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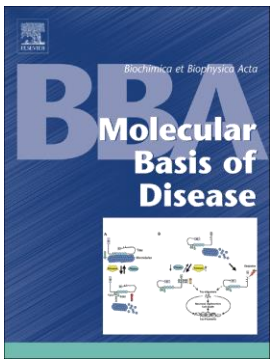

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The LMTK-family of kinases: Emerging important players in cell physiology and disease pathogenesis

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Abstract

Lemur Tail (former tyrosine) Kinases (LMTKs) comprise a novel family of regulated serine/threonine specific kinases with three structurally and evolutionary related members. LMTKs exercise a confusing variety of cytosolic functions in cell signalling and membrane trafficking. Moreover, LMTK2 and LMTK3 also reside in the nucleus where they participate in gene transcription/regulation. As a consequence, LMTKs impact cell proliferation and apoptosis, cell growth and differentiation, as well as cell migration. All these fundamental cell behaviours can turn awry, most prominently during neuropathologies and tumour biogenesis. In cancer cells, LMTK levels are often correlated with poor overall prognosis and therapy outcome, not least owned to acquired drug resistance. In brain tissue, LMTKs are highly expressed and have been linked to neuronal and glia cell differentiation and cell homeostasis. For one member of the LMTK-family (LMTK2) a role in cystic fibrosis has been identified. Due to their role in fundamental cell processes, altered LMTK physiology may also warrant a hitherto unappreciated role in other diseases, and expose them as potential valuable drug targets.

On the backdrop of a compendium of LMTK cell functions, I hypothesize that the primary role of LMTKs may dwell within the endocytic cargo recycling and/or nuclear receptor transport pathways.

1. Introduction: Salient features of LMTK-family kinases

All living organisms use protein modifications in order to adjust and regulate cell behaviour in response to external cues. Protein kinases are one such class of modulators that help to coordinate multi-tiered intracellular signalling and transport pathways by adding a phosphate group to their specific substrates. Phosphorylation causes conformational changes in the respective molecules leading to changes in their binding specificity and/or affinities. Phosphorylation often regulates plasma membrane residency and abundance of proteins/receptors through multiple endocytic pathways.

Interestingly, amino acid sequence alignments and evolutionary pedigree analysis have classified kinases into distinct families, e.g. the cell surface receptor tyrosine kinase (RTK) family, an intensively investigated group [1]. Less highlighted but nevertheless highly conserved, places the three members comprising Lemur Tail (former Tyrosine) Kinases (LMTKs) at a more recent branch of kinase evolution.

All LMTKs feature a short N-terminal segment followed by a kinase domain and a long C-terminal tail, taking their name in reference to the long tail of Lemurs (Figure1) [2]. Sequence analysis and experimental evidence indicate a N-terminal signal peptide and a transmembrane (TM) region. Indeed, topological studies using fluorescence protease protection experiments concluded that LMTK2 is an integral membrane protein with two transmembrane domains, positioning both the N-and C-terminals in the cytoplasm [2, 3].

Similar studies for LMTK1 and LMTK3 have not been conducted; therefore, their exact membrane topology remains elusive. However, LMTK1 is subjected to alternative splicing giving rise to two isoforms, LMTK1A without and LMTK1B with a presumed transmembrane domain [3]. However, three palmitoylated cysteines have been identified that localise LMTK1A to intracellular vesicle membranes of transferrin positive recycling endosomes in cortical cultured neurons and COS-7 cells [4].

The short N-terminal region is followed by a highly conserved kinase domain, initially suggestive of tyrosine kinase activity that had been deduced from sequence homology with other known tyrosine kinases [5]. However, *in vitro* pepscan, using the LMTK2 kinase fold attached to its adjacent proposed specificity conferring C-terminal segment, revealed that phosphorylation activity is strictly restricted to serine/threonine residues that are either preceded or followed by proline residues [6].

Another specific feature shared by LMTKs relates to their long C-terminal proline-rich region [5]. Additionally, LMTK3 contains three novel distinctive poly-proline motives whose function is unknown. Embedded in these poly-proline regions X-proline-proline-X-proline (where X represents an aliphatic amino acid) motifs are found that potentially can interact with SRC Homology 3 Domain (SH3) containing proteins. The SH3 interactome revealed that SH3 domain containing proteins significantly participate in endocytosis but also in the remodelling of the cytoskeleton and in signal transduction pathways [7, 8].

There is an abundance of recent review articles that provide comprehensive summaries of the many examples of RTKs that undergo nuclear translocation [9]. *In silico* sequence analysis predicts nuclear localisation and export signals for both LMTK2 and LMTK3. Indeed, although both kinases are predominantly part of cytoplasmic endomembranes, they are also detected in the nucleus (see below) [10]. Physiological relevance to nuclear localisation was given recently to LMTK2-androgen receptor (AR) complexes that reside in the nucleus (see below) [10]. In addition, it was found that under importin 1 knockdown conditions, nuclear levels of LMTK3 were reduced concomitant with increased cytoplasmic levels [11]. Subsequently, chromatin- immunoprecipitation experiments revealed chromatin association of LMTK3. These data together are suggestive of a role of either LMTK proteins in chromatin scaffolding and/or receptor mediated specific gene regulation [10, 11]. However, no such data about potential dual LMTK1 localisation are available.

Mechanisms that lead to nucleocytoplasmic equilibrium have yet to be untangled. For instance, it remains an intriguing question whether similar to RTKs, auto phosphorylation and/or protein cleavage occurs in spatiotemporal regulation of LMTKs (see below).

LMTK2 and LMTK3 but not LMTK1 knockout mice have been generated [12, 13]. LMTK2 -/- and LMTK3 -/- mice are viable and grossly normal suggesting that either protein is dispensable for embryonic and postnatal survival, however LMTK2 knockout mice produce sterile male progenies through the inhibition of spermatogenesis [13]. It is not known whether partly functional redundancy or other compensating mechanisms cause non-essentiality.

Finally, each LMTK family member has been described independently in different laboratories giving rise to multiple synonyms for each of the three LMTKs (see www.uniprot.org). Therefore, for practical reasons, we will refer to the family members as LMTK1-3.

2. LMTKs substrates and other binding partner(s)

Substrate specificity and spatio-temporal regulation is a pivotal characteristic of all kinases. Regulation often involves additional upstream acting kinases. In addition, substrate-binding kinases often summon molecules that together orchestrate particular transduction pathways.

2.1. LMTK substrates

To date, LMTK2 has been reported to bind to and phosphorylate threonine 320 of the serine/threonine protein phosphatase 1C (PP1C), a phosphatase often associated with its role in the turnover of membrane proteins/receptors [14]. Binding of PP1C to LMTK2 utilises a valine-threonine-phenylalanine motif in LMTK2. Cyclin-dependent kinase 5 (Cdk5) increases the phosphorylation activity of LMTK2 to PP1C that in turn attenuates PP1C phosphatase activity (see below) [14]. Additionally, PP1C is also subjected to regulation through LMTK2 dependent inhibitor-1 (Inh-1) binding

to PP1C [6]. Since PP1C has manifold cellular targets, LMTK2 regulation of PP1C/Inh-1 triggers coordinated responses affecting different targets and pathways, simultaneously.

Serine737 of cystic fibrosis transmembrane conductance regulator (CFTR) has also been described as another LMTK2 phosphorylated residue modulating residency and recycling of CFTR at the plasma membrane [see below) [15].

With the caveat of having used solely the kinase domain of LMTK3, estrogen receptor alpha (ER) has been identified as a LMTK3 substrate, although the phosphorylated amino acid residue has not yet been determined and the verification of the obtained *in vitro* results with a more complete recombinant LMTK3 protein is still pending [16](Figure 2).

Although not directly proven, LMTK3 loss-of-function experiments suggested that hepatocyte growth factor (HGF) induced LMTK3-dependent phosphorylation of serine 435 in Rab-Coupling Protein (RCP), a Rab11/14 GTPase effector protein involved in endocytic recycling [17].

Interestingly, amino acids juxtaposed to the LMTK2 in CFTR and LMTK3 in RCP phosphorylation sites show a high degree of sequence similarity (peptide corresponding to CFTR residues 733–741 (ERRLpSLVPD) and 430-443 RCP (ESRRSpSLLSLMTGK). Whether this is an indication for functional redundancy has not yet been scrutinised. Moreover, it is unknown whether additional substrate specific phosphorylation sites exist for either kinase [15, 17].

It has been reported that both LMTK1 and LMTK2 can also undergo autophosphorylation specifically on serine/threonine residues. In granule cells, LMTK1 becomes hypophosphorylated under depolarising culture conditions and hyperphosphorylated under apoptosis inducing conditions [18]. Mutations in serine 480, serine 558, and serine 566 suppressed hyperphosphorylation and apoptosis suggesting an involvement of these residues in apoptotic functions. *In vitro* experiments with LMTK2 kinase domain also revealed autophosphorylation, however, the identification of the affected residues awaits investigation [6]. Nevertheless, LMTK3 has not yet been reported to undergo

autophosphorylation. This autophosphorylation of LMTKs is a feature reminiscent of RTKs that undergo autophosphorylation upon pathway induction.

Finally, no direct substrate has as yet been determined for LMTK1. However, based on the conserved primary structure of the kinase domain, the assumption holds that serine/threonine specificity extends to all LMTK members including LMTK1.

2.2. Scaffolding functions of LMTKs

Despite substrate binding/phosphorylation, LMTKs can also serve as scaffolds that assemble regulatory and other molecules that act in concert leading to a common response.

One prominent example for a scaffolding function of LMTKs is exhibited by LMTK1. It has been suggested that LMTK1 inhibits the activation of the plasma membrane, osmoregulator Na-K-2Cl cotransporter, NKCC1, by scaffolding the inhibitory protein phosphatase 1 (PP1) in close proximity to the stimulatory serine-threonine kinase SPAK/WNK4, hence resulting in the dephosphorylation of SPAK [19, 20]. While the kinase activity of LMTK1 was dispensable for PP1 suppression of NKCC1 function, binding of LMTK2 led to the phosphorylation- mediated regulation of PP1C activity (see above). The discrepancy in the physiological role of kinase activity of LMTKs warrants future studies in the exact mechanisms of ATP binding/hydrolysis.

2.3. Interaction of LMTKs with regulatory kinases

Kinases as part of tiered signalling are often subjected to regulation that ultimately connect with intrinsic or external cavalcades. Several upstream kinases have been shown to affect and regulate LMTK kinase activities. Accordingly, LMTK1A and LMTK2 bind to the cyclin-dependent kinase (CDK5) activator protein p35 [14, 21]. P35 although preferentially expressed in neurons, is, however, also expressed in the breast cancer cell line MCF-7 (Wendler, unpublished data). LMTK1 and LMTK2 can undergo Cdk5/p35 dependent phosphorylation, with Serine 34 in LMTK1 and serine 1418 in LMTK2 serving as the respective phosphorylation sites [14, 21]. Interestingly, the p35-binding site for both

LMTK1 and 2 is placed on the C-terminal side adjacent to the kinase domain. However, the actual Cdk5 phosphorylation sites in LMTK1 and LMTK2 are located on opposite ends of the respective kinases. This fact renders it rather inconceivable that both Cdk5 phosphorylation sites are functionally related.
In silica analysis predicts additional CDK5 consensus phosphorylation sites in either protein and also in LMTK3. However, no evidence has been reported for Cdk5-dependent LMTK3 phosphorylation.

The non-RTK Src, a protein required for the internalisation of integrins and RTKs, focal adhesion turnover, and cell spreading and migration, was described as a kinase with potential regulatory function for LMTK1A [4, 22]. Src kinase directly interacts with N-palmitoylated LMTK1A and phosphorylates tyrosine 25 and tyrosine 46 juxtaposing the Cdk5 phosphorylation site at serine 34 [4]. It has been speculated that Cdk5 dependent phosphorylation prevents Src dependent phosphorylation and *vice versa,* regulating endocytic vesicle flow in axonal and dendritic cell protrusions [23].

Myosin VI, a plus-to-minus directed, actin-based, unconventional motor protein that acts in retrograde membrane transport interacts with LMTK2. A region, close to the kinase domain of LMTK2 that overlaps with CDK5/p35 binding, binds to the WWY motif containing C-terminal segment of myosin VI that in turn can also bind to disabled homolog 2 (Dab2), an adapter protein linking endocytosis with the cytoskeleton [24, 25]. The potential mechanistic relationship between LMTK2, CDK5/p35, myosin VI and potentially Dab2 in vesicle selection/transport remains to be interrogated.

Finally, the investigation of nuclear functions of LMTK3 revealed KRAB-associated protein 1 (KAP1) as a binding partner (see below) [11].

In conclusion, activities of LMTKs are subjected to regulation, by regulators such as Cdk5/p35 and Src, important players during cell differentiation. Moreover, binding proteins have been identified that link LMTKs to protein transport. Therefore, LMTKs might serve as regulated platforms for the turnover/recycling of surface proteins.

3. Regulated LMTKs functions in endocytic recycling pathways

Engagement of plasma membrane cargo with the intracellular transport machinery depends on the binding of pathway-specific adaptor molecules and coat proteins. Cargo modification such as phosphorylation plays a pivotal role in this process and in some instances triggers internalisation.

Co-localisation studies with Rab5 (EE, early endosome), Rab11 (RE, recycling endosome), and Rab7 (LE, late endosome) established that LMTK1-3 are integral to endosomal recycling but not to late endosomal/lysosomal degradation pathways (Figure 3). In addition, the LMTK regulators Src and Cdk5/p35 have been linked to endosomal vesicle transport [26, 27]. Therefore, it stands to reason that LMTK kinase activities may select/modulate substrate recycling. For instance, data from Cos-7 cells indicate that LMTK1A associates with p35 in early and recycling endosomes. Moreover, in PC12D cells, nerve growth factor (NGF) stimulated Cdk5/p35 dependent phosphorylation that in turn inhibited Src dependent tyrosine phosphorylation of LMTK1A suggesting a Cdk5/p35 Src interplay [23]. Subsequent reports by Takano *et al* placed LMTK1A upstream of Rab11A, regulating endocytic recycling in CHO-1 and mouse cortical neurons (see below) [28, 29].

LMTK2 is part of the signalling cascade leading to kinesin-1 dependent anterograde transport of axonal cargo along microtubules. Kinesin-1 is a heterotetrameric protein complex consisting of two identical motor protein subunits and two “light chains” (KLC). Glycogen synthase kinase-3β (GSK3 binds and phosphorylates un-phosphorylated KLC thus releasing cargo, which includes synaptic vesicles, amyloid precursor protein (APP) or other proteins such as mothers against decapentaplegic homolog 2 (Smad2) transcription factor, whose presence shifts from the cytoplasm to the nucleus upon TGF [30].

The TGF is manifoldly implicated in cell-fate determination and tissue re-generation. It has been reported that in a chain of events, Cdk5/p35 phosphorylates LMTK2 at serine 1418. Activated LMTK2 blocks PP1C activity through phosphorylation at threonine 320, which in turn

leads to enhanced inhibitory phosphorylation of GSK3β at serine 9 [14]. Inhibited GSK3β is no longer able to phosphorylate KLC leading to premature Smad2 release. Altered TGF /Smad2 signalling affects a wide range of cellular processes including the regulation of cell division and apoptosis, differentiation, and cell migration [31, 32].

As discussed above, LMTK2 physically interacts with myosin VI. Both LMTK2 and myosin VI have been attributed to EE and tubular RE suggesting that LMTK2 and myosin VI might act together in consecutive parts of the early recycling pathway, i.e. from the plasma membrane (PM) to the EE and the RE compartment.

For instance, apical PM surface density of CFTR, an ATP-gated anion channel depends on both LMTK2 and myosin VI [15]. Studies in human bronchial epithelial cells (HBE) revealed that endogenous LMTK2 physically interacts and phosphorylates CFTR (serine 737) at the apical cell membrane. Expressing a kinase domain truncated form of LMTK2 abolished this phosphorylation and consequently decreased CFTR internalisation. In addition, depletion of either myosin VI or LMTK2 resulted in a significant decrease in the recycling of CFTR to the PM [24]. LMTK2 regulation of CFTR recycling has severe physiological implications affecting CFTR-mediated chloride secretion in mice and human.

Interestingly, myosin VI not only acts in endocytic recycling but also displays nuclear functions through the binding to and the activation of RNA-polymerase II [33, 34]. Since the myosin VI-binding partner LMTK2 also partly resides in the nucleus, it will be of interest whether their functional relationship also extends to gene transcription regulation. In this context, it is noteworthy that LMTK2-androgen receptor (AR) complex is also detected in the nucleus, prompting the question for the existence of a tri-partite AR-LMTK2-myosin VI complex that could trigger the onset of AR specific gene transcription [10]. Nuclear myosin VI localisation is also crucial for ER dependent regulation of gene regulation [33]. Since LMTK3 binds to ER and also partly resides in the nucleus one could speculate

whether a tri-partite LMTK3/myosin VI/ ER [illegible]x exists and triggers transcriptional gene activation. However, LMTK3/myosin VI interaction has not been reported.

In conclusion, it will be of the utmost interest to understand the functional relations of LMTK2/LMTK3 with motor proteins such as myosin VI or possibly other cytoplasmic/nuclear myosins also; how they relate to each other and how nucleocytoplasmic distribution is achieved and regulated.

Another line of evidence suggested that LMTK3 might directly modulate endocytic trafficking by the phosphorylation of endocytic trafficking regulators. It was found that upon hepatocyte growth factor induction, LMTK3 phosphorylates the Rab11/14 effector protein RCP in H1299 cells, a non-small cell lung cancer cell line. Thereupon, phosphorylated RCP engages with Rab14 positive recycling vesicles that carry RTK ephrin receptor A2, shunting it to an alternative recycling pathway back to the PM that ultimately leads to cell:cell repulsion and enhanced cell migration [17].

Finally, cultured primary neuronal cells obtained from LMTK3 knockout mice accumulated Glu2NB-containing NMDA receptors that are preferentially transported through the endosomal recycling pathway. Although AP2 binding to LMTK3 was observed, it was not determined whether the AP2 or the NMDA receptor additionally become LMTK3 phosphorylated during transport [12].

To summarise, we conclude that LMTKs prime different proteins for intracellular transport. Their exact function in cargo selection/sorting remains elusive. However, the involvement of LMTKs in endocytic trafficking warrants a multitude of pleiotropic downstream consequences for cell physiology, architecture and behaviour, and ultimately for tissue function and development.

4. Neuronal cell functions of LMTKs

All LMTKs are highly expressed in brain tissue of the cortex, hippocampus and cerebellum. Consequently, all three family members have been reported to execute physiological functions in neuronal tissue development [12, 28, 35].

The major isoform of LMTK1, LMTK1A, is implicated in dendritic formation and anterograde membrane delivery during neurite outgrowth [29]. Cdk5/p35 that is highly enriched in neuronal cells plays a pivotal role in both these processes. In axons of *C. elegans,* a kinase-ablated form of Cdk5 promotes neurite outgrowth consistent with results obtained in cells with diminished LMTK1A levels, or where a form of LMTK1A in which the Cdk5/p35-phosphorylation site was mutated to an alanine is expressed [28, 29, 36]. Thus LMTK1A functions as a negative regulator of neurite outgrowth. Accordingly, primary neurons obtained from LMTK1A -/- mice increased the prevalence of anterograde transport of Rab11A positive vesicle membranes to the sites of growth.

However, contrasting results have been obtained in immature granule cells where overexpression of wild-type LMTK1A promoted and a kinase-defective mutant significantly inhibited neurite outgrowth suggesting a positive regulatory function for LMTK1A. On the same note, experiments in cerebellar and cortical neurons, and in retinal ganglion cells conclude that Cdk5/p35 inhibition can reduce neurite length [37-39]. Context specific roles of LMTK1A may explain these contrasting results and promote future research in the exact mechanistic relationship between both Cdk5/p35 and LMTK1A.

Despite its role in neuronal cell differentiation, overexpression of LMTK1 led to enhanced apoptotic cerebellar granule cells, which suggests a role in the establishment of proper neuronal connectivity during brain development and aging.

LMTK1 functions, however, are not only restricted to neuronal cells as its upregulation has also been detected during oligodendrocyte differentiation. It has been speculated that enhanced LMTK1A levels during differentiation could lead to the modulation of the levels of GPR17, a G-protein coupled receptor at the cell surface, through reduced degradation and enhanced receptor recycling, a key event during oligodendrocyte differentiation [40].

LMTK2 was also identified as a negative regulator, which when introduced as a kinase dead version into PC12 cells, enhanced neurite outgrowth during NGF induced neuronal cell differentiation, suggesting a role in NGF-TrkA signalling [41].

As discussed further above, LMTK2 also participates in the TGF /Smad2 signal transduction chain leading to the expression of TGF -responsive genes that are instructive during neuronal cell development [42]. In an Alzheimer's disease tau mouse model, impeded TGF Smad2 transport can be traced back to decreased LMTK2 expression levels. Furthermore, it has been speculated that decreased LMTK2 levels and/or kinase activity could potentially lead to aberrant tau hyperphosophorylation due to elevated GSK3 □□□□□□□□ (excellently discussed in [43])

Functional/mechanistic studies linking LMTK3 functions to neuronal cell development are limited. Primary neurons obtained from wild type and LMTK3 -/- knockout mice have not shown any significant difference in axon length and arborisation of dendrites. Most interestingly, however, mice lacking LMTK3 displayed significant behavioural differences compared to the control group, including hyperlocomotion, reduced anxiety and depression-like behaviour. Abnormal behaviour was supported by the increased turnover of dopamine and chemical imbalance in the brains of deficient mice, a phenomenon associated with decreased anxiety [12]

To summarise, we conclude that all three LMTK kinases exhibit functions during neuronal development that are maintained throughout adulthood. Moreover, imbalanced/decreased LMTK function/levels may play a part in neurodegenerative pathologies.

5. LMTKs and Cancer

As LMTKs play a part in intracellular trafficking, signalling and gene expression, it is not surprising that changes in their physiological levels/activities impact cell behaviour, including cell division, proliferation, migration and invasive capabilities.

A wealth of cancer related publications reported alterations in LMTK expression/protein levels. Accordingly, tumour cells derived from different tumour types can correlate either positively or negatively with LMTK expression/protein levels suggesting context specificity. Due to the overall low cellular abundance of LMTK proteins in cells including cancer cells, correlation is often based on mRNA levels, which might not necessarily correspond to translated protein: mRNA levels might be subjected to altered regulatory mechanism in the respective cancer cell context.

Consequently, much of the available experimental evidence describing a role of LMTKs in cancer cells is based on either loss-of- and/or gain-of- function (GOF) experiments whereas endogenous LMTK protein escapes detection. Results solely based on GOF experiments might produce new aberrant phenotypes and often skew the conclusions therefore.

With this framework in mind, I will focus on the cell physiological consequences caused by altered LMTK levels and/or functions.

Univocally, elevated levels of Src activity has been linked to cell growth during cancer progression promoting cell proliferation and survival, angiogenesis, and migration/invasion pathways in colon, liver, lung, breast, pancreas tumours and metastatic melanoma. Signal transduction pathway analysis in metastatic melanoma cell lines revealed an inverse correlationship between LMTK1 expression levels and activated Src. Occuring lower LMTK1 mRNA levels in metastatic melanoma cell lines led to increased levels of activated Src protein, whereas ectopic overexpression of LMTK1 in primary and metastatic derived melanoma cell lines decreased activated Src levels. Consequently, abrogated LMTK1 levels promoted cell migration while its overexpression resulted in a modest inhibition of cell proliferation and colony formation. Importantly, apoptosis was promoted when either LMTK1 overexpressing cell line was grown in phosphate-buffered saline alone suggesting a a role for LMTK1 in mediating coordinated cellular responses to nutrient supply [44]. However, no physical association between LMTK1 and Src has been detected in melanoma cells putting a question mark on the putative nature of the crosstalk between these two kinases.

LMTK2 is frequently mutated in lung adenocarcinoma and pulmonary sarcomatoid carcinoma [45, 46]. In addition, single-nucleotide polymorphisms (SNPs) in the LMTK2 gene have been identified in colorectal adenocarcinoma and prostate cancer that increases the susceptibility for prostate tumours more significantly in Asian men cohorts [47].

LMTK2 protein levels are also often down-regulated in human prostate cancer tissue in comparison to adjacent non-cancerous tissue [48]. One potential mechanism by which down-regulated LMTK2 may increase the susceptibility for prostate cancer might involve the regulation of AR functions. Under androgen-deprived conditions, LMTK2-AR complexes are found primarily in the cytoplasm. In the presence of the synthetic androgen, R1881 AR-LMTK2 complexes are also detected in the nucleus (see also above). Furthermore, experiments in the androgen-dependent prostate cancer cell line, LNCaP, indicated that loss of LMTK2 results in ligand enhanced transcription of androgen-responsive genes professed to reduce AR degradation and enhanced transcription [10].

Nevertheless, the exact mechanisms through which LMTK2 affects AR responses remain unknown. The further above discussed Cdk5/p35 dependent LMTK2 regulation of PP1C phosphatase activity might provide a promising link. AR-responsive genes are upregulated under LMTK2 depletion conditions. On the other hand, PP1C-dependent dephosphorylation of serine 650 in AR causes a decrease in AR-transcriptional activity. Since LMTK2 phosphorylation of PP1C leads to PP1C phosphatase inhibition, these discordant results prevent an obvious LMTK2/ PP1C link in the regulation of AR activities [49].

Lastly, LMTK2 silencing also increased the sensitivity of immortalized epithelial and cancer cells to TNF-related apoptosis-inducing ligand (TRAIL) treatment through down-regulation of the BCL2-family anti-apoptotic proteins and the reciprocal up-regulation of the pro-apoptotic protein in a GSK3β- and PP1A-dependent manner suggesting a role of LMTK2 in drug resistance [Conto, 2017, Cancer letter].

Ample data have been gathered implying LMTK3 as a survival factor in different forms of cancers. For instance, LMTK3 has been identified as a positive regulator in the Wnt3a induced Wnt/β-catenin signalling cascade but has not been further investigated [50]. Deregulated Wnt/ β-catenin signalling contributes to the progression of various cancers including colorectal, hepatocellular carcinoma and breast cancer [51].

Furthermore, a siRNA kinome screen identified LMTK3 as a positive regulator of ERα-dependent transcriptional activity in MCF-7 breast cancer cells [16, 52]. LMTK3 enhances both ERα expression and protein stability presumable through direct phosphorylation of ERα. Interestingly, this places LMTK3 in analogy to LMTK2 for a role in steroid hormone receptor stability/-dependent gene regulation (Figure 4).

LMTK3 is also often indirectly involved in numerous cellular signalling pathways, although the exact mechanisms by which regulation is achieved often remains obscure. For instance, Giamas *et al* drew a model in which LMTK3 acts by decreasing protein kinase C (PKC)-dependent kinase B (PKB/AKT) phosphorylation and activity that also contributes to ERα stability. Additionally, decreased PKB/AKT prevents forkhead box O3 (FOXO3) degradation. As a consequence FOXO3 stability is enhanced leading to an upregulation of ERα-gene expression [16].

Finally, overexpression of LMTK3 in the low LMTK3 expressing breast cancer cell line led to a chain of events that ultimately resulted in enhanced transcription factor serum response factor (SRF), and induced increase of integrin α5 and β1 subunits expression. Changes in the cytoskeleton promote invasive cell behaviour, thus it was delineated that integrin subunit expression was dependent on LMTK3 interaction with growth factor receptor-bound protein 2 (GRB2). Interaction activated CDC42 Rho family guanosine triphosphatase that in turn promoted the RAS downstream signalling cascade, eventually increasing gene expression of integrins α5 and β1 [53]. Notably, Grp2 is a SH3 domain containing protein that might bind to putative SH3 binding motifs in LMTK3. Moreover, Grp2 also binds to dynamin, a guanosine triphosphatase receptor, opening the possibility that LMTK3 regulates Grp2

cell surface residency and therefore activity. A more detailed investigation of the interplay between LMTK3 and Grp2 receptor recycling will be instructive to elucidate the exact mechanism on how LMTK2 affects metastatic behaviour.

On the other hand, Caswell *et al* have shown that blocking the adhesive cell properties promoted the simultaneous association of integrin α5/ β1 and EGFR1 to RCP resulting in coordinated PM recycling of this tri-partite receptor complex. This modified signaling downstream of EGFR1 increased its autophosphorylation and resulted in the activation of the proinvasive kinase PKB/Akt [54]. Since RCP is a known LMTK3 substrate (see above) it together suggests that LMTK3 mediated phosphoregulation of endocytic recycling and LMTK3-dependent cross-talk affect transcription of integrin α5/ β1 contribute to cell migration.

In a nuclear context, LMTK3 interacts with KRAB-associated protein 1 (KAP1), a master regulator of dynamic chromatin structure. LMTK3/KAP1 interaction was stabilised by protein phosphatase 1 . This complex suppressed KAP1 phosphorylation specifically at LMTK3-associated chromatin regions. Interaction led to transcriptional repression of tumour suppressor-like genes. Moreover, LMTK3 tethered chromatin to the nuclear periphery that resulted in gene silencing [11]. Therefore, nuclear LMTK3 acts as a scaffolding protein in chromatin reorganisation.

Lastly, LMTK3 has been implicated in drug resistance insofar as its overexpression lead to changes in the transcription of numerous genes promoting DNA repair, cell viability and tumorigenesis processes / pathways in the breast cancer cell line MCF-7 [55]. In addition, in a subset of melanoma, the overexpression of LMTK3 overcame drug resistance in tyrosine kinase KIT mutations that led to KIT-inhibitors induced drug resistance through the modulation of the translation rate of KIT [56].

Overall, LMTK family members have been heavily implicated in cancer insofar as they affect directly/indirectly numerous signalling pathways that impact cell behaviour. Whether LMTK implication in several signalling pathways results from endocytic cargo recycling is one of the most

pressing questions whose answer might shed light into the mechanism and hierarchy of LMTK related effects.

6. Conclusion and Outlook

The family of LMTKs share multiple structural, functional and cell biological features. Moreover, current data reveal similarities in the regulation of LMTK kinase activities that will considerably extend over the coming years considering the number of *in silico* predicted phosphorylation sites. Based on available functional data, the hypothesis emerges that LMTKs regulate endosomal cargo recycling. In addition, LMTKs may shunt steroid receptors from the endocytic recycling pathway to the nucleus where they take part in receptor-specific gene transcription. If and how those two functions in recycling and nuclear transport, respectively, relate to each other will be a matter of future investigations. Functional mutations or deregulation of the expression of LMTK-family members result in a variety of pleiotropic downstream signalling defects that may corroborate neuropathological diseases, diverse cancers and other partly not yet acknowledged diseases. One of the challenges in the field will be to resolve the 3D structure of the kinase domain/regulatory segment in order to design modulators of LMTK functions. It is this nuanced picture of diverse phenotypes that renders LMTKs attractive drug targets.

Figures

Figure1. Depicted is the overall structure of LMTK proteins. LMTK kinases are characterised by a N-terminal signal peptide followed by a predicted transmembrane (TM) region with one or two transmembrane domains. LMTK1 occurs in an additional isoform in which the TM region is omitted by alternative splicing and membrane anchoring of the protein product is achieved through palmitoylation instead. A highly conserved kinase domain followed by a long C-terminal tail completes the primary structure. Embedded in the c-terminal tail LMTK3 features two poly-proline stretches (not shown in scheme)

Figure2. Steady-state distribution of LMTKs LMTK1 and LMTK2 have both been attributed to the early (EE) and recycling endosomal (RE) pathways where they sequentially colocalise with Rab5 (EE), Rab11 and transferrin receptor (both RE), and myosin VI (EE). Although LMTK3 is present on cytoplasmic membranes its steady-state distribution has not yet been reported in detail. Additionally, LMTK2 has been localised to the plasma membrane and similar to LMTK3 also to the nucleus.

Figure3. Similar functional competence of LMTKs in response to sex steroid ligands (SSLs) LMTK1A has not been brought into connection with SSLs. LMTK2 and LMTK3 have been implicated in androgen and estrogen receptor (AR and ER) trafficking, respectively. It remains elusive whether either can be activated by the respective SSLs androgen and estrogen, respectively. LMTK1 and LMTK2 are both substrates for Cdk5/p35. Although predicted, LMTK3 has not yet been confirmed as a Cdk5 substrate with either p35 or p25 as Cdk5 coactivators.

Figure4. Putative role of LMTK3 in ER trafficking ER has been identified as a potential substrate of LMTK3. In this hypothetical model, phosphorylated ER might prevent receptor degradation through Hsp27/Hsp90 chaparon binding and receptor palmitoylation (via PAT activity). Palmitoylated ER may subsequently bind to caveolin-1-positive vesicular structures resulting in either the recycling of ER where it can engage with estrogen ligand shunt ER where it participates in a ligand-independent transcriptional response Alternatively, Hsp27/Hsp90 itself might be the phosphorylation target of LMTK3 triggering alternative ER Analogous pathways involving HSPs may exist for other steroid androgen receptor trafficking [57, 58].

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References

1. Robinson, D.R. et al. (2000) The protein tyrosine kinase family of the human genome. Oncogene 19 (49), 5548-57.

2. Nixon, A. et al. (2013) Determination of the membrane topology of lemur tyrosine kinase 2 (LMTK2) by fluorescence protease protection. Am J Physiol Cell Physiol 304 (2), C164-9.

3. Tomomura, M. et al. (2007) Structural and functional analysis of the apoptosis-associated tyrosine kinase (AATYK) family. Neuroscience 148 (2), 510-21.

4. Tsutsumi, K. et al. (2008) Palmitoylation-dependent endosomal localization of AATYK1A and its interaction with Src. Genes Cells 13 (9), 949-64.

5. Gaozza, E. et al. (1997) AATYK: a novel tyrosine kinase induced during growth arrest and apoptosis of myeloid cells. Oncogene 15 (25), 3127-35.

6. Wang, H. and Brautigan, D.L. (2002) A novel transmembrane Ser/Thr kinase complexes with protein phosphatase-1 and inhibitor-2. J Biol Chem 277 (51), 49605-12.

7. Bar-Sagi, D. et al. (1993) SH3 domains direct cellular localization of signaling molecules. Cell 74 (1), 83-91.

8. Tonikian, R. et al. (2009) Bayesian modeling of the yeast SH3 domain interactome predicts spatiotemporal dynamics of endocytosis proteins. PLoS Biol 7 (10), e1000218.

9. Mills, I.G. (2012) Nuclear translocation and functions of growth factor receptors. Semin Cell Dev Biol 23 (2), 165-71.

10. Shah, K. and Bradbury, N.A. (2015) Kinase modulation of androgen receptor signaling: implications for prostate cancer. Cancer Cell Microenviron 2 (4).

11. Xu, Y. et al. (2015) LMTK3 Represses Tumor Suppressor-like Genes through Chromatin Remodeling in Breast Cancer. Cell Rep 12 (5), 837-49.

12. Inoue, T. et al. (2014) LMTK3 deficiency causes pronounced locomotor hyperactivity and impairs endocytic trafficking. J Neurosci 34 (17), 5927-37.

13. Kawa, S. et al. (2006) Azoospermia in mice with targeted disruption of the Brek/Lmtk2 (brain-enriched kinase/lemur tyrosine kinase 2) gene. Proc Natl Acad Sci U S A 103 (51), 19344-9.

14. Manser, C. et al. (2012) Cdk5/p35 phosphorylates lemur tyrosine kinase-2 to regulate protein phosphatase-1C phosphorylation and activity. J Neurochem 121 (3), 343-8.

15. Luz, S. et al. (2014) LMTK2-mediated phosphorylation regulates CFTR endocytosis in human airway epithelial cells. J Biol Chem 289 (21), 15080-93.

16. Giamas, G. et al. (2011) Kinome screening for regulators of the estrogen receptor identifies LMTK3 as a new therapeutic target in breast cancer. Nat Med 17 (6), 715-9.

17. Gundry, C. et al. (2017) Phosphorylation of Rab-coupling protein by LMTK3 controls Rab14-dependent EphA2 trafficking to promote cell:cell repulsion. Nat Commun 8, 14646.

18. Tomomura, M. and Furuichi, T. (2005) Apoptosis-associated tyrosine kinase (AATYK) has differential Ca2+-dependent phosphorylation states in response to survival and apoptotic conditions in cerebellar granule cells. J Biol Chem 280 (42), 35157-63.

19. Gagnon, K.B. et al. (2007) Apoptosis-associated tyrosine kinase scaffolding of protein phosphatase 1 and SPAK reveals a novel pathway for Na-K-2C1 cotransporter regulation. Am J Physiol Cell Physiol 292 (5), C1809-15.

20. Rodan, A.R. and Jenny, A. (2017) WNK Kinases in Development and Disease. Curr Top Dev Biol 123, 1-47.

21. Honma, N. et al. (2003) Apoptosis-associated tyrosine kinase is a Cdk5 activator p35 binding protein. Biochem Biophys Res Commun 310 (2), 398-404.

22. Kaplan, K.B. et al. (1992) Association of p60c-src with endosomal membranes in mammalian fibroblasts. J Cell Biol 118 (2), 321-33.

23. Tsutsumi, K. et al. (2010) Phosphorylation of AATYK1 by Cdk5 suppresses its tyrosine phosphorylation. PLoS One 5 (4), e10260.

24. Chibalina, M.V. et al. (2007) Myosin VI and its interacting protein LMTK2 regulate tubule formation and transport to the endocytic recycling compartment. J Cell Sci 120 (Pt 24), 4278-88.

25. Morris, S.M. et al. (2002) Myosin VI binds to and localises with Dab2, potentially linking receptor-mediated endocytosis and the actin cytoskeleton. Traffic 3 (5), 331-41.

26. Kawauchi, T. (2014) Cdk5 regulates multiple cellular events in neural development, function and disease. Dev Growth Differ 56 (5), 335-48.

27. Reinecke, J. and Caplan, S. (2014) Endocytosis and the Src family of non-receptor tyrosine kinases. Biomol Concepts 5 (2), 143-55.

28. Takano, T. et al. (2012) LMTK1/AATYK1 is a novel regulator of axonal outgrowth that acts via Rab11 in a Cdk5-dependent manner. J Neurosci 32 (19), 6587-99.

29. Takano, T. et al. (2014) LMTK1 regulates dendritic formation by regulating movement of Rab11A-positive endosomes. Mol Biol Cell 25 (11), 1755-68.

30. Batut, J. et al. (2007) Kinesin-mediated transport of Smad2 is required for signaling in response to TGF-beta ligands. Dev Cell 12 (2), 261-74.

31. Massague, J. and Gomis, R.R. (2006) The logic of TGFbeta signaling. FEBS Lett 580 (12), 2811-20.

32. Massague, J. et al. (2005) Smad transcription factors. Genes Dev 19 (23), 2783-810.

33. Fili, N. et al. (2017) NDP52 activates nuclear myosin VI to enhance RNA polymerase II transcription. Nat Commun 8 (1), 1871.

34. Majewski, L. et al. (2018) Myosin VI in the nucleus of neurosecretory PC12 cells: Stimulation-dependent nuclear translocation and interaction with nuclear proteins. Nucleus 9 (1), 125-141.

35. Baker, S.J. et al. (2001) Characterization of an alternatively spliced AATYK mRNA: expression pattern of AATYK in the brain and neuronal cells. Oncogene 20 (9), 1015-21.

36. Juo, P. et al. (2007) CDK-5 regulates the abundance of GLR-1 glutamate receptors in the ventral cord of Caenorhabditis elegans. Mol Biol Cell 18 (10), 3883-93.

37. Hahn, C.M. et al. (2005) Role of cyclin-dependent kinase 5 and its activator P35 in local axon and growth cone stabilization. Neuroscience 134 (2), 449-65.

38. Nikolic, M. et al. (1996) The cdk5/p35 kinase is essential for neurite outgrowth during neuronal differentiation. Genes Dev 10 (7), 816-25.

39. Paglini, G. et al. (1998) Evidence for the participation of the neuron-specific CDK5 activator P35 during laminin-enhanced axonal growth. J Neurosci 18 (23), 9858-69.

40. Jiang, C. et al. (2018) AATYK is a Novel Regulator of Oligodendrocyte Differentiation and Myelination. Neurosci Bull 34 (3), 527-533.

41. Kawa, S. et al. (2004) Involvement of BREK, a serine/threonine kinase enriched in brain, in NGF signalling. Genes Cells 9 (3), 219-32.

42. Meyers, E.A. and Kessler, J.A. (2017) TGF-beta Family Signaling in Neural and Neuronal Differentiation, Development, and Function. Cold Spring Harb Perspect Biol 9 (8).

43. Bencze, J. et al. (2018) Biological function of Lemur tyrosine kinase 2 (LMTK2): implications in neurodegeneration. Mol Brain 11 (1), 20.

44. Ma, S. and Rubin, B.P. (2014) Apoptosis-associated tyrosine kinase 1 inhibits growth and migration and promotes apoptosis in melanoma. Lab Invest 94 (4), 430-8.

45. Liu, X. et al. (2016) Next-Generation Sequencing of Pulmonary Sarcomatoid Carcinoma Reveals High Frequency of Actionable MET Gene Mutations. J Clin Oncol 34 (8), 794-802.

46. Seo, J.S. et al. (2012) The transcriptional landscape and mutational profile of lung adenocarcinoma. Genome Res 22 (11), 2109-19.

47. Eeles, R.A. et al. (2008) Multiple newly identified loci associated with prostate cancer susceptibility. Nat Genet 40 (3), 316-21.

48. Harries, L.W. et al. (2010) Alterations in LMTK2, MSMB and HNF1B gene expression are associated with the development of prostate cancer. BMC Cancer 10, 315.

49. Chen, R.Q. et al. (2009) Kinome siRNA screen identifies SMG-1 as a negative regulator of hypoxia-inducible factor-1alpha in hypoxia. J Biol Chem 284 (25), 16752-8.

50. Naik, S. et al. (2009) Vascular Endothelial Growth Factor Receptor-1 Is Synthetic Lethal to Aberrant {beta}-Catenin Activation in Colon Cancer. Clin Cancer Res 15 (24), 7529-7537.

51. Clevers, H. (2006) Wnt/beta-catenin signaling in development and disease. Cell 127 (3), 469-80.

52. Stebbing, J. et al. (2011) Lemur tyrosine kinase-3 (LMTK3) in cancer and evolution. Oncotarget 2 (6), 428-9.

53. Xu, Y. et al. (2014) The kinase LMTK3 promotes invasion in breast cancer through GRB2-mediated induction of integrin beta(1). Sci Signal 7 (330), ra58.

54. Caswell, P.T. et al. (2008) Rab-coupling protein coordinates recycling of alpha5beta1 integrin and EGFR1 to promote cell migration in 3D microenvironments. J Cell Biol 183 (1), 143-55.

55. Stebbing, J. et al. (2018) LMTK3 confers chemo-resistance in breast cancer. Oncogene 37 (23), 3113-3130.

56. Klug, L.R. et al. (2018) LMTK3 is essential for oncogenic KIT expression in KIT-mutant GIST and melanoma. Oncogene.

57. Echeverria, P.C. and Picard, D. (2010) Molecular chaperones, essential partners of steroid hormone receptors for activity and mobility. Biochim Biophys Acta 1803 (6), 641-9.

58. Razandi, M. et al. (2010) Heat shock protein 27 is required for sex steroid receptor trafficking to and functioning at the plasma membrane. Mol Cell Biol 30 (13), 3249-61.

Conflict of Interest

No conflict of interests

Highlights

- LMTK family of proteins comprises three kinase regulated serine/threonin kinases LMTK kinases regulate membrane trafficking/recycling and signalling
- Altered regulation/function of LMTKs leads to defects in cell development and can cause acquired drug resistance

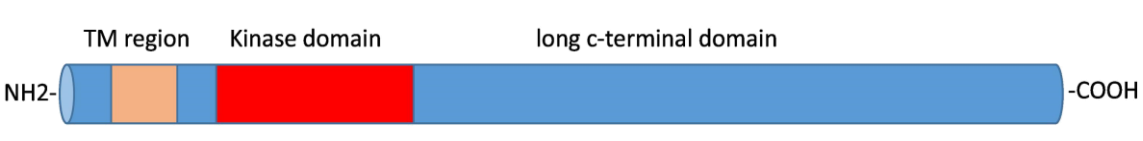

TM region
Kinase domain
long c-terminal domain
NH2-
-COOH

Figure 1

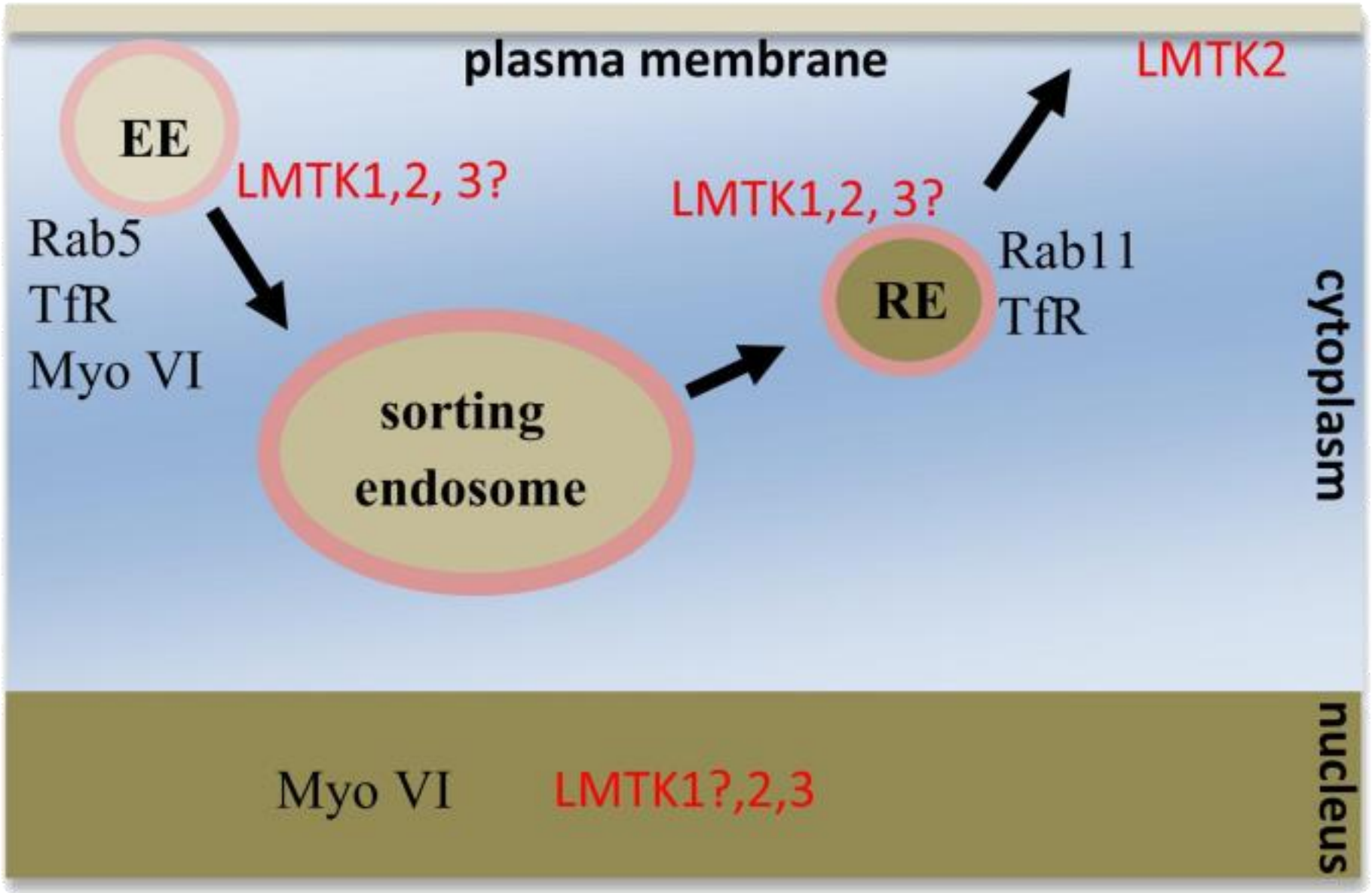
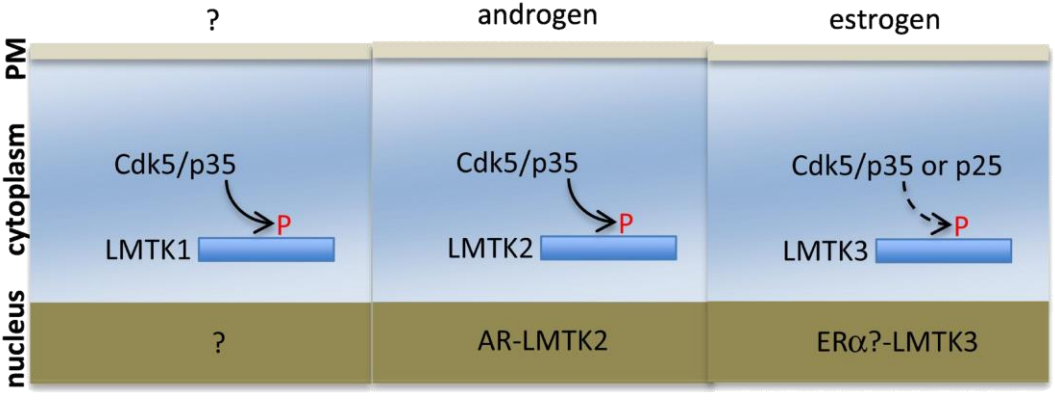

plasma membrane
LMTK2
EE
LMTK1,2, 3?
LMTK1,2, 3?
Rab5
TfR
Myo VI
RE
Rab11
TfR
sorting
endosome
cytoplasm
Myo VI
LMTK1?,2,3
nucleus

Figure 2


?
androgen
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Cdk5/p35
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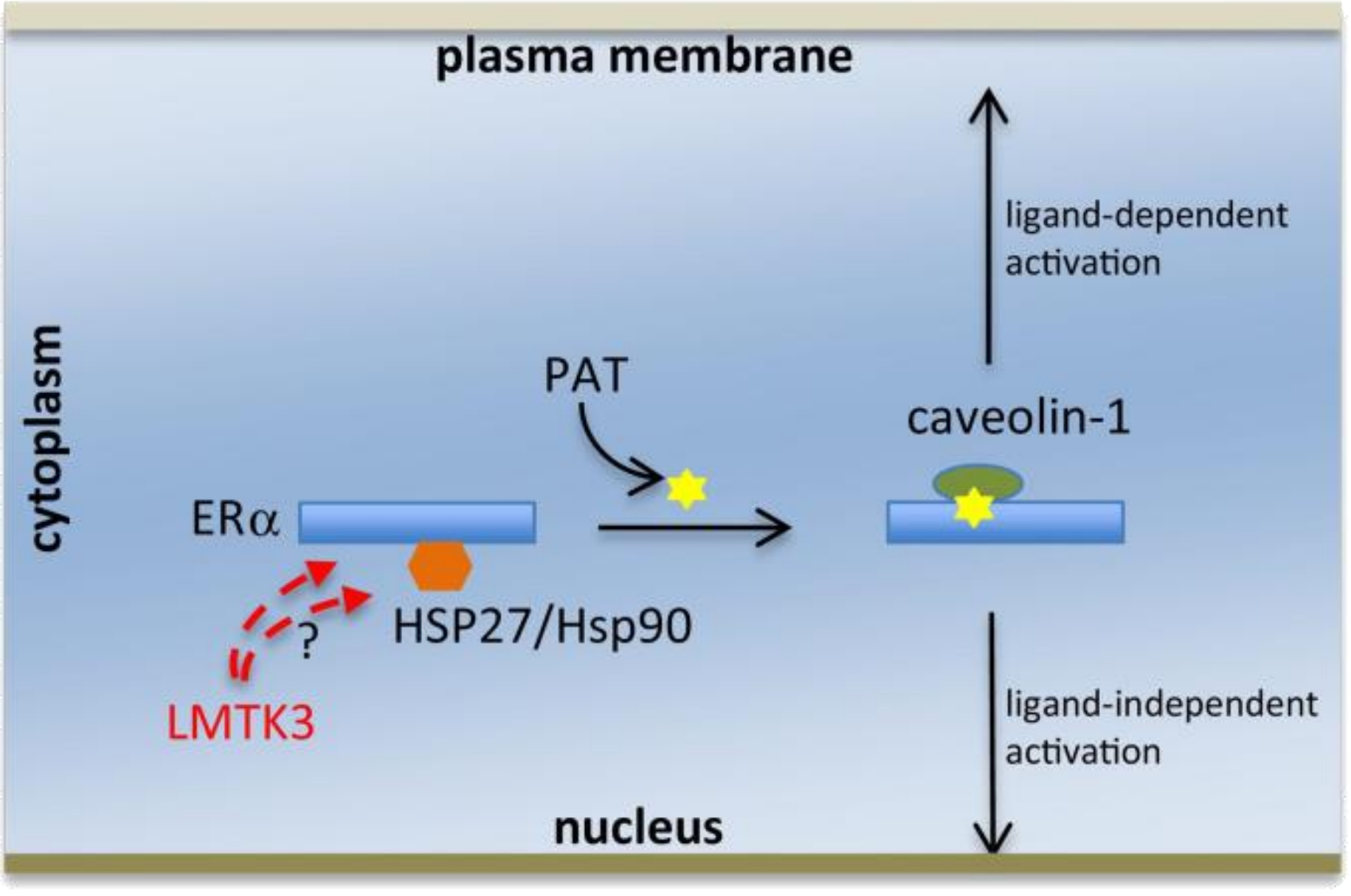
Figure 3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ligand-dependent
activation
cytoplasm
PAT
caveolin-1
ERα
?
HSP27/Hsp90
LMTK3
ligand-independent
activation
nucleus

Figure 4