STE20-related Kinase Adaptor Protein α (STRADα) Regulates Cell Polarity and Invasion through PAK1 Signaling in LKB1-null Cells

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LKB1 is a Ser/Thr kinase, and its activity is regulated by the pseudokinase, STE20-related adaptor α (STRADα). The STRADα-LKB1 pathway plays critical roles in epithelial cell polarity, neuronal polarity, and cancer metastasis. Though much attention is given to the STRADα-LKB1 pathway, the function of STRADα itself, including a role outside of the LKB1 pathway, has not been well-studied. Data in Caenorhabditis elegans suggest that STRADα has an LKB1-independent role in regulating cell polarity, and therefore we tested the hypothesis that STRADα regulates cancer cell polarity and motility when wild-type LKB1 is absent. These results show that STRADα protein is reduced in LKB1-null cell lines (mutation or homozygous deletion) and this partial degradation occurs through the Hsp90-dependent proteasome pathway. The remaining STRADα participates in cell polarity and invasion, such that STRADα depletion results in misaligned lamellipodia, improper Golgi positioning, and reduced invasion. To probe the molecular basis of this defect, we show that STRADα associates in a complex with PAK1, and STRADα loss disrupts PAK1 activity via Thr423 PAK1 phosphorylation. When STRADα is depleted, PAK1-induced invasion could not occur, suggesting that STRADα is necessary for PAK1 to drive motility. Furthermore, STRADα overexpression caused increased activity of the PAK1-activating protein, rac1, and a constitutively active rac1 mutant (Q61L) rescued pPAKThr423 and STRADα invasion defects. Taken together, these results show that STRADα-rac1-PAK1 pathway regulates cell polarity and invasion in LKB1-null cells. It also suggests that while the function of LKB1 and STRADα undoubtedly overlap, they may also have mutually exclusive roles.

LKB1 is a serine/threonine kinase (also known as STK11;(1)) that contains two nuclear localization sequences, a central kinase domain, and a C-terminal farnesylation motif (2). LKB1 ranks as the 3rd highest mutated gene in lung adenocarcinoma (3–5) and functions in epithelial cell polarity, neuronal polarity, energy stress, and cancer metastasis. LKB1 is localized to the cytoplasm and nucleus in mammalian cells, and its localization is regulated by its cofactor STE20-related adaptor (STRAD)2 (6–9).

STRAD is a pseudokinase that activates LKB1 in an allosteric manner where ATP binding to STRAD transitions the LKB1 kinase domain to an active-like kinase conformation (10) resulting in phosphorylation of LKB1 and STRAD (7). There are two known isoforms of STRAD, STRADα and STRADβ, and while both enhance LKB1’s kinase activity, only STRADα is involved in LKB1 nucleo-cytoplasmic shuttling (11). Studies show that STRADα and another LKB1-regulating protein, MO25α, influence the subcellular localization of wild-type, but not mutant LKB1, such that STRADα overexpression induces LKB1 nuclear export into the cytoplasm (7). Mutational analysis of human cancers shows that out of 34 LKB1 point mutants, 12 of these mutants fail to interact with STRADα-MO25, suggesting that the LKB1-STRADα-MO25 interaction is functionally significant (12).

Studies investigating STRADα-LKB1 function have linked it to two major pathways: the AMP kinase (AMPK) energy stress pathway and the cell polarity program (reviewed in Ref. 13). In the latter, LKB1 activation via STRADα binding causes cell autonomous polarization such that LKB1-activated cells fully polarize even in the absence of junctional cell-cell contacts, traditionally a prerequisite for polarization (6). In lung cancer, LKB1 is critical for cell polarity where LKB1 rapidly translocates to the cellular leading edge in motile cells to regulate cdc42 activity, a small Rho GTPase in the cell polarity pathway and activator of p21-activated kinase 1 (PAK1) (14, 15). PAK1 is a Ser/Thr kinase that associates with actin and lamellipodia

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proteins to mediate actin rearrangements, polarity, and adhesion (16–20). Mouse knock-out studies support a role for LKB1 in lung cancer invasion and metastasis, since in a mutant k-ras driven mouse model of lung cancer, LKB1 inactivation led to lung carcinomas with more frequent metastasis compared with tumors lacking p53 or Ink4a/Arf (21). Thus, these data support a role for STRADα-LKB1-inactivation in the invasion and metastasis of lung tumors.

To date, most studies have focused on LKB1 function and its role in cancer progression, motility, and metastasis. However, the molecular details of how STRADα itself functions and how its function is regulated have not been well described. A report in Caenorhabditis elegans shows an LKB1-independent function of STRADα (22), suggesting that STRADα could have roles outside of the LKB1 pathway in human cancer. Therefore, we tested the hypothesis that STRADα can function in cancer cell motility when wild-type LKB1 is absent. To do this, STRADα expression, regulation, and functionality were examined in LKB1 wild-type and null cell lines. These data show that STRADα protein is reduced in all LKB1 mutant cell lines tested compared with LKB1 wild-type cells, and this partial degradation occurs through the canonical Hsp90-dependent proteasome pathway. The remaining STRADα participates in maintaining proper cell polarity during cancer cell motility through a rac1-PAK1 cell polarity/motility pathway. Based upon these data, we conclude that STRADα regulates PAK1 in LKB1-null cells to oversee cancer cell polarity and invasion.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—All cell lines were maintained at 37 °C in a humidified chamber with 5% CO₂, ATCC-recommended media (RPMI 1640 or DMEM) were used for each cell line, supplemented with 10% FBS and 1% penicillin/streptomycin. Keratinocyte Serum Free Media supplemented with Bovine Pituitary Extract (BPE) and Epidermal Growth Factor (EGF) was used for Beas-2b epithelial lung cells. Human plasma Pituitary Extract (BPE) and Epidermal Growth Factor (EGF) were used for H9262 lung carcinoma cells. Human plasma was supplemented with Bovine Serum Free Media supplemented with 10% FBS and 1% penicillin/streptomycin. Media (RPMI 1640 or DMEM) were used for each cell line, supplemented with 10% FBS and 1% penicillin/streptomycin.

**Antibodies**—Antibodies against LKB1 (Abcam, Cambridge, MA), STRADα (Santa Cruz Biotechnology, Santa Cruz, CA), ubiquitin (Cell Signaling/Millipore, Billerica, MA), HA (Invitrogen, Carlsbad, CA), GAPDH (Cell Signaling), actin (Sigma), tubulin (Millipore), and pPAKThr423 (Cell Signaling), anti-GFP (Invitrogen) were used for Western blotting and immunofluorescence.

**Transfections and Drug Treatments**—Lipofectamine 2000 (Invitrogen) was used to transfect cells with the GFP:LKB1, GFP:LKB1 K78M, racQ61L, FLAG-STRADα, and the myc-PAK1 according to the manufacturer’s protocol. Cells were harvested 48 h post-transfection. Oligofectamine (Invitrogen) was used for small interfering RNA (siRNA) transfections according to the manufacturer’s protocol. LKB1 siRNA and STRADα siRNA were used at 100 nmol/liter in two successive 24-hour transfections. The LKB1 siRNA was a different sequence than the LKB1 shRNA. To inhibit protein translation, cells were treated with 100 μM cycloheximide (Acros Organics, Morris Plains, New Jersey) solubilized in water, at various time points. Proteasome inhibition was accomplished using MG-132 (Peptide Institute, Osaka, Japan) dissolved in DMSO. MG-132 was added to cells at 50 μM and incubated at 37 °C for 6 h, or 5–10 μM for 24 h. The Hsp90 inhibitor geldanamycin (Enzo Life Sciences, Farmingdale, NY) was used at 10 μM.

**Western Blotting**—Cells were harvested and lysed in TNS solution (50 mM Tris pH 7.5, 100 mM NaCl, 2 mM EDTA, 1% Nonidet P-40, Roche Complete Protease Inhibitor Mixture) at 4 °C. When preparing soluble and insoluble fractions, a high-salt lysis buffer (1% Nonidet P-40, 10% glycerol, 20 mM HEPES pH 7.6, 150 mM NaCl, 2 mM Na₃VO₄, 2 mM sodium molybdate, 2 mM Na₂P₂O₇, and Complete Protease Inhibitors) was added to cells and incubated for 30 min at 37 °C. The soluble fraction was collected after a 30 min at 2100 × g spin at 4 °C, and the pellet (insoluble fraction) was sonicated in an equivalent volume of high salt lysis buffer to disrupt the aggregate. Cell lysates were boiled for 5 min with protein loading buffer prior to SDS-PAGE. Protein concentrations were determined by the bichinchoninic acid protein assay kit (Pierce). Equal concentrations of protein from whole-cell lysates were solubilized in SDS sample buffer and separated on SDS 12.5% polyacrylamide gels. Proteins were transferred to polyvinylidene difluoride membrane, and the resultant membrane was blocked in 10% milk, then probed with primary antibodies diluted in 5% BSA for a minimum of 1 h at room temperature up to overnight at 4 °C. This was followed with the appropriate secondary horseradish peroxidase-conjugated antibody and visualized by chemiluminescence.

**Immunofluorescence, Confocal Microscopy, and Image Analysis**—Cells were fixed on coverslips with PHEMO buffer (68 mmol/liter PIPES, 25 mmol/liter HEPES, 15 mmol/liter EGTA, 3 mmol/liter MgCl₂, 10% DMSO) with 3.7% formaldehyde, 0.05% glutaraldehyde, and 0.5% Triton X-100. After washing in PBS, coverslips were blocked in 10% BSA for 30 min and processed according to Ref. 14. Cells were imaged using a Zeiss LSM 510 META confocal microscope using a 20× Plan-Apo objective (NA = 0.75). Image analysis of the LKB1 and STRADα signal was performed on confocal images using Meta-morph software. DAPI fluorescence was used to identify the nucleus and used to create an object of the nucleus. A region of interest was then created by the software outlining the nucleus, which was transferred to the corresponding LKB1 or STRADα image. The average fluorescence intensity was then quantified within this region of interest from thresholded images to generate nuclear intensity. To determine cytoplasmic intensity the nuclear object was subtracted from the corresponding LKB1 or STRADα image. The resultant cytoplasmic mean fluorescence intensity was quantified from the thresholded image. All images in the same cell line were acquired with identical settings and thresholded the same.

**Cell Polarity Measurements**—Cell polarity measurements were done as previously described in Ref. 14.

**Quantitative Real-Time PCR**—Total RNA was isolated from cells using Qiagen’s RNeasy Mini Kit and subsequently reverse-transcribed with M-MLV Reverse Transcriptase (Invitrogen). The resultant cDNA was amplified using primers specific for
LKB1 and STRADα, and analyzed by quantitative real-time PCR using SYBR Green detection. Reactions (25 μl) contained 1 μl of DNA, 0.2 μm primers, and 12.5 μl of IQ SYBR Green Supermix (Bio-Rad). The reaction protocol began with a 3 min hot start at 95 °C and followed with cycles of 95 °C, 10 s; 55 °C, 60 s. Melt curve analysis verified a single product. Relative quantities were calculated, standardized by comparison to 18 S rRNA (small ribosomal subunit)(23). Primers used were as follows: STRADα (forward-GCCATGTCCCCCTTAAAGGAT, reverse-TCATGGTCAGCTCTCTCAGC), 18 S (forward-GAGGGAGCTGAGAAGCCG, reverse-GTCCGGAAGTGGTAAATTCGC).

Invasion Assays—BD Biocoat Matrigel invasion chambers (BD Biosciences, Sparks, MD) were loaded with 0.5 × 10^5 cells in serum-free media that had been treated with control or LKB1-null cells (mutation causing an early stop codon or homozygously deleted) cancer cell lines. In the four lung cancer cell lines (H23, H460, H1944, and A549) and Hela cells tested, restoration of STRADα expression was mediated at the transcriptional level, quantitative real-time PCR was performed for STRADα in control H1299 pLKO.1 cells and H1299-LKB1 shRNA cells. In this case, STRADα mRNA levels remained unchanged (Fig. 1E); therefore, STRADα transcription is unaffected by LKB1 depletion, suggesting that these effects are mediated at the protein level and not transcriptionally.

To determine if reduced STRADα protein expression is mediated at the transcriptional level, quantitative real-time PCR was performed for STRADα in control H1299 pLKO.1 cells and H1299-LKB1 shRNA cells. In this case, STRADα mRNA levels remained unchanged (Fig. 1E); therefore, STRADα transcription is unaffected by LKB1 depletion, suggesting that these effects are mediated at the protein level and not transcriptionally.

To determine if LKB1 re-expression in LKB1-null cell lines restores STRADα protein expression, wild-type FLAG-LKB1 and a FLAG-LKB1 K78M kinase-dead mutant were transfected into LKB1-null cell lines. In the four lung cancer cell lines (H23, H460, H1944, and A549) and Hela cells tested, restoration of both wild-type FLAG-LKB1 and the K78M kinase-dead LKB1 mutant restored soluble STRADα levels to varying degrees (Fig. 2). Taken together, LKB1 re-expression restores STRADα levels in a kinase-independent manner, suggesting that LKB1 kinase activity is not critical for maintaining STRADα protein levels, and that LKB1-STRADα oligomerization serves as a protective mechanism for STRADα deletion.

STRADα Stability Is Regulated by Hsp90-dependent Proteasome Degradation—To assess how STRADα protein stability is regulated, protein synthesis was halted with cycloheximide, and STRADα levels were assessed in LKB1 wild-type H1299 lung cancer cells. Western blotting shows that STRADα is rapidly degraded and 45 min post-cycloheximide treatment, STRADα is nearly absent. In contrast, LKB1 is more stable and shows minor degradation beginning at around 75 min (Fig. 3A). The rate of STRADα degradation was similar in LKB1 wild-type H1299 lung cancer cells relative to isogenic control H1299 pLKO.1 cells; however, overall STRADα levels were significantly less (supplemental Fig. S1).
used alone and in combination with geldanamycin. Both the lysis buffer-soluble and insoluble fractions were obtained, since degraded protein often is insoluble after lysis. Geldanamycin-induced degradation of STRADα/H9251 could be partially reversed by co-treatment with the proteasome inhibitor MG-132 as evidenced by the re-appearance of the STRADα/H9251 protein band in the soluble and insoluble fraction (Fig. 3D). This suggests that Hsp90 drives STRADα/H9251 degradation through the proteasome and when the proteasome is inhibited, both soluble and insoluble STRADα accumulate. MG-132 treatment also resulted in a higher molecular weight STRADα band in both the insoluble and soluble fractions (Fig. 3D) that would be consistent with ubiquitinated STRADα, since MG-132 treatment leads to an accumulation of ubiquitinated protein (Fig. 3E, ubiquitin blot). The basal levels of this potentially ubiquitinated STRADα may also vary between cell lines since some cell lines show a prominent higher molecular weight STRADα band (Fig. 2).

To determine if STRADα degradation occurs in a similar manner when LKB1 levels are reduced, LKB1 was depleted in the H1299 lung cancer cell line and BEAS-2B lung epithelial cell line in the presence and absence of MG-132. Western blotting shows that when LKB1 is depleted, STRADα levels decrease as shown previously; however, this can be partially reversed with MG-132 treatment predominantly resulting in a higher molecular weight STRADα band (Fig. 3E). This suggests that when LKB1 is absent, STRADα is partially degraded through the proteasome.
**STRADα Regulates Cell Polarity**

**STRADα Regulates Cell Polarity in LKB1-null Cell Lines**—Results show that STRADα is partially degraded when LKB1 is absent; however, a significant fraction of STRADα still remains (Figs. 1–3). Therefore, we wanted to determine if the remaining STRADα has a functional role in LKB1-null cells. We investigated the role of STRADα in cancer cell polarity since STRADα regulates cell polarity independently of LKB1 in *C. elegans* (22). To test this, a Golgi polarization assay was performed to quantify cell polarity defects (14, 29, 30) in control and STRADα-depleted cells. This assay uses Golgi re-alignment as a functional marker of the cell polarity program, where the Golgi re-aligns between the nucleus and the leading edge of the cell when polarity is intact. To quantitate this, the region adjacent to the nucleus is sub-divided into three 120° regions (see “Experimental Procedures”), such that random alignment would occur 33% of the time (Fig. 4A) and in general proper alignment occurs in greater than 60% of cells. To determine how STRADα impacts cell polarity in LKB1-null cells, STRADα was successfully depleted in all cell lines using STRADα-specific siRNA (Fig. 4B). Control siRNA-treated cells show intact polarity such that 63% of H157 cells have proper alignment. In A549 cells, 81% of A549 cells have proper polarity, and 60% of HeLa and H460 cells have proper polarity (Fig. 4, C and D). In STRADα-depleted cells, the Golgi were unable to properly align in H157 and Hela cells, with only 35% and 37% of cells having proper alignment, respectively. In A549 cells, only moderate alignment was observed, with 54% of cell showing proper Golgi positioning, and in H460 only 37% had proper polarity. In all cell lines where STRADα was depleted, mis-directed and shorter lamellipodia were also observed (Fig. 4D), which is consistent with defective cell polarity. These results suggest that STRADα functions autonomously from LKB1 in LKB1-null cell lines to regulate cell polarity.

**STRADα Regulates Cancer Cell Invasion in LKB1-null Cell Lines**—Since cell polarity is necessary to establish directionality during cancer cell invasion, invasion assays were performed to determine if STRADα depletion affects cancer cell invasion. The data showed that in all four cell lines tested, STRADα depletion significantly reduced invasion, whereby cells lacking STRADα had significantly less invaded cells than control (Fig. 5A). Next, the converse experiment was performed to determine how STRADα overexpression (Fig. 5B) impacted cancer cell invasion. In these cases, FLAG-STRADα overexpression led to increased invasion in all cell lines tested. Specifically, in H460 and HeLa cells, STRADα overexpression significantly increased invasion 2.0-fold and 4.0-fold, respectively (Fig. 5C). In A549 and H157 cells, the increase in invasion was not significant; however, both cases clearly trended to increased invasion. Thus, taken together, these results show that in cell lines deficient of wild-type LKB1, STRADα depletion reduced cancer cell invasion, whereas its overexpression increased cancer invasion.

**STRADα Regulates PAK1 Thr423 Phosphorylation in the Absence of LKB1 and Complexes with PAK1**—To determine how STRADα regulates cell polarity and invasion, we focused on one of the key regulators of the cell polarity and motility program, PAK1. When activated by the small rho GTPases cdc42 or rac1, PAK1 undergoes a conformational change leading to Thr423 PAK1 phosphorylation (18, 31). We wanted to
determine if STRAD regulates PAK1 phosphorylation when wild-type LKB1 is absent. These results showed that STRAD depletion caused decreased Thr423 PAK1 phosphorylation in both LKB1 mutant (H157) and deleted (HeLa) cell lines (Fig. 6, A and B). Total PAK1 levels did not change in either case. Therefore, based upon these data we conclude that STRAD regulates Thr423 PAK1 phosphorylation in LKB1-null cell lines.

Next, we determined whether STRAD can complex with PAK1 using a co-immunoprecipitation approach. To do this, myc-PAK1 was expressed in H157 cells and endogenous STRAD was immunoprecipitated. Western blotting shows that endogenous STRAD forms a complex with PAK1. Based upon the data in Fig. 3, we also tested
whether STRADα associated with Hsp90 and this result showed that STRADα was not associated with Hsp90 in LKB1 deficient cells (Fig. 6C). An IgG immunoprecipitation is shown as a negative control, which did not result in a PAK1 signal.

To determine if this STRADα-PAK1 pathway is linked to cancer invasion, PAK1 overexpression was assessed in control and STRADα-depleted cells. In control HeLa and H157 cells, PAK1 overexpression resulted in increased invasion as expected (Fig. 6D); however, wild-type PAK1 overexpression in STRADα depleted cells led to no significant change in invasion (Fig. 6D), suggesting that STRADα is necessary for PAK1 to enhance invasion.

**STRADα Regulates rac1 Activity during Cancer Cell Invasion**—Because we show that STRADα regulates pPAK1Thr423 in the absence of LKB1 and associates in a complex with PAK1 (Fig. 6), we next wanted to determine how this pathway is mediated.
Since PAK1 is primarily activated by the rac1 or cdc42 small Rho GTPases, activation assays were done to determine if FLAG-STRAD/H9251 overexpression induced activation of cdc42 or rac1. FLAG-STRAD/H9251 overexpression did not result in a significant increase in cdc42 activation (not shown); however, FLAG-STRAD/H9251 overexpression in both HeLa and H157 cells led to a significant increase in GTP-bound rac1 (active rac1; Fig. 7A). This result suggests that STRAD/H9251 regulates PAK1 activity through rac1.

We then tested whether rac1 activation could rescue pPAK1Thr423 and invasion defects in STRADα-depleted cells by utilizing a rac1 Q61L mutant that is always in the active state (32, 33). Transfection of rac1 Q61L mutant rescued pPAK1Thr423 defects induced by STRADα depletion (Fig. 7B). Furthermore, it also rescued cell invasion in STRADα-depleted cells (Fig. 7C). Specifically, STRADα depletion resulted in decreased invasion as expected; however, transfection of rac1 Q61L into STRADα-depleted cells rescued defective invasion (Fig. 7C). Therefore, this result showed that an active rac1 can restore pPAK1Thr423 levels and STRADα invasion defects in STRADα-depleted cells, further suggesting that STRADα signals via rac1 to activate PAK1.

**DISCUSSION**

Motile cells polarize and generate functionally distinct cellular compartments to drive directional movement. These morphological changes are due to a dynamic and coordinated interplay of signaling molecules and the cytoskeleton (34, 35). We show that STRADα loss in LKB1-null cell lines disrupts cell polarity during motility, where cells fail to properly align their Golgi and have misdirected lamellipodia (Fig. 4). On the molecular level, this observation is associated with reduced PAK1Thr423 phosphorylation. PAK1Thr423 is the critical site regulating PAK1 activity (18), allowing PAK1 to associate with actin and lamellipodial proteins to mediate actin rearrangements, polarity, and adhesion through downstream signaling cascades (36). We show that STRADα complexes with PAK1, suggesting that this STRADα-PAK1 complex is regulating
PAK1\textsuperscript{Thr423} phosphorylation (Fig. 6). It is likely that STRAD\(\alpha\) regulates PAK1 activity via Rac1, since overexpression of STRAD\(\alpha\) causes increased Rac1 activity and a constitutively active Rac1 mutant restores STRAD\(\alpha\)-depletion signaling and invasion defects (Fig. 7). Interestingly, LKB1 itself is implicated in regulating pPAK1 in cancer cell polarity and also complexes with PAK1 (14, 37); however, since these cell lines are LKB1-null, these results show that STRAD\(\alpha\) functions independently of LKB1 to regulate cancer cell invasion and polarity via PAK1.

Studies in C. elegans support this result by showing that STRAD\(\alpha\) can function in an LKB1-independent manner to oversee neuronal polarity (22). In this case, STRD-1 (STRAD\(\alpha\)) and PAR-4 (LKB1) work independently, whereby STRAD\(\alpha\) directly interacts with SAD-1 but PAR-4 does not (22). Despite different model systems used by our group and by Kim et al., in both cases aberrant polarity is observed when STRAD\(\alpha\) functionality alone is compromised. Additional data also from C. elegans, show that LKB1 can function autonomously from STRAD\(\alpha\), such that PAR-4 (LKB1) requires STRD-1 (STRAD\(\alpha\)) to phosphorylate AMPK under energy stress conditions but PAR-4 can promote phosphorylation of PAR-1 (MARKs) in a STRD-1 independent manner (38). Taken together, these results show that STRAD\(\alpha\) and LKB1 are not always essential binding partners, and while their functions undoubtedly overlap, they also have mutually exclusive roles.

Cancer invasion is impeded when STRAD\(\alpha\) is depleted, but is enhanced when STRAD\(\alpha\) is overexpressed (Fig. 6). This reduced invasion in STRAD\(\alpha\)-depleted cells may be a conse-

![FIGURE 7. STRAD\(\alpha\) regulates rac1 activity during cancer cell invasion.](image-url)
quence of the aberrant polarity observed in these cells (Fig. 4) and is likely tied to the PAK pathway, since PAK1 can only induce invasion when STRADα is present (Fig. 6D). Furthermore, STRADα is likely regulating PAK through rac1 since overexpression of STRADα led to activation of rac1 (Fig. 7) and constitutively active rac1 rescues STRADα-depletion pPAKThr423 and invasion defects (Fig. 7). Based upon these data, we conclude that STRADα is required for lung cancer invasion likely through rac1-PAK1 signaling, and thus could serve a pro-metastatic role when wild-type LKB1 is absent. This is in contrast to LKB1, which when dephosphorylated knock-out in a mouse model induces metastasis (39, 40), thereby serving as a metastasis suppressor. The question then arises, could STRADα be playing dual roles, such that it represses invasion when associated with LKB1, but stimulates invasion when LKB1 is null. Other proteins can play seemingly opposing roles, such as the growth factor TGFβ, which can promote invasion and metastasis, but also induce growth suppression depending on the cell type and environment (41). If this is indeed the case with STRADα, this result suggests that metastasis induced by wild-type LKB1 loss or mis-localization in patients (27, 28, 40) could be driven by, in part, a pro-invasive role of STRADα.

The data presented here also show that STRADα protein stability is LKB1-dependent, such that loss of LKB1 results in reduced STRADα protein levels (Figs. 1–3). In LKB1 wild-type cells, STRADα is degraded through the proteasome in an Hsp90-dependent manner but turnover is enhanced when LKB1 is mutant. This pathway is similar to the LKB1 degradation pathway where LKB1 interacts with the molecular chaperones Hsp90 and Cdc37/p50 to regulate LKB1 stability and proteasomal degradation (42, 43). Based upon our data, we propose a model that when LKB1 is present, STRADα is degraded via the proteasome in an Hsp90-dependent manner but when LKB1 is mutant or absent, the rate of STRADα degradation through this pathway is increased.

Overall, these data show that STRADα can function independently of LKB1 to regulate cell polarity and invasion via PAK1 when LKB1 function is compromised. The question of whether an LKB1-independent, STRADα-rac1-PAK1 pathway exists when wild-type LKB1 function is intact is difficult to determine since LKB1 itself can alter PAK1 phosphorylation (14, 37); thus, discerning the effects of LKB1-STRADα-PAK1 from the effects of a separate STRADα-PAK1 pathway is difficult with standard approaches. Nevertheless, future studies will attempt to determine if these signaling events unfold in LKB1 wild-type cells and whether LKB1 loss triggers a pro-metastatic STRADα signaling pathway.

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FIGURE S1- STRADα degradation rate is similar in H1299 pLKO.1 control cells compared to H1299 LKB1 shRNA cells. Western blot of STRADα and GAPDH showing time points post-cyclohexamide treatment in H1299 pLKO.1 control cells and H1299 LKB1 shRNA cells.
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