIM domain is an important determinant of this process, as mutation of conserved zinc-binding cysteine and histidine residues significantly impairs LPP targeting to adhesions (Petit et al. 2003). The most severe phenotype occurs when both LIM 1 and 2 are mutated, while mutations in the third LIM domain only modestly diminish adhesion targeting (Petit et al. 2003). These results are in agreement with the observation that the LIM 3 sequence of LPP is more divergent than LIM 1 and 2 (Petit et al. 2003). Others have since described co-localization of LPP with vinculin in rat aortic cells (Gorenne et al. 2003), palladin in smooth muscle tissues (Jin et al. 2007), and protein phosphatase 2A (PP2A) in HT1080 fibrosarcoma cells (Janssens et al. 2016). The precise functional roles of LPP within adhesions have yet to be elucidated, although recent evidence strongly suggests an important role in cell migration and invasion (Ngan et al. 2013, 2017).

LPP interaction partners

LPP is a highly modular protein with numerous protein-protein interaction motifs (Fig. 1). Binding sites for α -actinin, LIM and SH3 protein (LASP), palladin, PP2A, scrib, supervillin and vasodilator-stimulated phosphoprotein (VASP) have implicated LPP in multiple biological and cellular processes.

α -Actinin: α -actinin is an actin-binding protein that localizes to the leading edge of cells (Knight et al. 2000), lamellipodia (Langanger et al. 1984), filopodia (Xue et al. 2010), adhesions (David-Pfeuty and Singer 1980; Wehland et al. 1979), invadopodia (Hirooka et al. 2011), and stress fibers (Feramisco 1979; Langanger et al. 1984). While α -actinin lacks G-actin binding and actin initiation/nucleation ability, it functions to crosslink actin, promote cell migration (Foley and Young 2014; Le Clainche and Carlier 2008), stimulate adhesion maturation (Choi et al. 2008), and promote invadopodia formation (Yamaguchi et al. 2017). It interacts with LPP through an α -actinin binding domain (ABD) in the PRR (amino acid residues 41–57) (Li et al. 2003; Petit et al. 2003). Six core residues (FAPVVA in human LPP and YAPVVA in mouse LPP) allow LPP to bind the central-rod region of α -actinin, which contains spectrin-like repeats 2 and 3 (Li et al. 2003). Overexpression of α -actinin isoforms has been identified in many different cancers, including breast cancer (Honda 2015).

LASP: LASP is a nebulin-family LIM containing protein involved in actin binding and cytoskeletal re-organization. It was first identified from a cDNA library of metastatic axillary lymph nodes and since then LASP overexpression has been implicated in breast and ovarian cancers (Grunewald and Butt 2008). LASP interacts with an XPPPP motif within the PRR of LPP via its SH3 domain (Keicher et al. 2004). Interestingly, mouse embryonic fibroblasts (MEFs) that lack LASP (LASP^{-/-}) migrate more rapidly and exhibit increased adhesion dynamics. It has been postulated that these effects are due to compensation by LPP, which is upregulated by 2-fold in the absence of LASP (Zhang et al. 2009).

Palladin: Palladin is a member of the palladin/myotilin/myopalladin family of actin-associated scaffolds responsible for cell shape, adhesion, motility, contraction and invadopodia formation (Najm and El-Sibai 2014; Otey et al. 2005; Parast and Otey 2000). Like LPP, palladin is abundantly expressed in mesenchymal cells and has been found in stress fibers, adhesions and cell-cell junctions (Parast and Otey 2000). In human iliac vein SMCs, palladin is found to co-localize with LPP and α -actinin during cell spreading (Jin et al. 2007). Palladin interacts with the first and second LIM domains of LPP through an N-terminal region (Jin et al. 2007). LPP and palladin expression appear to be co-regulated, as focal adhesion kinase (FAK) null cells exhibit decreased LPP and palladin levels; however, their expression can be rescued by paired-related homeobox gene-1 (PRX1) and angiotensin II (Jin et al. 2007).

PP2A: PP2A is a ubiquitously expressed serine/threonine phosphatase that regulates many cellular processes including cell proliferation, signal transduction, cell

motility and cytoskeletal dynamics (Seshacharyulu et al. 2013). Its core structure is composed of a 36 kDa catalytic C subunit (PPP2CA/B, α and β isoforms) and a 65 kDa structural/scaffold A subunit (PR65 or PPP2R1A/B, α and β isoforms), which is frequently associated with a regulatory B subunit (B, B', B'' or B''') that dictates subcellular localization and substrate specificity (Bononi et al. 2011; Eichhorn et al. 2009). It has recently been shown that the B'' subunit PR130 interacts with the LIM domains of LPP (Janssens et al. 2016). PP2A activity is required for efficient cell migration on collagen I as siRNA-mediated knockdown of PR130 impairs wound closure. Interestingly, PR130 is absent from mature (non-dynamic) adhesions where LPP accumulates. This suggests that PR130 must dissociate from the LIM domains of LPP for adhesion stabilization to occur (Janssens et al. 2016). Alterations of PP2A subunits and binding proteins have been implicated in many cancers including brain, breast, liver and lung (Perrotti and Neviani 2013; Seshacharyulu et al. 2013).

Scrib: Scrib is a member of the leucine-rich repeat and PDZ (LAP) family of proteins involved in cell shape, adhesion and polarity (Petit et al. 2005). LPP-scrib interactions are mediated through the C-terminal region of LPP and the PDZ domain of scrib (Petit et al. 2005). Both proteins are co-localized at cell-cell contacts; however, scrib is dispensable for LPP targeting. Likewise, LPP is dispensable for targeting scrib to cell-cell contacts and adhesions (Petit et al. 2005). In zebrafish, the proteins cooperate to mediate convergence and extension movements during gastrulation (Vervenne et al. 2008). Scrib has been found to act as either a tumor suppressor or tumor promoter depending on the context, and has also been implicated in skin carcinogenesis (Pearson et al. 2015), lung cancer (Elsum et al. 2014) and prostate cancer (Pearson et al. 2011). Its mislocalization or dysregulation has been implicated in breast cancer formation and metastasis (Anastas et al. 2012; Feigin et al. 2014; Savi et al. 2014).

Supervillin: Supervillin (SVIL) belongs to the gelsolin superfamily of proteins responsible for actin organization (Silacci et al. 2004). It was first characterized as a tightly bound peripheral membrane protein that interacts with actin filaments (Pestonjamas et al. 1997). Further studies showed that SVIL and other family members (gelsolin, adseverin, advillin, villin, CapG and flightless I) sever actin filaments by weakening non-covalent bonds between individual actin molecules (Silacci et al. 2004; Sun et al. 1999). In doing so, SVIL can regulate actin assembly and disassembly within the cytosol. Gelsolin family members have been implicated in numerous cellular processes including, but not limited to, cell migration and invasion. SVIL can interact

directly with the LIM domains of LPP and TRIP6, but not zyxin, through residues 342–571 (Takizawa et al. 2006). SVIL localization to TRIP6 containing adhesions enhances their assembly and disassembly rates (Takizawa et al. 2006) while SVIL binding to myosin II mediates cellular contractility and podosome dynamics (Bhuwania et al. 2012). SVIL can also increase Rac1 activation to promote cell spreading (Son et al. 2015). Overexpression of SVIL has been found to enhance gelatin degradation and invadopodia formation through binding to cortactin (Crowley et al. 2009). Indeed, SVIL is observed in cortactin/Tks5/cdc42 labeled podosomes and invadopodia (Crowley et al. 2009).

VASP: VASP is a member of the Ena/VASP family of proteins comprised of VASP, Mena and Evl. VASP is often found in lamellipodia, filopodia and adhesions (Krause et al. 2002), and plays a role in cell adhesion, cell motility and actin polymerization (Holt et al. 1998; Krause et al. 2003; Kwiatkowski et al. 2003; Reinhard et al. 2001). LPP has 2 ActA repeats (FPPPP) located in its PRR that bind the EVH1 domains of VASP family members (Hansen and Beckerle 2006). VASP also interacts with G and F-actin, and regulates actin assembly, cellular contraction and invadopodia formation (Albiges-Rizo et al. 2009). Moreover, VASP increases the rate of actin polymerization, promotes F-actin elongation, and blocks the barbed end of growing actin filaments from binding capping proteins (Hansen and Mullins 2010). Overexpression and phosphorylation of Ena/VASP family members have been implicated in the formation of breast (Di Modugno et al. 2010; Doppler et al. 2015; Gertler and Condeelis 2011; Hu et al. 2008; Philippart et al. 2008), colon (Ali et al. 2015; Toyoda et al. 2009; Zuzga et al. 2012) and lung cancers (Dertsiz et al. 2005).

Biological functions of LPP

LPP's complex domain structure and diversity of interacting partners allow it to regulate distinct biological processes relevant to cell migration and invasion. As previously mentioned, LPP is abundantly expressed in SMCs (Gorenne et al. 2003). LPP knockdown in SMCs results in decreased cell migration (Gorenne et al. 2006), while transfection of eGFP-LPP causes a 2-fold increase in EGF-induced migration (Jin et al. 2007). Moreover, exogenous expression of LPP rescues cell spreading and EGF-stimulated motility in FAK null embryonic fibroblasts (Jin et al. 2007). In MDCK cells, LPP co-localizes to adhesions in the basal plane and cell-cell contacts in the apical plane (Van Itallie et al. 2014). Knockdown of LPP diminishes E-cadherin dependent cell-cell adhesion and tight junction

formation, but increases cell-substrate adhesion (Van Itallie et al. 2014). LPP localization to cell-cell junctions likely occurs through interactions with α -actinin (Gregory Call et al. 2011).

Knockout/knockdown studies in model organisms demonstrate the cumulative effect of changes in LPP dependent cell-cell and cell-ECM adhesion. Morpholino-mediated knockdown of LPP in zebrafish impairs convergence & extension movements during gastrulation due to reduced directed migration of paraxial cells (Vervenne et al. 2008). Interestingly, LPP expression is significantly diminished in zebrafish treated with morpholinos targeting Wnt11. Loss of LPP in female C57BL/5 J mice results in partial embryonic lethality (Vervenne et al. 2009). While male LPP^{-/-} mice exhibit normal fertility, females in LPP^{-/-} x LPP^{-/-} crosses produce low numbers of offspring. LPP^{-/-} mice that reach adulthood do not display any overt phenotypes; however, LPP^{-/-} MEFs migrate more slowly and exhibit decreased viability compared to LPP^{+/+} MEFs. Furthermore, LPP^{-/-} MEFs have reduced expression of LPP binding partners, including α -actinin, PR130 and scrib (Vervenne et al. 2009).

Recent studies implicate LPP as a mechanosensitive protein capable of responding to extracellular forces. In the myocardium, increased LPP expression is observed in the hearts of aortic-banded rats (pressure overload induced cardiac hypertrophy), suggesting that hemodynamic load can regulate LPP levels (Hooper et al. 2012). Nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide synthase (NOS), significantly increases LPP expression in cardiac myocytes (Hooper et al. 2012, 2013). Conversely, cyclic mechanical stretching of myocytes, which mimics *in vivo* mechanical stress, causes a decrease in LPP expression and membrane localization (Hooper et al. 2012, 2013). In cultured cells, LPP expression and subcellular localization also appear to be regulated by substrate stiffness. LPP localizes to long, broad adhesions at the periphery of SMCs cultured on rigid surfaces. In contrast, cells grown on compliant matrices show LPP localization to small adhesions and punctate structures (Jin et al. 2009).

Taken together, these observations implicate LPP as a mechanosensor that responds to diverse cues in physiological conditions such as myofibrillogenesis (Hooper et al. 2012, 2013) and vascular injury (Jin et al. 2009). This is not unexpected as the role of LIM domain-containing proteins in sensing mechanical changes in the ECM is now starting to emerge (Smith et al. 2014). While zyxin serves as the “prototype” mechanosensor (Hirata et al. 2008a, b; Hoffman et al. 2012; Smith et al. 2013; Uemura et al. 2011; Yoshigi et al. 2005), considerably less is known about the mechanobiology of LPP. It would be interesting to determine whether LPP expression and localization are correlated with ECM composition, protein density and mechanical stiffness. An intriguing hypothesis is that LPP expression and localization dictate cellular modes of migration in response to matrix stiffness.

Functional roles of LPP in cancer

LPP augments the malignant phenotypes of cancer cells

The first associations between LPP and cancer emerged with the discovery of HMGA2-LPP fusion proteins in lipoma and other mesenchymal tumors (Petit et al. 2000, 2003). Indeed, exogenous expression of HMGA2-LPP induces malignant transformation of NIH3T3 cells while wild-type HMGA2 does not. Consequently, HMGA2-LPP transformed cells grow in soft agar and induce tumors in athymic mice (Fedele et al. 1998).

LPP was subsequently identified as one of the most upregulated proteins in invasive endometrial carcinomas (Colas et al. 2012). In this context, it was found to enhance cell migration and invasion by acting as a transcriptional regulatory partner of ETV5. ETV5 is a member of the PEA3 subfamily of ETS transcription factors. It activates the zinc finger E-box binding transcription factor Zeb1, which represses E-cadherin to facilitate an epithelial-to-mesenchymal transition (EMT) (Colas et al. 2012). LPP responds to extracellular epidermal growth factor (EGF) stimulation in an ETV5-dependent manner and induces a complete re-organization of cell-cell and cell-substrate contacts to promote tumor invasion (Colas et al. 2012).

Most recently, LPP has been described as an important driver of cell migration, invasion and metastasis in breast cancer (Ngan et al. 2013, 2017). The ErbB2 receptor tyrosine kinase belongs to the EGF receptor (EGFR) family (Yarden and Sliwkowski 2001). Activation of ErbB family members through homo- or heterodimerization leads to autophosphorylation of the cytoplasmic tail residues and recruitment of various signaling molecules. Signaling pathways initiated from the ErbB2 receptor mediate cell proliferation, differentiation, motility, adhesion and cellular transformation (Dankort et al. 2001a, b; Jones et al. 2006; Yarden and Sliwkowski 2001). The cooperation between transforming growth factor beta (TGF β) and ErbB2 signalling pathways has been investigated in multiple breast cancer models. Collectively, these studies demonstrate that TGF β can modulate the actin cytoskeleton to enhance cell migration and invasion of ErbB2-overexpressing breast cancer cells (Northey et al. 2008, 2013; Seton-Rogers et al. 2004; Ueda et al. 2004; Wang et al. 2006, 2008, 2009). The functional crosstalk between TGF β and ErbB2 signaling pathways has also been shown to enhance breast cancer metastasis in transgenic mouse models (Muraoka et al. 2003; Siegel et al. 2003). In this regard, LPP is required for TGF β -induced migration and invasion of ErbB2-expressing cells (Ngan et al. 2013). Cells expressing endogenous levels of LPP exhibit an increase in cell migration and invasion following EMT, while shRNA-mediated knockdown of LPP abolishes this effect (Ngan et al. 2013). Furthermore, TGF β enhances adhesion targeting of LPP, which may permit faster assembly and disassembly of

these structures (Ngan et al. 2013). Indeed, LPP constructs harboring a mutant LIM domain (LPP-mLIM1) or a dysfunctional α -actinin binding domain (LPP- Δ ABD) fail to support TGF β -induced cell migration and invasion (Ngan et al. 2013). These data reveal an important role for LPP in mediating breast cancer cell migration and invasion. However, further investigation is required to understand the precise molecular mechanisms at play.

Invadopodia are structures that enable invasive carcinoma cells to (1) escape the primary tumor, (2) intravasate into the circulatory and lymphatic systems, (3) extravasate into the parenchyma of distant tissues, and (4) initiate the formation of metastasis (Eddy et al. 2017; Paterson and Courtneidge 2017). Sophisticated imaging approaches are now producing direct evidence of their involvement during tumor cell intravasation and extravasation *in vivo* (Carmona et al. 2016; Harney et al. 2015, 2016; Leong et al. 2014). Furthermore, *ex vivo* models demonstrate that breast carcinoma cells form invadopodia when seeded onto urinary bladder-derived basement membrane (BM) (Parekh et al. 2011) and invasive colorectal cells form invadopodia that breach BM barriers through physical and enzymatic mechanisms (Schoumacher et al. 2010). There is also evidence to suggest a direct correlation between the propensity of cancer cells to form invadopodia and poor prognosis in breast cancer patients (Blouw et al. 2015). Recent data shows that LPP, while dispensable for tumor growth, is required for invadopodia formation, breast cancer cell extravasation, and the establishment of lung metastases (Ngan et al. 2017). *In vitro* gelatin degradation assays indicate that knockdown of LPP significantly impairs invadopodia formation in response to TGF β without influencing metalloproteinase (MMP) expression or activity. Furthermore, LPP co-localizes with actin and Tks5, an obligate component of invadopodia (Blouw et al. 2015), at sites of gelatin degradation. The ability of LPP to support invadopodia formation requires an intact LIM1 and α -actinin binding domain. *Ex ovo* imaging of LPP and Tks5 in a chick chorioallantoic membrane assay shows that breast cancer cells harboring LPP-mLIM1 and LPP- Δ ABD fail to extravasate out of the vasculature (Ngan et al. 2017). Consequently, the ability of LPP-mLIM1 and LPP- Δ ABD mutants to rescue the formation of lung metastases is significantly impaired compared to wild-type LPP (Ngan et al. 2017). LPP is a Src substrate and phosphorylation of three tyrosine residues (Y245/Y301/Y302) is required for TGF β -induced breast cancer cell invasion. *In vivo* data demonstrates that simultaneous mutation of Y245/Y301/Y302 significantly decreases the number of surface metastatic lesions, albeit not to the same extent as knockdown of LPP (Ngan et al. 2017). Interestingly, these phosphorylation events are dispensable for TGF β -induced increases in breast cancer cell migration (Ngan et al. 2017). Taken together, these data support a novel role for LPP in modulating two fundamental processes linked to breast cancer

metastasis: (1) increased cell migration via growth factor stimulation and (2) enhanced invasion through the promotion of invadopodia formation (Fig. 2).

LPP as a suppressor of malignant cancer phenotypes

Interestingly, studies in other cancer models have implicated LPP as a negative regulator of cancer progression. In lung cancer, MMP15 is a direct transcriptional target of LPP and ETV5, which degrades N-cadherin to weaken cell-cell interactions (Kuriyama et al. 2016). Knockdown of LPP and ETV5 in PC14PE6 cells increases cell-cell adhesion, which fosters collective cell migration and enhances lung cancer cell dissemination (Kuriyama et al. 2016). Thus, loss of LPP, ETV5 or MMP15 is a prognostic marker for increased malignancy in this context.

In a similar fashion, LPP was identified as a predictor of better survival in B-cell lymphoma (Jais et al. 2008). LPP, LMO2, MME and FOXP1 are part of a cell-of-origin signature in diffuse large B-cell lymphoma. When highly expressed, this signature is correlated with better overall survival and progression free survival in patients treated with R-CHOP (Rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone) (Jais et al. 2008). These results indicate that the ability of LPP to modulate the malignant phenotypes of cancer cells can be highly context dependent.

Future perspectives – LPP as a mechanosensor during metastasis

During cell migration, the actin cytoskeleton couples to adhesions in a tangential orientation that leads to the generation of traction forces (Albiges-Rizo et al. 2009). The formation of invadopodia requires the perpendicular alignment of actin filaments with respect to the underlying ECM to create protrusive forces as MMPs degrade matrix (Albiges-Rizo et al. 2009). How cancer cells sense the biophysical microenvironment and convert stimuli into either migratory or invasive responses remains largely unexplored.

LPP regulates malignant cancer phenotypes, in part, through its localization to adhesions (migration) and invadopodia (invasion). Interestingly, LPP is emerging as a mechanosensory and potentially mechanoregulatory protein, which is consistent with the fact that both adhesions and invadopodia are mechanosensory cellular structures (Parekh and Weaver 2016; Schiller and Fassler 2013). The notion that biophysical cues can direct LPP to adhesions or signal LPP-mediated invadopodia formation is an exciting prospect. Increasing data showing that ECM substrate/tissue rigidity promotes breast cancer cell migration and invasion (Chaudhuri et al. 2014; Paszek et al. 2005) aligns well with data supporting LPP as a key player in cancer progression (Ngan et al. 2017).

Fig. 2 Schematic illustrating distinct points within the metastatic cascade that rely on LPP-regulated cellular functions (*left panel*). LPP is localized to adhesions, which are cellular structures on the ventral surface of cells that are critical for their migration. LPP is situated where the actin cable connects to components of the adhesion (*upper right panel*). Invadopodia

are actin-rich structures with associated proteolytic activity that are responsible for localized degradation of extracellular matrix components (*lower right panel*). Recently, LPP has been shown to be a constituent of cellular adhesions and also required for efficient invadopodia formation, cancer cell invasion and metastasis

Extracellular matrix stiffness and cancer progression It is well documented that breast density is positively correlated with cancer development (Boyd et al. 2014; Li et al. 2005; McCormack and dos Santos Silva 2006). Normal fatty breast tissue is characterized by a Young's modulus of 5–7 kPa while breast parenchyma has a modulus of 30–50 kPa (Athanasίου et al. 2010). Tissue stiffness increases during disease progression, with benign lesions measuring 45 ± 40 kPa and malignant lesions reaching Young's moduli of 147 ± 40 kPa (Athanasίου et al. 2010). Tumor stiffness is highly variable (64–181 kPa); however, this parameter increases along with the grade of ductal carcinoma in situ (DCIS) (Bae et al. 2017) and with larger tumors of higher grade (Evans et al. 2012). Collagen crosslinking and ECM stiffening accompany tumorigenesis, which together promote adhesion formation/maturation (Levental et al. 2009; Provenzano et al. 2009). Increased matrix stiffness induces an EMT and enables a

mesenchymal cell-like mode of cancer cell migration and invasion (Wei et al. 2015). In addition, increasing tissue stiffness is associated with thickening and linearization of interstitial collagen adjacent to the invasive tumor front (Acerbi et al. 2015). Collectively, these changes enhance tumor progression by inducing cell migration and invasion. Indeed, in vivo experiments show that increased stromal collagen within mouse mammary tissue significantly increases tumor formation and lung metastasis (Provenzano et al. 2008), while increased breast cancer cell invasion correlates with increased ECM stiffness in human breast tumors (Acerbi et al. 2015).

Substrate stiffness and cellular migration Many physiological processes require cells to migrate towards stiffer ECM in a process known as durotaxis (Lo et al. 2000; Sunyer et al. 2016; Vervenne et al. 2008). In vitro experiments demonstrate that force fluctuations within adhesions are converted into

biochemical signals through a FAK/phosphopaxillin/vinculin signaling pathway (Plotnikov et al. 2012). Although durotaxis is beneficial in development and wound healing, it is particularly problematic in pathological conditions such as cancer (Friedl and Alexander 2011; Ulrich et al. 2009).

While these observations indicate that substrate stiffness is a stimulus for adhesion maturation and cell migration, an optimal range likely exists that promotes maximal cell migration. Indeed, a biphasic relationship between substrate stiffness and migration appears to exist: very soft substrates fail to support traction forces required for cell migration while very stiff substrates induce maturation and hyper-stabilization of adhesions, which impedes cellular migration (Galbraith et al. 2002; Lo et al. 2000). Mathematical models of steady-state cell speed suggest that fibroblasts migrate best on substrates with stiffness's of ~30–40 kPa (Dokukina and Gracheva 2010; Pelham and Wang 1997). In agreement with these models, traction force mapping shows that IMR-90 lung fibroblasts treated with TGF β exhibit greater root-mean-square traction on elastic moduli greater than 13 kPa when compared with untreated controls (Marinkovic et al. 2012). Stiffer substrates cause greater cell spreading, induce the formation of more cell-matrix adhesions, and consequently result in slower migration (Lange and Fabry 2013). It has been observed that cells plated on stiff, highly cross-linked substrates (~30–100 kPa) exhibit stable adhesions (Discher et al. 2005). These data suggest that intermediate substrate stiffness might be favorable for migration (Pathak and Kumar 2012), with soft or very stiff substrates both resulting in a lack of cell migration.

LPP has been shown to act as a mechanosensitive protein that can modulate adhesion dynamics and promote cancer cell migration. Thus, it is possible that LPP may emerge as an essential protein in regulating cellular responses to a key range of substrate stiffness. Several outstanding questions remain regarding how LPP may function in sensing biophysical cues that stimulate cell migration. How is LPP targeted to adhesions in response to changes in substrate stiffness? Do the binding partners of LPP change as a function of stiffness? What are the molecular mechanisms engaged by LPP that facilitate cell migration in response to optimal ranges of substrate/tissue stiffness?

Substrate stiffness and cellular invasion Invadopodia are also mechanosensory structures that form optimally on substrates of varying stiffness (Kai et al. 2016; Parekh et al. 2011). Indeed, breast carcinoma cells have been shown to demonstrate peak invadopodia-associated ECM degradation on 30 kPa and 2 GPa substrates, with significantly less activity on intermediate rigidities (Parekh et al. 2011). Other studies have shown that soft matrices (0.1–0.4 kPa) can increase MMP secretion, promote MMP activity, and induce invadopodia formation (Gu et al. 2014). It has been suggested

that a minimum stiffness of 0.165 kPa is required for invadopodia formation (Aung et al. 2014); however, cancer cells exhibit significantly less invadopodia on 0.36 kPa substrates compared to 3.3 kPa (Alexander et al. 2008). Similarly, head and neck squamous cell carcinoma cells show less ECM degradation on rigidities of 1.0 kPa when compared to 22.7 kPa (Jerrell and Parekh 2014). These data suggest that invadopodia may optimally form on softer substrates relative to those that induce peak cell migration. Interestingly, very stiff substrates that impair cell migration may re-engage invadopodia for effective cell invasion.

Conclusions

LPP regulates the ability of cells to migrate, invade and metastasize through its actions in the nucleus, its role in controlling adhesion dynamics, and its ability to promote invadopodia formation and function. Further studies will be needed to (1) understand how cancer cells sense changes in substrate stiffness and (2) define how such biophysical cues instruct cancer cells to preferentially engage migratory or invasive programs. Two confounding aspects of this emerging field are the different methods/approaches used to model changes in substrate stiffness and the large variations in stiffness explored. Systematic studies that compare the effects of a dynamic range of substrate stiffness on migration (adhesion dynamics) and invasion (invadopodia formation) within the same experimental system are clearly needed. Whether LPP mediates the switch between cell migration and invasion in response to changing biophysical cues is an intriguing possibility that requires rigorous testing.

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