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Review Article

Cell Adhesion and Transcriptional Activity — Defining the Role of the Novel Protooncogene *LPP*

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Abstract

Integrating signals from the extracellular matrix through the cell surface into the nucleus is an essential feature of metazoan life. To date, many signal transducers known as shuttle proteins have been identified to act as both a cytoskeletal and a signaling protein. Among them, the most prominent representatives are zyxin and lipoma preferred (translocation) partner (LPP). These proteins belong to the LIM domain protein family and are associated with cell migration, proliferation, and transcription. LPP was first identified in benign human lipomas and was subsequently found to be overexpressed in human malignancies such as lung carcinoma, soft tissue sarcoma, and leukemia. This review portrays LPP in the context of human neoplasia based on a study of the literature to define its important role as a novel protooncogene in carcinogenesis.

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Genetic Background

Specialized cell adhesion sites not only play a role in the architectural organization and polarity of the cell but also are dynamic units directly involved in communicational processes. Adhesion receptors and their cytoskeletal partners can regulate nucleocytoplasmatic trafficking of signaling proteins and are thereby capable of influencing gene expression [1–4]. One such protein that may be involved in this process is the LIM-containing protein LPP (lipoma preferred (translocation) partner) [1].

Petit et al. [5] initially described LPP as the preferred translocation partner in a cytogenetic subgroup of lipomas that is characterized by translocations that mainly involve chromosome 12. Several chromosomes were identified as translocation partners of chromosome 12 with 3q27-q28 being preferentially involved. Moreover, it was shown that the high-mobility group (HMG) protein gene *HMGA2* at 12q15 is consistently rearranged as a consequence of these translocations. Fusion transcript analysis of *HMGA2* in the lipoma cell line Li-501/SV40 unmasked ectopic genetic sequences that originated from the chromosome segment 3q27-q28. These results subsequently yielded in the identification and characterization of the chromosome 3 translocation partner gene named *lipoma preferred (translocation) partner* (*LPP*) gene. Northern blot analysis detected a messenger RNA (mRNA) of more than 10 kb in a variety of human tissues [5,6]. The gene was found to span a genomic region of more than 400 kb. Detailed sequencing analysis of *LPP* revealed an open reading frame of 1836 nucleotides. The main promoter of the *LPP* gene is located in intron 2 leading to a full-length LPP protein of a highly modular organization [5]. An alternative promoter was found in murine intron 7 leading to a short form of LPP specifically expressed in testis [7]. Alternative LPP variants are also assumed to exist in human because high levels of two smaller *LPP* gene transcripts have also been detected specifically in the testis [5]. However, these smaller human *LPP* transcripts have not been characterized in all detail yet.

Functional Organization, Structure, and Binding Partners of LPP

LPP encodes an 80-kDa protein that was characterized as a novel member of group 3 proteins in the LIM family [5]. On the basis of the arrangement, position, and high sequence similarity of the LIM domains of LPP with those of zyxin, LPP was classified as a zyxin family member. This protein family consists of zyxin, ajuba, LIMD1,

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thyroid receptor–interacting protein 6 (TRIP6, also termed as zyxin-related protein 1), WT1-interacting protein, migfilin, and LPP [8–14], all of which are strongly involved in cellular motility, proliferation, and tumorigenesis [12,13,15,16].

The *LPP* gene encodes a proline-rich protein containing a leucine zipper motif in its amino-terminal region and three LIM domains at the carboxy-terminal end (Figure 1) [5]. LIM domains are cysteine- and histidine-rich domains that form two zinc fingers capable of mediating protein-protein interactions. Through binding to other partners, LIM proteins participate in diverse cellular processes [17,18].

LPP mRNA is ubiquitously expressed in virtually all types of tissues with emphasis on organs of the reproductive tract [19,20]. In contrast to the ubiquitous LPP mRNA presence, the protein is selectively expressed in smooth muscle cells (SMCs), especially in the uterus, stomach, corpus cavernosum, portal vein, aorta, bladder, and ileum [21].

LPP not only is colocalized with vinculin at sites of cell adhesion but also translocalizes into the nucleus [1]. In various benign and malignant tumors, a mutant form of LPP is permanently present in the nucleus [22,23]. Considering the size of LPP, it is unlikely that the protein translocates into the nucleus by mass action and free diffusion. Thus, an active and selective import mechanism seems to be more likely [1,21,22,24].

All three LIM domains of LPP cooperate to provide robust targeting to focal adhesions, with the linker region between LIM domains 1 and 2 playing a pivotal role. Overexpression of the LIM domains results in the depletion of endogenous LPP and vinculin from focal adhesions [1]. Thus, the C-terminal LIM domains of LPP are required for targeting the protein to points of cell-cell and cell-matrix contacts. In contrast, the LIM domains are dispensable for nuclear LPP localization [1]. Beyond its structural function, the C-terminal LIM domains 2 and 3 exhibit transcriptional activity by enhancing transcription factors [25].

The proline-rich pre-LIM region of LPP harbors an intrinsic nuclear export signal [1,22,26] and contains binding sites for α-actinin, vasodilator-stimulated phosphoprotein (VASP), and LIM and SH3 domain protein 1 (LASP-1) [5,26,27]. LPP and its family member zyxin differ in their absolute and functional relevant number of their VASP binding repeats (FPPPPP repeats). Whereas LPP harbors two proline-rich motifs, of which only one binds VASP *in vitro* [22], zyxin features four functional active FPPPPP repeats [28–30].

VASP has been proposed to increase actin polymerization and force cell protrusion. By binding to the proline-rich repeats of LPP and zyxin, VASP is recruited to specific cellular locations, thus directing changes in actin dynamics [30]. The LIM region of zyxin and LPP acts as a negative autoregulatory domain that normally masks the VASP-recruiting function of the FPPPPP repeats, thereby preventing VASP incorporation into actin networks [31].

Similar to zyxin, LPP was found to bind to α-actinin *in vitro* and *in vivo* [26,32]. Studies using the three-hybrid system indicated that zyxin and LPP compete for the same binding site in the central rod of α-actinin containing spectrin-like repeats 2 and 3. On the LPP site, a conserved motif present at the N-terminus is involved in the interaction with α-actinin. Quantitative data obtained with the two- and three-hybrid systems suggested that LPP has a lower affinity for α-actinin than zyxin does. It is likely that this difference leads to slightly variant roles played by LPP and zyxin during the assembly and disassembly of focal adhesions [26].

Although both proteins localize at cell adhesions, zyxin is more prominently distributed along stress fibers. Moreover, there is a difference in the relative abundance of the two proteins. In fibroblasts, the level of zyxin is approximately five times higher than that of LPP, whereas epithelial cells show no significant difference in zyxin/LPP protein levels [22]. Despite the high sequence homology of zyxin and LPP, the observed differences in localization and affinity for the same binding partners might be due to the minor variants in their amino acid sequences.

Recently, it was demonstrated that, LPP-VASP binding is required for anchoring α-actinin at cell-cell contacts, whereas zyxin localization and function at cell-cell contacts is independent of the α-actinin binding site [32], leading to the assumption that zyxin is recruited to cell-cell contacts by other docking proteins, e.g., LASP-1 [33,34].

With its C-terminal tail, LPP interacts directly with the tumor-suppressor Scrib. Interestingly, although all zyxin family members share high sequence homology, only LPP and TRIP6 bind to Scrib [24]. LPP/Scrib will be discussed in more detail later.

Using the C-terminus of LPP as bait in a yeast two-hybrid system, another interaction partner of LPP and a key player in focal adhesion organization was identified — palladin. The palladin-interacting

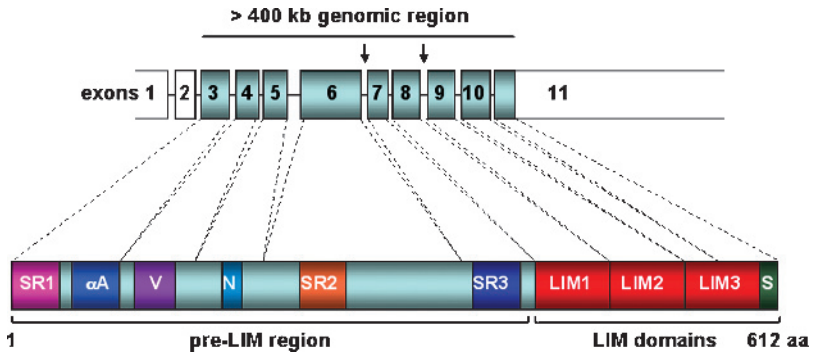

Figure 1. Schematic illustration of the *LPP* gene (upper part) and LPP protein (lower part). *LPP* spans over a genomic region of >400 kb. The gene product consists of 612 amino acids. Several binding sites for other proteins are located within the N-terminal pre-LIM region: the first of two LPP/TRIP6 similar regions (SR1) followed by binding motifs for α-actinin (αA), VASP (V), a nuclear export signal (N), the second LPP/TRIP6 similar region (SR2), and a zyxin/LPP/TRIP6/LIMD1 similar region (SR3). The LIM domain region consists of three tandem LIM domains, each of which is equipped with a double zinc finger structure. The Scrib-binding motif (S) is located at the very C-terminal tail of LPP.

Table 1. Overview of the Putative LPP Functions in the Context of Intracellular LPP Localization.

Location	Function	Reference(s)
Focal contacts/cell-cell contacts	Modulation of cytoskeleton and linker between membrane and cytoskeleton	[21,37]
	Scaffolding and adaptor protein	[26]
	SMC migration	[21,37]
	Interaction partner for VASP, zyxin, palladin, Scrib, α-actinin, and LASP-1	[24,26,27,30,32,35,51]
	Recruitment of VASP to cell-cell adhesions	[30,32]
Nucleus	Independent transcription factor	[1,22,25,51]
	Transcriptional coactivator of transcription factors such as PEA3 and ER81	[25,40]

region of LPP was mapped to the first and second LIM domains, whereas the N-terminus of palladin interacts with LPP, both *in vitro* and *in vivo*. Like LPP, palladin is highly expressed in differentiated smooth muscle (SM) and localized at focal adhesions, at isolated lamellipodia, and at dense bodies [35]. Both LPP and palladin enhance cell migration and spreading [36], and their expression is markedly decreased in migration-defective focal adhesion kinase (FAK)–null cells [21,37].

Recently, LPP was identified as a substrate of the protein-tyrosine-phosphatase 1B, a negative regulator of multiple signaling pathways downstream of receptor tyrosine kinases and functionally linked to Ras signaling [38,39].

The modular organization of LPP and its multiple interaction sites for cytoskeletal proteins suggest an important role in focal adhesion architecture as a versatile scaffolding and adaptor protein.

An overview of the putative functions and binding partners of LPP is given in Tables 1 and 2, respectively.

LPP and Its Effect on Gene Transcription

LPP has been shown to shuttle into the nucleus and to possess a Crm1-dependent nuclear export signal. The protein exhibits two domains harboring transcriptional activation capacity that coincides with the LIM domains and the proline-rich region of the LPP protein [1,22]. Within the nucleus, LPP is recruited to PEA3-dependent promoter regions and acts as a coregulatory protein enhancing the transactivational potential of PEA3 [40]. PEA3-binding sites have been identified in the regulatory regions of many genes associated with tumorigenesis as well as embryogenesis. A significant fraction of target genes encodes proteases required for degradation of the extracellular matrix (ECM), for example, serine proteases, urokinase plasminogen activator, COX-2, and several matrix metalloproteinases (MMPs) [41,42].

In a recent article, *vascular endothelial growth factor* was also identified as a potential PEA3 target gene [43]. Matrix metalloproteinases in turn contribute to metastatic dissemination of tumor cells by degrading the ECM, and their deregulated expression has been associated with the capability of tumor cells to metastasize [41,44,45].

LPP was shown to substantially increase the reporter gene activity of the target gene *COX-2* in a dose-dependent manner when cotransfected with PEA3 and mitogen-activated kinase/ERK kinase [40]. However, the role of the mitogen-activated protein kinase (MAPK) pathway in controlling the activity of the PEA3-LPP complex is still under investigation.

LPP also interacts with the related ETS transcription factor ER81 by enhancing its transactivational potential [40]. Like PEA3, ER81 has been shown to play an important role in breast tumor metastasis as well as human epidermal growth factor receptor 2/*neu*–mediated mammary oncogenesis [46]. In contrast, less effect of LPP is seen on the third

Table 2. LPP Binding Partners and Their Putative Roles in Human Cancer.

Protein	Expression	Localization	Putative Physiological Function	Putative Role in Human Cancer	Reference(s)
LASP-1	Ubiquitous	cc, fa, nuc	scaffolding protein, zyxin-recruitment to fa and cc, NCST	Overexpression in breast, ovarian and liver cancer increases motility and proliferation and is associated with metastasis	[33,34,90–95]
VASP	Ubiquitous	cc and fa	Regulation of actin polymerization, involved in cellular migration	Overexpression in lung cancer is positively correlated with grading and staging Overexpression in breast cancer cells enhances migration	[96–100]
Palladin	Ubiquitous	cc, fa, Z-discs	Actin organization, involved in cell motility, embryonic development, scar formation in the skin, neuronal development	Palladin mutation causes familial pancreatic cancer Overexpression is found in spontaneous pancreatic cancer Overexpression in breast cancer cells enhances migration	[101–105]
Scrib	Ubiquitous	cc, fa, cy	Tumor suppressor; essential for cell shape, polarity, and directed cell migration in early embryonic development	Deregulation leads to breast cancer formation Invasive cervical carcinomas show decrease in Scrib due to ubiquitin-mediated degradation by the high-risk papilloma virus E6 proteins Scrib is downregulated in colon cancer	[51,106–109]
PEA3	Epithelia	nuc	ETS transcription factor regulated by Ras and MAPK pathway	Transcription of prometastatic genes such as serine proteases, urokinase plasminogen activator, COX-2, and several MMPs in various cancers	[40–44,110]
ER81	Mesenchyme	nuc	Related ETS transcription factor of the PEA3 group	Role in breast tumor metastasis as well as HER-2/*neu*–mediated mammary oncogenesis	[40,44–46]
α-actinin	Ubiquitous Z-discs, stress-fibers	cc, fa, F-actin	Scaffolding protein in fa and cc, recruitment of LPP and other signaling proteins involved in cellular motility, actin filament cross-linking, links cytoskeleton to transmembrane proteins	Overexpression of α-actinin isoforms is associated with worse prognosis of patients with astrocytomas, ovarian, breast, lung colorectal, and esophageal cancers	[32,111–115]

All LPP binding partners are dysregulated in various cancer entities. Many of them contribute to tumor invasiveness and migration.
cc indicates cell-cell contact; *cy*, cytoplasm; *fa*, focal adhesion; *NCST*, nucleocytoplasmatic signal transduction; *nuc*, nucleus.

member of this subfamily, ets-related molecule, suggesting a specificity of action [40]. However, the knowledge about the effects of ER81 and its target genes in the context of LPP coactivation is fragmentary.

Recent work has shown that LPP can also act as an independent transcription factor with or without binding to other transcription factors. Moreover, HMGA2/LPP fusion proteins (see also LPP Fusion Genes section) retain the transactivational functions of the LPP LIM domains and thus function as genuine transcription factors [25,47,48].

LPP in Early Embryonic Development

LPP is localized at focal adhesions and cell-cell contacts and is involved in the regulation of SMC migration [49]. A known interaction partner of LPP in human is the tumor suppressor protein Scrib [11,24].

Knockdown of Scrib expression during zebrafish embryonic development results in defects of convergence and extension (C&E) movements, which appear during gastrulation and cause elongation of the anterior-posterior body axis. During vertebrate gastrulation and neurulation, C&E reflects the medial migration and intercalation of mesodermal and neuroectodermal cells. The mediolateral cell polarization mediated through C&E is controlled by the noncanonical *wingless* (*Wnt*) signaling pathway, which represents the vertebrate planar cell polarity pathway [50–52].

Interestingly, silencing of LPP in zebrafish embryos also results in impaired C&E movements, phenocopying noncanonical *wingless-type MMTV integration site family member 11* (*Wnt11*) signaling mutants. The defective dorsal convergence movements are associated with a reduced ability of embryonic cells to migrate along straight paths, affecting both mesodermal and neuroectodermal structures. The embryos with decreased LPP expression showed a shortened body axis, a phenotype also observed for other genes involved in gastrulation [51,53,54]. Furthermore, the expression of LPP is significantly reduced in embryos with morpholino-mediated knockdown of Wnt11 and in embryos overexpressing Wnt11 or a dominant-negative form of Rho kinase 2 (ROK-2), a downstream effector of Wnt11. These data suggest that LPP expression is dependent on noncanonical Wnt signaling and that LPP acts downstream of Wnt11 and ROK-2 [51]. Likewise, an involvement of LPP in ROK-dependent signaling pathways was demonstrated in human iliac vein SMCs [37].

Surprisingly, *Lpp* knockout mice do not present defects of C&E during embryonic development. Moreover, $Lpp^{-/-}$ mice show no increased mortality compared with wild-type littermates and reach adulthood without displaying any obvious abnormalities [7]. This could be in part explained by the functional redundancy of zyxin family proteins because *zyxin* knockout mice also lack any macroscopic abnormalities [55]. However, Scrib and α-actinin mRNA levels were significantly reduced in $Lpp^{-/-}$ mice. In addition, $Lpp^{-/-}$ murine embryonic fibroblasts show reduced migration capacity and viability [7].

LPP in SM Physiology

Little is known about the physiological role of LPP and its operations to ensure normal protein signaling, but to date, LPP is best studied in SMCs [21,36,37,49,56].

Immunofluorescence microscopy demonstrated the almost selective expression of LPP in vascular and visceral SMC [21,37]. Consistently, single nucleotide polymorphisms in the *LPP* gene are associated with severe villus atrophy of the small intestine in celiac disease [57]. *LPP* has also been detected among a core of computationally predicted SM-specific genes from expressed sequence tag data [58] supporting the idea of LPP as a novel marker for SMC differentiation [37].

In other mature (noncultured) tissues, including heart and skeletal muscle, the protein is barely present, and its concentration correlates with the levels of the SM marker α-actin. LPP is present at 100-fold higher protein level in SM-rich tissues, including bladder, uterus, ileum, and aorta, than in non-SM organs, such as liver, heart, or brain. In healthy SM tissues, Gorenne et al. [21] reported detection of LPP in punctate foci at the cell surface where it colocalized with vinculin in peripheral membrane-dense bodies involved in actin filament attachment sites. Overexpression of LPP increased epidermal growth factor–stimulated migration of vascular SMCs, suggesting the participation of LPP in cell motility [21]. Conversely, *LPP* knockout led to reduced migration of murine embryonic fibroblasts [7].

trans-Retinoic acid treatment of A404 cells was shown to significantly increase LPP levels as well as SM α-actin, myosin heavy chain and smoothelin mRNA levels in a ROK-dependent manner. Treatment with the ROK inhibitor Y-27632 led to dissociated focal adhesions as well as reduced LPP staining at the cell periphery. Moreover, enhanced nuclear accumulation of LPP is observed after cell incubation with nuclear export inhibitor leptomycin B [37]. Similarly, treatment of human iliac vein SMCs (HIVS) with leptomycin B caused accumulation of LPP in the nucleus [21]. Therefore, LPP apparently has a potential for relocating to the nucleus through a shuttling mechanism that is sensitive to inhibition of ROK. Cytoplasmic-nuclear shuttling has also been demonstrated for zyxin [59] and TRIP6 [60], suggesting this as a common property for the group 3 LIM proteins.

Thus, it is intriguing to consider a possible signaling role for LPP in SMCs, possibly in the transcriptional feedback control of cytoskeletal proteins.

In adult pig hearts, intact coronary arteries exhibit strong immunostaining for LPP that colocalized with SM α-actin and smoothelin in the tunica media. In contrast, no expression of LPP was found in the adventitial layer. In injured vessels 28 days after stent implantation, neointimal cells migrating around the stent lesion were positive for LPP and SM α-actin but not for smoothelin [37]. Adventitial cells remained negative, whereas LPP expression could be detected in the walls of microvessels located in the adventitial layer [37]. Similar findings were reported for remodeling of injured rat aortic arteries [36]. These dynamic expression patterns in injured arteries suggest that LPP may act in concert with multiple players to facilitate cytoskeletal remodeling events needed to accomplish the transition from stationary to migrating SMCs *in vivo* [36,49,56].

Overexpression of myocardin, a well-known cofactor of the serum response factor (SRF) [61], significantly increased LPP mRNA expression in A404 cells [37]. Interestingly, inactivation of RhoA decreased myocardin mRNA expression in retinoic acid–treated A404 cells and HIVS. In addition, LPP silencing significantly decreased SMC migration. LPP expression was also markedly reduced in *FAK-null* cells known to show impaired migration [36]. Consistently, overexpression of LPP in cultured HIVS increased epidermal growth factor–stimulated cell migration by approximately 2.5-fold in a transwell chemotaxis assay. Moreover, expression of LPP in migration-defective *FAK-null* cells led to a significant increase in cell spreading on a fibronectin matrix. This is in agreement with data demonstrating an up-regulation of LPP in $FAK^{-/-}$ cells with restored FAK expression [36].

These results suggest that levels of LPP in SMCs are controlled by FAK signaling and raise questions about the role of FAK and LPP as determinants of SMC phenotype [21,37].

Recently, Gorenne et al. [37] reported the *LPP* gene being responsive to RhoA-mediated signaling pathways that activate myocardin-dependent transcription in SMC differentiation. In an extending work, Petit et al. [62] focused on the transcriptional regulation of the *LPP* gene by myocardin. The murine *LPP* gene contains three evolutionarily conserved CArG boxes. One box is part of an alternative promoter in intron 2. Petit et al. [62] showed that this promoter is directly regulated by SRF and myocardin, which thereby modulate transcription of LPP in SMC. Overexpression of myocardin resulted in an approximately three-fold increase of porcine *LPP* gene expression after 48 hours [37]. Thus, *LPP* was classified as a novel myocardin/SRF target gene [37,62]. In conclusion, LPP seems to be a SRF/myocardin– and RhoA/ROK–dependent SMC differentiation marker that plays a role in regulating SMC migration [21,37,62].

LPP in Tumorigenesis and Cancer

After the discovery of LPP in lipoma, many other human tumors have been identified, which take advantage of the physiological LPP properties to increase their malignant potential. To date, three different strategies have been identified, which allow tumors to usurp the physiological LPP signaling mechanism to enhance their runaway proliferation:

A) Formation of oncogenic LPP fusion genes
B) Overexpression of LPP leading to the disruption of the normal LPP signaling pathway
C) Abuse of the LPP shuttle mechanism for oncogenic signaling to the nucleus

A) LPP Fusion Genes

LPP is a frequent fusion partner for several oncogenes. The most prominent genes are members of the "high mobility group AT hook" HMGA protein family consisting of four proteins: HMGA1a, HMGA1b, HMGA1c, and HMGA2. All are nuclear factors characterized by three DNA-binding domains, called AT hooks, and an acidic carboxy-terminal tail. The proteins are architectural transcription factors that both positively and negatively regulate the transcription of a variety of genes [63–66], thereby influencing a considerable number of cellular processes including cell growth, proliferation, differentiation, and apoptosis. HMGAs do not directly activate transcription but rather regulate gene expression by changing DNA conformation [63].

Both HMGA1 and HMGA2 are barely detectable in normal adult tissue but are abundantly and ubiquitously expressed during embryonic development [63,65,66]. In malignant epithelial tumors as well as in leukemia, however, expression of HMGA1 is again strongly upregulated to embryonic levels, thus leading to ectopic expression of (fetal) target genes. HMGA2 overexpression has a causal role in inducing neoplasia and a malignant phenotype [5,63,67].

HMGA genes are often involved in chromosomal rearrangements. Such translocations are mostly detected in benign tumors of mesenchymal origin and are believed to be one of the most common chromosomal rearrangements in human neoplasia [63,68–70]. In most cases, *HMGA2* alterations involve breaks within the third intron of the gene resulting in aberrant transcripts carrying exons 1 to 3, which encode the three DNA-binding domains, fused to ectopic sequences [64].

The *LPP* gene is the most frequent translocation partner of *HMGA2* in a subgroup of lipomas. Moreover, *LPP* is also frequently rearranged in cases without cytogenetic detectable involvement of 3q27-q28. To date, two alternative *HMGA2/LPP* hybrid transcripts have been characterized. These two transcripts differ in the number of either two or three LIM domains in the predicted HMGA2/LPP fusion protein [71–73] (Figures 2 and 3, *A* and *B*). Interestingly, both forms are expressed only in the nucleus [22]. A truncated form of HMGA2 carrying the three DNA-binding domains of HMGA2 and the LIM domains of the *LPP* gene caused malignant transformation of NIH3T3 cells, whereas the wild-type HMGA2 does not exert any transforming activity [67]. These findings indicate that, specifically, the fusion of *HMGA2* together with *LPP* achieves oncogenic potential [22,67].

The HMGA2/LPP fusion protein activates transcription through the well-characterized PRDII element, which is a part of the interferon-β (IFN-β) enhancer and known to bind to HMGA2. It was also shown that HMGA2/LPP activates transcription through the HLA-B associated transcript 1 element of the rhodopsin promoter, an HMGA1-binding element. Finally, in a number of lipomas, HMGA2/LPP and HMGA2 are coexpressed with wild-type HMGA2 even augmenting the transactivational functions of ectopic HMGA2/LPP. These results support the concept that the transactivational functions of the novel HMGA2/LPP transcription factor contribute to lipomagenesis [25,64,74].

Recent work showed that transforming growth factor β (TGF-β)–induced expression of HMGA2 was associated with the development of epithelial-mesenchymal-transition (EMT) [75]. Epithelial-mesenchymal-transition occurs during embryogenesis, carcinoma invasiveness, and metastatic dissemination and can be regulated by TGF-β signaling through intracellular Smad transducers. The specific molecular mechanisms that control the initiation of EMT are, to date, widely unknown. Transcriptomic analysis, however, revealed that the Smad pathway induces the *HMGA2* gene during EMT. Endogenous HMGA2 mediates EMT by TGF-β, whereas ectopic HMGA2 causes irreversible EMT characterized by severe E-cadherin suppression. HMGA2 controls the expression of four known regulators of EMT, the zinc-finger proteins Snail and Slug, the basic helix-loop-helix protein Twist, and inhibitor of differentiation 2 [75]. Thus, ectopic overexpression of a hybrid *HMGA2/LPP* gene possibly provides a mechanistic explanation for a disruption of the physiological HMGA2 pathway resulting in tumor development.

The existence of identical fusion genes in different types of tumors is a very common strategy of these cells to promote proliferation. Consistently, *HMGA2/LPP* fusion transcripts have been reported in lipoma, pulmonary chondroid hamartoma (PHC), and soft tissue chondroma [47,48,71–73,76–78]. The high frequency of t(3q27-28;12q14-15) in lipomas and PCHs renders the *HMGA2/LPP* fusion gene the most common fusion gene in human tumors [77,79]. All tumors characterized by this *HMGA2/LPP* gene product share the same structure, that is, a protein composed of the AT hooks of HMGA2 and the LIM domains of LPP. Its common occurrence in PCHs indicates the absence of a larger deletion of the *LPP* locus accompanying the translocation [79,80], as described in a lipoma [73,74]. Moreover, in primary central nervous system lymphomas (PCNSLs, diffuse large B-cell lymphomas confined to the brain), *LPP* was fused to the *BCL6* gene [81]. *BCL6* is a protooncogene encoding a nuclear transcriptional repressor, with pivotal roles in germinal center formation and regulation of lymphocyte function, differentiation, and survival. BCL6 suppresses p53 in germinal center B

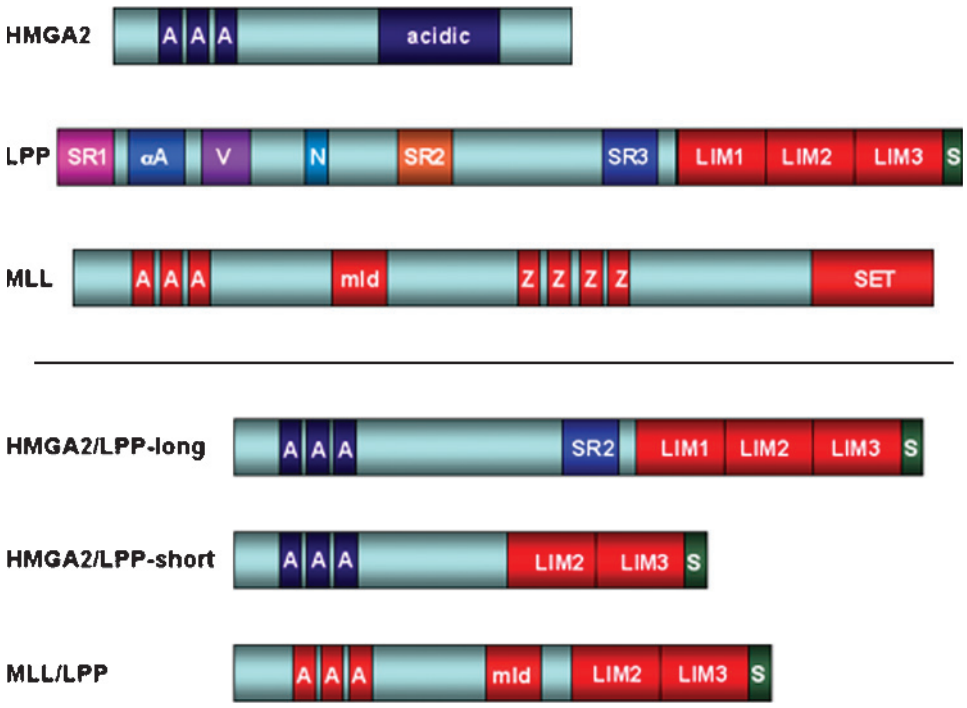

Figure 2. Schematic illustration of the HMGA2, LPP, and MLL proteins (upper panel) and the pathogenic fusion proteins found in human neoplasia to date (lower panel). AT hooks (A) are specialized DNA-binding motifs, acidic domain (acidic), α-actinin binding site (αA), VASP binding sites (V), nuclear export signal (N), LPP/TRIP6 similar region 1 (SR1), LPP/TRIP6 similar region 2 (SR2), zyxin/LPP/TRIP6/LIMD1 similar region (SR3), methyl-transferase–like domain (mld), PHD zinc fingers (Z), set domain (SET), and Scrib-binding motif (S).

cells and its constitutive expression can protect B-cell lines from apoptosis induced by DNA damage [82]. Little is known about chromosomal aberrations underlying PCNSLs. However, fluorescence *in situ* hybridization analysis of 41 PCNSLs revealed that 14 tumors (34%) carried a breakpoint in the *BCL6* locus. All breakpoints were located within the *BCL6* major translocation cluster. In some cases, a deletion in 3q leads to the loss of an 837-kb fragment extending from the first intron of *BCL6* to the third intron of the *LPP* gene. This deletion may bring the *BCL6* gene under the control of regulatory elements of the *LPP* gene. DNA sequencing analysis of the junctional sequences provided evidence that aberrant class switch recombination or somatic hypermutation may be involved in the generation of *BCL6* translocations [81]. In addition, in a more recent study, a high LPP expression was associated with better treatment response of patients with B-cell lymphomas. This improved clinical outcome was possibly because of LPP-induced expression of genes responsible for the increased differentiation of the leukemic B cells [83].

Another gene, which was found to be rearranged with *LPP*, is *MLL*, the mixed lineage leukemia gene. MLL/LPP fusion transcripts were detected in a patient with secondary leukemia (AML-M5, FAB classification) after treatment with DNA topoisomerase II inhibitors. Fluorescence *in situ* hybridization and Southern blot analyses identified a rearrangement in the *MLL* gene because of a novel t(3;11)(q28;q23) chromosomal translocation in AML cells 3 years after chemotherapy for a follicular lymphoma. Through inverse polymerase chain reaction, the *LPP* gene on 3q28 was identified as the *MLL* fusion partner. The predicted MLL/LPP fusion protein included the AT hook motifs and methyltransferase domain of *MLL* joined to the two last LIM domains of *LPP*. The reciprocal LPP/MLL transcript, predicted to include the proline-rich and leucine zipper motifs, and the first LIM domain of *LPP* were also detected by reverse transcription–polymerase chain reaction. The new tumor-specific fusion proteins MLL/LPP and LPP/MLL contain many features present in other *MLL* rearrangements [84] (Figure 2) and, as such, could be used as promising novel drug targets owing to their unique presence in such tumors and absence in normal tissue. Interestingly, all these tumor-specific fusion proteins, composed of AT hooks from other proteins, are mainly localized within the nucleus [22] and possibly contribute to altered LPP transcriptional targeting [63,64] (Figure 3*B*).

B) Overexpression of LPP Disrupts Normal LPP Signaling

LPP is highly upregulated by gains on 3q in squamous cell lung carcinomas turning LPP into a potential candidate for pathogenesis and diagnosis of lung cancer [85]. Amplification of the 3q region accompanied by a 20-fold overexpression of LPP was detected in primary sarcomas and sarcoma cell lines pointing to an essential role of LPP overexpression in tumorigenesis not only of epithelia but also of tissues of mesenchymal origin [86].

LPP is a coregulatory binding partner for PEA3 [40]. PEA3 is a member of an ETS domain transcription factor subfamily and regulated by a number of signaling cascades including the MAPK pathways and Ras. PEA3 activates gene expression and is thought to play an important role in promoting tumor metastasis [44,45]. LPP forms a complex with PEA3 and is found to be associated with PEA3-regulated promoters. By manipulating LPP levels, it is possible to upregulate the transactivation capacity of PEA3 in a dose-dependent manner [40]. Therefore, overexpression of LPP leads to an up-regulation of PEA3 target genes. In a similar fashion, LPP can functionally interact with the related PEA3 family member ER81. Thus, beyond its own function as an independent transcription factor, LPP has

an additional nuclear function as a transcriptional coactivator [40] (Table 1).

Among the PEA3 target genes are several MMPs [40]. Matrix metalloproteinases in turn contribute to metastatic dissemination of tumor cells by degrading the ECM, and their deregulated expression has been associated with the capability of tumor cells to metastasize. In fact, PEA3 group genes are often overexpressed in different types of cancer that also overexpress MMPs and that display a disseminating phenotype [41,44,45].

Hence, it is tempting to speculate that overexpression of LPP could cause an up-regulation of PEA3 targets such as MMPs resulting in an increased invasion and metastasis potential (Figure 3*C*). In support of this hypothesis, nonmetastatic breast cancer cells become metastatic when PEA3 is ectopically overexpressed [44,45]. LPP might be the link communicating changes in cytoskeleton or ECM contacts associated with invasion and metastasis into PEA3-mediated changes in gene expression profiles.

In comparison to LPP, zyxin overexpression has also been associated with increased invasiveness and migrational abilities of human hepatocellular carcinoma cells [87]. However, zyxin gene transfer into an Ewing sarcoma model resulted in the inhibition of tumor growth accompanied by reconstitution of zyxin-rich focal adhesions, reorganization of the actin cytoskeleton, and decreased cell motility [88]. Thus, overexpression does not represent *per se* a consistent mechanism for tumorigenesis but seems to depend on the cellular context.

C) LPP, an Abused Shuttle Protein

As LPP continually shuttles between the cell periphery and the nucleus, it represents a potential novel link between cell surface events and changes in gene expression [1]. LASP-1, another focal adhesion protein, that is found to be overexpressed in human breast and ovarian cancer, is known to interact with zyxin and LPP [27,33,34,89]. Although LASP-1 has no classic nuclear import signal of its own, it translocates into the nucleus. Therefore, it was speculated that LASP-1 might bind to shuttle proteins such as zyxin and LPP to use their transportation potential into the nucleus [2]. Hypothetically, overexpression of LPP binding partners such as LASP-1 in human neoplasia could interfere with normal LPP signaling, resulting in an altered transcriptional signature of LPP that in turn could contribute to tumorigenesis (Figure 3*D*). A summary of the putative roles of LPP binding partners in human cancer is depicted in Table 2.

Conclusions and Future Prospects

In synopsis, it seems to be a common mechanism for tumors showing a high rate of genetic instability to fuse protooncogenes such

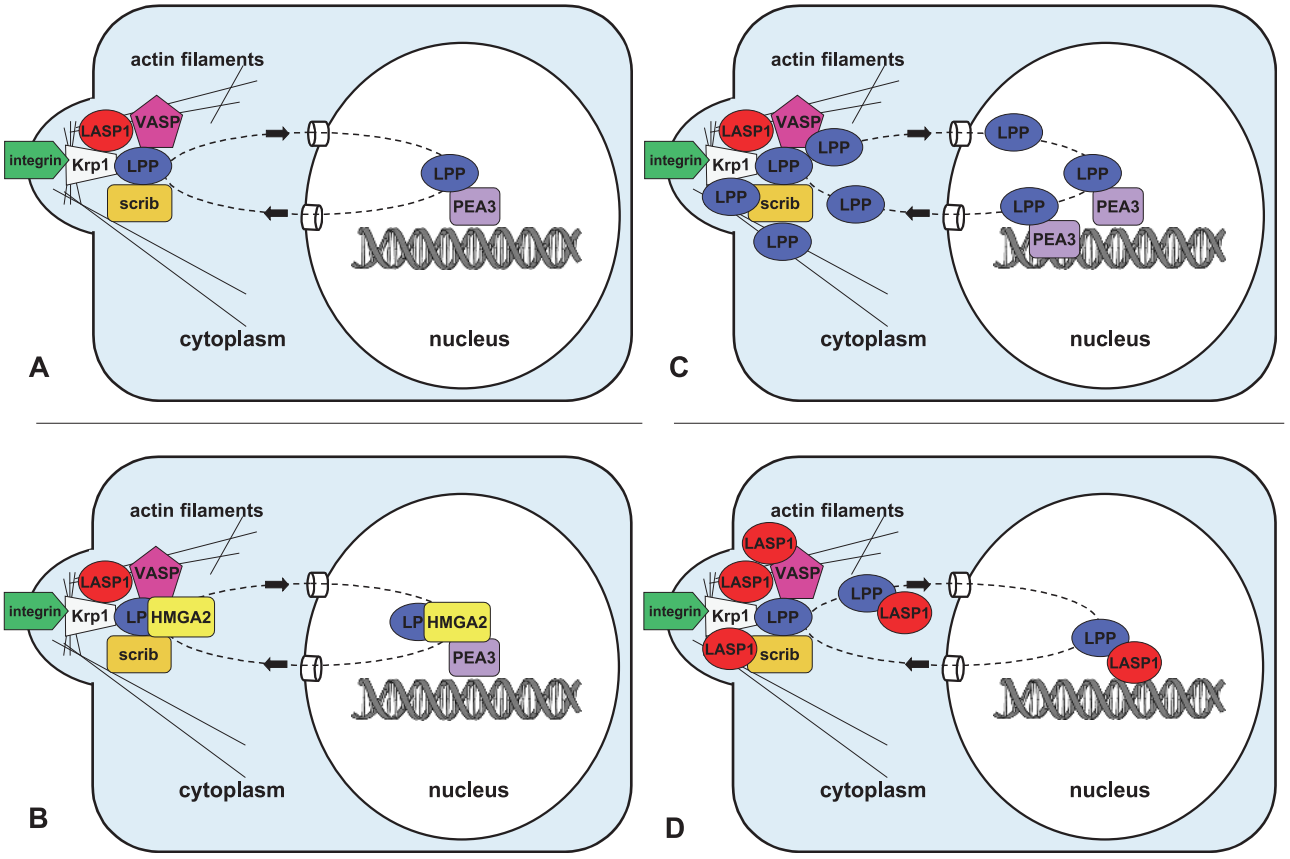

Figure 3. Schematic illustration of the hypothetical, physiological (A), and pathological (C–D) LPP signaling in human neoplasia. (A) Physiological LPP signaling: LPP permanently shuttles from cytosol into the nucleus and back, thereby transducing signals from the cell surface to the transcription machinery and possibly vice versa. (B) Abnormal LPP signaling through formation of fusion proteins with altered transcriptional signature. As an example, the most common fusion protein of the truncated HGMA2 with the LIM domains of LPP is shown. (C) LPP overexpression leads to enhanced and possibly changed transcription of LPP target genes. (D) Overexpression of LPP binding partners: overexpression of, for example, LASP-1 in tumor cells may lead to enhanced protein binding to LPP, increased nuclear transportation of the shuttle partners, and potentially pathophysiological gene regulation.

as *HMGA2*, *MLL*, and *BCL6* with the *LPP* gene. These translocations lead to the formation of novel fusion gene products that are highly specific for tumor cells, turning these fusion proteins into interesting candidates for the rational design of specific antitumor medication. Furthermore, overexpression of LPP itself or its interacting binding partners seems to be another mechanism for tumorigenesis through interference with normal LPP signaling.

To date, there is an increasing line of evidence that LPP plays an important role in the formation of a variety of human cancers. The physiological role of LPP, however, remains largely unknown. Thus, more work has to be undertaken to reveal the physiological function of LPP and to delineate the functional differences between normal and altered LPP signaling.

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