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Expression and transcriptional profiling of the LKB1 tumor suppressor in cervical cancer cells

Xiaoli Zhang^{a,b}, Hanxiang Chen^a, Xiao Wang^c, Weiming Zhao^{a,*}, and Jason J. Chen^{b,d,*}

^aDepartment of Microbiology, School of Medicine, Shandong University, Jinan, Shandong, China

^bDepartment of Medicine, University of Massachusetts Medical School, Worcester, MA 01532, USA

^cDepartment of Pathology, School of Medicine, Shandong University, Jinan, Shandong, China

^dCancer Research Center, School of Medicine, Shandong University, Jinan, Shandong, China

Abstract

Objectives—To characterize the biological activities of LKB1, examine LKB1 protein expression and identify LKB1-regulated genes that may serve as therapeutic targets in cervical cancer.

Methods—Proliferation of cervical cancer HeLa cells expressing LKB1 was examined. LKB1 expression in normal cervical tissues and cervical cancers was assessed by immunohistochemistry. Gene expression profiles of cervical cancer HeLa cells stably expressing LKB1 were analyzed by microarray. Differentially expressed genes were analyzed using Gene Ontology (GO) terms and the Kyoto Encyclopedia of Genes and Genomes (KEGG) PATHWAY database. Quantitative RT-PCR was used to validate the microarray data. The expression of lipid phosphatase inositol polyphosphate 4-phosphatase type II (INPP4B) was confirmed by western blotting.

Results—Expression of LKB1 inhibited HeLa cell proliferation and activated AMPK and was lost in more than 50% of cervical carcinomas. More than 200 genes were differentially expressed between HeLa cells with and without LKB1. Bioinformatics analysis with GO annotation indicated that LKB1 plays a role in receiving diverse stimuli and converting them into molecular signals. KEGG PATHWAY analysis showed that 8 pathways were significantly regulated. These include arginine and proline metabolism and inositol phosphate metabolism. The differential expression of 7 randomly selected genes was confirmed by quantitative RT-PCR. Furthermore, the steady-state level of INPP4B protein was up-regulated in LKB1-overexpressing cells.

* **Corresponding authors:** Jason J. Chen, Department of Medicine, University of Massachusetts Medical School, 364 Plantation Street, Worcester, MA 01605, USA, Cancer Research Center, School of Medicine, Shandong University, No. 44, Wenhua West Rd., Jinan, Shandong, China, 250012. Phone: +001-508-856-1857; Fax: +001-508-856-6796; Jason.chen@umassmed.edu. Weiming Zhao, Department of Microbiology, School of Medicine, Shandong University, No. 44, Wenhua West Rd., Jinan, Shandong, China, 250012. Phone: +86-531-88382579; Fax: +86-531-88382502; zhaowm@sdu.edu.cn.

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Conflict of interest statement

There are no conflicts of interest to report.

Conclusions—This study establishes LKB1 as an important tumor suppressor in cervical cancer and sheds light on a novel signaling pathway regulated by LKB1.

Keywords

LKB1; cervical carcinoma; gene expression profiling; INPP4B; phosphatidyl inositol signaling

1. Introduction

Cervical cancer is one of the most common cancers in women worldwide. Infection with high-risk types of human papillomavirus (HPV) is strongly associated with the development of cervical cancer. Although HPVs play significant roles in the initiation of cervical neoplasia, they are not sufficient for cervical carcinogenesis. Therefore, for HPV-infected cells to become cancerous, additional genetic changes must take place.

Liver kinase B1 (LKB1) (also known as STK11) was originally identified as the causative gene of Peutz-Jeghers syndrome (PJS) [1]. Patients with PJS have increased cancer predisposition. Importantly, somatic LKB1 mutations are frequently found in a significant number of sporadic non-small cell lung carcinomas (approximately 30%) [2]. A study of primary cervical tumors identified 20% harboring somatically acquired mutations in LKB1, which were associated with accelerated disease progression [3]. However, the Cancer Genome Atlas (TCGA) studies only identified LKB1 mutation in 2% of cervical cancer samples (Reviewed in [4]). A more recent whole-exome sequencing analysis identified LKB1 mutation in 4% of cervical squamous carcinoma [5]. LKB1 is a master upstream kinase that forms a complex with a pseudokinase STRAD and the scaffold protein MO25. This complex can phosphorylate at least 13 serine-threonine kinases [6]. The best-characterized substrate of LKB1 is AMP-activated protein kinase (AMPK), the master regulator of cellular metabolism [7]. AMPK subsequently activates the TSC2 complex or mTOR-binding partner raptor, which negatively regulates mTOR signaling [8; 9]. LKB1 is involved in response to diverse signals under physiological and pathological conditions via AMPK [10]. In addition, LKB1 exerts effects on cell polarity, proliferation, migration, senescence, apoptosis, DNA damage response and differentiation [11].

Although AMPK has been identified as an important substrate for LKB1 in metabolism and cell growth, the tumor suppressor activity of LKB1 is not fully understood. Given the importance of LKB1 in tumor suppression and its potential role in cervical cancer, we characterized the biological activities of LKB1, performed the first immunohistochemical (IHC) study for LKB1 in cervical cancers and performed a microarray experiment to identify LKB1-regulated genes.

2. Material and methods

2.1. Materials

Antibodies against AMPK α , phospho-AMPK α (Thr¹⁷²), LKB1 and INPP4B were purchased from Cell Signaling (Beverly, MA). Anti-LKB1 for immunohistochemistry, anti- β -actin, anti-GAPDH, G418 and hygromycin were obtained from Sigma-Aldrich (St. Louis,

MO). The plasmid pc-DNA3-flag-LKB1 was bought from Addgene (Cambridge, MA). Lipofectamine 2000 transfection reagent was purchased from Invitrogen (Carlsbad, CA).

2.2. Cell culture and transfection

The human cervical cancer cell lines HeLa and Ca Ski were purchased from American Type Culture Collection (ATCC). The HeLa cells stably transfected with pMEP4-GFP or pMEP4-GFP-LKB1 plasmids were kindly provided by Dr. Zhi-Xiang Xu, University of Alabama at Birmingham. The cells were cultured in medium containing hygromycin (100 µg/mL) to select for cells with the plasmids. All cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with L-glutamine and 10% fetal bovine serum (FBS) in a humidified incubator containing 5% CO₂ at 37°C. For transient expression of LKB1, HeLa cells were transfected with plasmids pcDNA3 and pcDNA3-flag-LKB1, selected by G418 (400 µg/mL) for two weeks, then used for cell proliferation assays. For bromodeoxyuridine (BrdU) experiments, transfected HeLa cells were collected 48 hrs after transfection. For RNA interference, small interfering RNA (siRNA) oligonucleotides for the non-silencing control siRNA (NS-siRNA), LKB1-siRNA01 and LKB1-siRNA02 were purchased from Guangzhou RiboBio Co., LTD (Guangzhou, China) and used at a final concentration of 20 nM in Ca Ski cells.

2.3. Cell proliferation assay

Cells were seeded in 96-well plates at the density of 2000 cells per well. Cell viability was determined by the Cell Counting Kit-8 (CCK-8, DOJINDO, Japan) and the Infinite M200 PRO microplate reader (Tecan Group Ltd.) at a wavelength of 450 nm.

2.4. Patients and tissue samples

Paraffin-embedded, anonymous and de-identified tissue blocks were obtained from Qilu Hospital, Jinan, Shandong, China. Twenty-five normal cervical tissues were collected from patients with uterine fibroids who had undergone total hysterectomy. The details of patients with cervical cancer and disease characteristics are described in Supplemental data S1. Each participant had signed an informed consent document. The study was approved by the Institutional Research Ethics Committee at Shandong University School of Medicine.

2.5. Immunohistochemistry

Immunohistochemistry (IHC) was performed as we previously described using the streptavidin–peroxidase–biotin (SP) IHC method [12]. As the stain was uniformly positive or negative in all tumor tissue samples, the staining index was simply based on the staining intensity, which was graded as “–”, no staining; “+”, weak staining; “++”, moderate staining; and “+++”, strong staining. Tumor samples that scored + to +++ were considered positive. All stained slides were observed and scored by 2 independent investigators. If the staining interpretation differed between the 2 investigators, the data for the slide were discarded.

2.6. Flow cytometry analysis

Cells were fixed in 70% ethanol, treated with 50 µg/mL RNase A plus 50 µg/mL propidium iodide, and analyzed by fluorescence-activated cell sorting (FACS, Becton Dickinson). For BrdU labeling, the FITC BrdU Flow Kit (BD Biosciences, San Diego, CA) was used.

2.7. Gene expression profiling

For induction of LKB1 expression, the LKB1-stably transfected cells were treated with 100 µM zinc for 20 hrs before being collected. RNA was extracted using TRIZOL reagent (Life Technologies, Inc., Carlsbad, CA) according to the manufacturer's recommendations. RNA was further purified using the RNeasy Mini Kit (Qiagen Inc., Valencia, CA). Three biological replicates were carried out and analyzed. Microarrays were performed at the UMass Medical School Genomics Core Facility using Affymetrix Human Gene 1.0 ST array chip (Affymetrix, Inc., Santa Clara, CA). The unpaired *t*-test, false discovery rate (FDR) and 2-fold change were used to identify differentially expressed genes.

2.8. Real-time PCR

cDNA was synthesized with Superscript VILO cDNA synthesis kit (Invitrogen). The primer sequences are listed in Supplemental data S2. iTaq Universal SYBR Green Supermix (Bio-Rad) was used for real-time PCR (qRT-PCR) in the Bio-Rad CFX96 Touch Real-Time PCR Detection system. Data were analyzed using the 2^{-C_t} method. Spearman's rank correlation was used to estimate the degree of association between microarray and qRT-PCR data.

2.9. Bioinformatics and statistical analysis

Functional classification of differentially expressed genes was analyzed by gene ontology (GO) via the WEB-based Gene Set Analysis Toolkit (Web Gestalt, <http://bioinfo.vanderbilt.edu/webgestalt/>). Pathway analysis was performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) PATHWAY database via Web Gestalt. For GO and KEGG analyses, at least two genes needed to be present in a cluster, and a probability (*P*) value of < 0.05 was considered. For additional statistical analyses, Student's *t*-test was used. *P* < 0.05 was considered significant.

3. Results

3.1. Characterization of LKB1-expressing cervical cancer cells

To examine the effects of LKB1 expression on cervical cancer cells, we transfected a plasmid encoding LKB1 into HeLa cells. Upon transfection, LKB1 became readily detectable (Fig. 1A). As shown in Fig. 1B, HeLa cells expressing LKB1 proliferated significantly slower than the vector control cells. We also examined DNA replication by BrdU labeling. As shown in Fig. 1C, overexpression of LKB1 significantly reduced the number of cells in S-phase. These results demonstrate that expression of LKB1 inhibits cervical cancer cell proliferation and are consistent with previous observations [13; 14]. Next we examined proliferation of HeLa cells stably expressing LKB1. In contrast to transiently transfected cells, stably transfected cells did not exhibit a significant difference in

proliferation (not shown) or cell cycle profile compared with vector control cells (Supplemental data S3). Nonetheless, LKB1 was readily detected and appeared to be active in these cells, as AMPK phosphorylation was significantly increased (Fig. 1D). These results indicate that LKB1 did not have a major effect on cell proliferation or S-phase entry when stably expressed, suggesting that the cells adapted to LKB1 expression after prolonged culture.

3.2. Loss of LKB1 expression in the majority of cervical carcinomas

So far, LKB1 protein expression in cervical cancer tissues has not been reported according to the NCBI database. To characterize LKB1 protein in cervical cancer, we performed IHC analysis on a series of cervical carcinoma tissues as well as normal controls. We used a mouse monoclonal antibody against LKB1, which detected a band in Ca Ski (known to be LKB1 positive) but not in HeLa cells [15] (Fig. 2A) and stained positive in normal skeletal muscle tissue cells that are known to be LKB1 positive [16] (Fig. 2B). Among the normal cervical tissues, five columnar cell epithelia (Fig. 2C) and 13 squamous cell epithelia (Fig. 2D) stained negative for LKB1, while the other 7 squamous cell epithelia showed a weak stain for basal cells, largely in the nucleus (Fig. 2E and 2F). The observation of weak or no LKB1 protein expression in normal cervix is consistent with what was observed in some other studies in normal colorectal and pancreas tissues [17; 18], which could be due to lack of stimuli to LKB1 in normal cells. Among cervical adenocarcinoma tissues, 14 showed positive cytoplasmic LKB1 stain (11 “+” and 3 “++”), while 11 (44%) stained negative (Fig. 2G–I). Among squamous cell carcinomas of the cervix, 32 out of 53 (60.4%) were negative for LKB1, and 21 showed positive stain (largely in the cytoplasm; 12 “+” and 9 “++”) (Fig. 2J–L). A summary of LKB1 IHC staining is provided in Supplemental data S4. LKB1 protein expression was not associated with clinical stage ($p > 0.05$, Supplemental data S5) or prognosis ($p > 0.05$, data not shown).

3.3. Gene expression profiles

We were interested in identifying genes differentially expressed between cells with and without LKB1 by a genome-wide approach. Such a study has never been performed in cervical cancer cells. We used the HeLa cells stably expressing LKB1 for microarray analysis to avoid the potential multitude of transient transfection-induced, proliferation-associated gene expression changes. In HeLa cells stably expressing LKB1, the expression of 222 genes (105 up-regulated and 117 down-regulated) was changed by at least 2-fold (varying between 2.1-fold and 42.6-fold). The top 5 (according to fold change) down- and up-regulated genes in LKB1-expressing cells are shown in Table 1. The rest of these genes are shown in Supplemental data S6. Interestingly, the cyclic GMP-inhibited phosphodiesterase A (PDE3A) was down-regulated in LKB1-expressing HeLa cells by over 40-fold. PDE3 catalyzes the synthesis of GMP and AMP [19]. The latter can turn on AMPK. Inhibition of PDE3A by LKB1 may therefore add another layer of regulation to AMPK.

Next we performed qRT-PCR to confirm the results obtained by the microarray. We picked 7 genes, ABCC2, AKAP12, GLP2R, PLK2, KYNU, STK11 (LKB1) and MAL2. The qRT-PCR data were consistent with the microarray data for these genes (Supplemental data S7) ($p < 0.05$ and $r > 0.8$ for all the verified genes). Thus, the qRT-PCR data suggest that the

probe sets used in the microarray experiments were likely to accurately measure the levels of transcripts.

3.4. Bioinformatic analysis of genes differentially expressed in LKB1-expressing HeLa cells

We first analyzed the differentially expressed genes in LKB1-expressing HeLa cells using the GO system, a gene function classification system that provides a comprehensive description of gene properties. Based on the GO annotation, the 222 differentially expressed genes could be classified into multiple functional groups (Fig. 3 and Supplemental data S8). Notably, half of the genes in the “biological processes” category belonged to the “responses to stimulus” functional group. These results are consistent with the known LKB1-AMPK function of reacting to a variety of stimuli [10; 20; 21]. Interestingly, consistent with the known functions of LKB1, the “signaling”, “signal transduction”, “single organism signaling” and “cell communication” functional groups each consisted of more than a third of the genes. These results indicate that LKB1 plays a role in receiving diverse stimuli and converting them into molecular signals. Additional functional groups that were consistent with known activities of LKB1 included “regulation of developmental process”, “tissue development”, “regulation of cell proliferation” and “cell proliferation” (Fig. 3A).

In the “molecular function” category, nearly half of genes belonged to the “protein binding” group. Other common groups were “signal transducer activity”, “molecular transducer activity”, “receptor activity” and “receptor binding” groups (Fig. 3B). Based on the above categories, we tentatively conclude that quite a few LKB1-regulated genes are involved in protein-protein interactions and signal transduction. Notably, the “actin binding” group was identified to be regulated by LKB1. LKB1 regulates actin filament assembly, which contributes to cell polarization [22]. Interestingly, in the “cellular component” category, half of the genes were in the “membrane” group. Specifically, these genes belonged to the “membrane part”, “cell periphery”, “plasma membrane” or “intrinsic to membrane” group (Fig. 3C). LKB1 induces epithelial cell polarization through the sorting of membrane proteins [23]. These observations are consistent with the role of LKB1 in cell polarity.

We then searched the differentially expressed genes against KEGG, a collection of pathway maps representing knowledge on the molecular interaction and reaction networks. Eight biological pathways were identified to be significantly regulated in LKB1-expressing HeLa cells (Table 2). Consistent with a role of LKB1 in metabolism, two metabolic pathways, arginine/proline metabolism and inositol phosphate metabolism, were represented. Interestingly, genes associated with axon guidance pathway were also significantly regulated. This is consistent with the report that suggested LKB1 is involved in axon development [24]. In addition, the hematopoietic cell lineage pathway was regulated by LKB1. Another study implicated that LKB1 plays important roles in hematopoietic stems cell development and maturation [25]. Notably, the phosphatidylinositol signaling pathway, which converges with the LKB1-AMPK pathway at mTOR to regulate cell growth and metabolism [26], was also identified.

3.5. The lipid phosphatase INPP4B is up-regulated by LKB1

Notably, inositol polyphosphate 4-phosphatase type II (INPP4B), an inositol phosphatase in the phosphatidylinositol signaling pathway and a potential tumor suppressor, was identified from our microarray as an LKB1-up-regulated gene (Supplementary data S6 and Table 2, Lines 33 and 5, 8 respectively). We therefore focused on INPP4B for further analysis. qRT-PCR confirmed that INPP4B mRNA increased after LKB1 expression in HeLa cells (Fig. 4A). The steady-state level of INPP4B protein also increased (1.4-fold) in the HeLa cells stably expressing LKB1 (Fig. 4B). These results confirm the microarray results regarding LKB1 up-regulation of INPP4B at the protein level. Furthermore, INPP4B was increased (for 1.9-fold) in the HeLa cells transfected with the LKB1-encoding plasmid (Fig. 4C). Moreover, knock-down of LKB1 in the cervical carcinoma Ca Ski cells, which have an intact LKB1-AMPK pathway [27], reduced the steady-state level of INPP4B (Fig. 4D). Although the full-length, 106-kDa INPP4B protein was only modestly reduced, most likely a result of signal saturation, the 85-kDa, cell type-specific and functional INPP4B isoform [28] was significantly reduced (to 0.5-fold) after LKB1 knock-down. These results provide further evidence for INPP4B as a target for LKB1 and suggest an important role for INPP4B in functioning downstream from LKB1.

4. Discussion

Loss of LKB1 expression in a significant number of cervical cancer tissues (Fig. 2) is consistent with the notion that LKB1 is a tumor suppressor. It is generally believed that HPV infection is necessary but not sufficient for cervical carcinogenesis. Additional factors, including gene mutation or altered expression, play important roles. LKB1 is likely to be such a factor. Notably, Wingo et al. identified LKB1 somatic mutation in 20% of cervical cancer samples and found that median survival was only 13 months for patients of cervical cancer with LKB1 mutation, but >100 months for those with LKB1-wild type [3]. The percentage of LKB1 protein expression loss in cervical carcinoma detected in this study was much higher than that of somatic LKB1 mutation [3; 4]. Lack of LKB1 expression in cervical cancers can be a result of deletion, epigenetic inactivation, protein instability, etc. While LKB1 promoter hypermethylation has been observed in primary tumors associated with PJS [29], Wingo et al. found no evidence of *LKB1* promoter hypermethylation in cervical cell lines or primary cervical tumor samples examined [3], suggesting that *LKB1* promoter hypermethylation is not a major mechanism responsible for loss of expression. It remains to be examined the extent to which LKB1 protein instability or promoter histone modifications in cervical cancer tissues. Our study found no significant difference between LKB1 expression and non-expression group regarding FIGO staging and prognosis. This could be due to the fact that most of our samples were from patients with stage I cervical cancer, the prognosis of these patients are better than those in late stages. However, we do not know whether LKB1 is active in cervical carcinomas that express it. For some tumor suppressor genes, such as p53, proteins can be expressed in tissues with mutations [30].

It is surprising that LKB1 expression, as a tumor suppressor, was detected in a sub-set of cervical carcinomas but was not detected in normal cervix or was relatively weak and restricted to the basal cells in the normal cervix (Fig. 2). Notably, LKB1 expression

appeared to be higher in some cervical tumor tissues than in normal tissues or normal cells surrounding tumors (Fig. 2), although its expression was lost in other tumor tissues. Our findings are consistent with what was observed in colorectal mucosa, while stronger expression was observed in colorectal carcinomas but not in normal mucosa [17]. In contrast, in a subset of pancreatic intraductal papillary mucinous neoplasms, the normal ducts strongly expressed LKB1 protein, whereas the neoplastic cells did not [18]. Differences in tissues may account for the discrepancies in these observations. Our observations are also consistent with the earlier finding that at the mRNA level, LKB1 expression was increased in cells undergoing continuous or rapid division [31]. We speculate that higher LKB1 expression in tumor cells is a cellular response to tumorigenesis-associated stresses such as hypoxia. Alternatively, elevated expression of LKB1 in a sub-set of cervical carcinomas may reflect its potential role in the cervix as an oncogene at certain conditions or stages. Although LKB1 has been well established as a tumor suppressor, it exhibits oncogenic activities in some circumstances. For example, loss of LKB1 prevents Ras/Large-T-induced transformation in mouse embryonic fibroblasts [32].

Notably, Jimenez et al. investigated gene expression profiling by transiently overexpressing LKB1 in lung adenocarcinoma cells [14]. The study was conducted more than 10 years ago, and the microarray study focused on cancer and several related genes. One hundred genes were found to be deregulated by LKB1. The common observation shared by that study and ours is the regulation of the phosphatidylinositol signaling pathway by LKB1. Interestingly, while they found an up-regulation of PTEN after restoration of LKB1, we observed up-regulation of INPP4B. Both PTEN and INPP4B are phosphatases involved in the metabolism and homeostasis of phosphoinositides, inhibiting PI3K and converging at mTOR [4; 33]. Other studies have also shown that LKB1 regulates phosphatidylinositol signaling by interacting with PTEN [34; 35]. However, it was also reported that concurrent loss of LKB1 and PTEN leads to bladder tumor and ovarian cancer [36; 37], suggesting that they function in independent pathways in these tumors. Recently, INPP4B has received attention as a potential tumor suppressor in several cancers because it inhibits PI3K/Akt signaling. Notably, most of the LKB1-deregulated genes from the Jimenez et al. study are represented in cell proliferation, apoptosis and cell adhesion. Although we also identified several genes involved in cell proliferation, we did not find any genes related to apoptosis or cell adhesion.

INPP4B inhibits AKT phosphorylation and thereby inhibits cell proliferation [38]. However, loss of INPP4B by itself is not associated with high phospho-Akt levels, and concurrent loss of PTEN and INPP4B is required for Akt hyperactivation [37]. Consistent with this notion, ablation of the gene for INPP4B does not result in spontaneous tumor development in mice up to 4 months of age [39]. In addition to AKT, INPP4B may target TAPP1 and TAPP2 to modulate cellular motility via actin cytoskeletal remodeling [40]. In addition, INPP4B may regulate hexokinase 2 to regulate radio-resistance in cancer [41]. Currently, we do not know how LKB1 up-regulates INPP4B. As LKB1 can function as a transcriptional coactivator or repressor [42; 43], it may directly or indirectly up-regulate INPP4B gene transcription. Currently, little is known about how the INPP4B gene is regulated in general. One study showed that androgen receptor induces the expression of INPP4B in prostate cancer cells

[44]. Future studies should examine how LKB1 regulates INPP4B and what biological functions of LKB1 are mediated by INPP4B.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

We performed the first immunohistochemical analysis on LKB1 in cervical carcinomas and demonstrated loss of LKB1 expression in more than 50% tumors.

We performed genome-wide expression profiling study and identified more than 200 LKB1 regulated genes, including the emerging tumor suppressor INPP4B.

Fig. 1.

Characterization of HeLa cells expressing LKB1. (A) HeLa cells were transfected with a plasmid encoding LKB1 (pc-DNA3-flag-LKB1) or vector and were subjected to G418 selection for two weeks. LKB1 was detected by western blot. GAPDH was used as a loading control. (B) The viability of LKB1-transfected HeLa cells was determined by CCK8 assay. Error bars represent standard deviations. Data are from a representative experiment of two independent experiments, each conducted in sextuplicate. * $p < 0.05$; ** $p < 0.01$. (C) Representative flow cytometry of BrdU staining profiles from transiently LKB1-transfected

HeLa cells is shown (left panel). The percentage of BrdU-positive cells is indicated. The mean percentage of BrdU-positive cells from two experiments is summarized (right panel). Error bars reflect the standard deviations. (D) LKB1, AMPK, and p-AMPK were detected in HeLa cells stably expressing LKB1 by western blot. β -Actin was used as a loading control. A representative blot of 3 independent experiments is shown.

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Fig. 2.

LKB1 protein expression in cervical cancer cell lines and tissues. (A) Western blot of LKB1 in HeLa and Ca Ski cells. GAPDH was used as a loading control. Representative IHC images of LKB1 in (B) normal human skeletal muscles, (C) normal cervical columnar epithelium (negative), (D) normal cervical squamous epithelium (negative), (E) normal cervical squamous epithelium (nucleus), (F) high-magnification of corresponding areas in the box from E, (G) cervical adenocarcinoma (negative), (H) cervical adenocarcinoma (cytoplasm), (I) high-magnification of corresponding areas in the box from H, (J) cervical squamous cell carcinoma (negative), (K) cervical squamous cell carcinoma (cytoplasm) and

(L) high-magnification of corresponding areas in the box from K. Original magnification, 20×. High-magnification, 40×. Arrows indicate examples of LKB1-positive cells.

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Fig. 3. GO ontology categories of differentially expressed genes. (A) “Biological process” category. Seventeen groups (with > 30 genes) out of 40 are listed. (B) “Molecular function” category. Eleven groups (with > 10 genes) out of 23 are listed. (C) “Cellular component” category. Twelve groups (with > 20 genes) out of 20 are listed.

Fig. 4. Induction of INPP4B expression by LKB1 in HeLa cells. HeLa cells stably expressing LKB1 or empty vector were treated with 100 μ M zinc for 20 hrs. (A) The mRNA level of INPP4B was examined by qRT-PCR, $**p < 0.01$. (B) The steady-state level of INPP4B protein was measured by immunoblot. (C) INPP4B was examined by immunoblot after transfection with LKB1-expressing plasmid or empty vector, followed by G418 selection for two weeks. (D) Ca Ski cells were transfected with siRNA targeting LKB1. The steady-state levels of LKB1 and INPP4B were measured by immunoblot. GAPDH was used as a loading control. The results from 1 of the 2 siRNAs targeting LKB1 used are presented. Data from a representative experiment of 2 independent experiments are shown.

Table 1

Top 5 down- and up-regulated genes in LKB1-expressing cells compared with control cells.

Probe Set ID	Gene Symbol	Ratio of Vector/LKB1 (down-regulation)	Ratio of LKB1/Vector (up-regulation)	Description
7954293	PDE3A	42.6		phosphodiesterase 3A, cGMP-inhibited
8123864	TFAP2A	20.4		transcription factor AP-2 alpha (activating enhancer binding protein 2 alpha)
8048026	CPS1	18.2		carbamoyl-phosphate synthase 1, mitochondrial
8045539	KYNU	17.6		kynureninase (L-kynurenine hydrolase)
7929779	ABCC2	15.0		ATP-binding cassette, sub-family C (CFTR/MRP), member 2
7946071	OR51B5		25.0	olfactory receptor, family 51, subfamily B, member 5
7934979	ANKRD1		21.1	ankyrin repeat domain 1 (cardiac muscle)
7946067	OR51B4		13.7	olfactory receptor, family 51, subfamily B, member 4
7957260	GLIPR1		11.5	GLI pathogenesis-related 1
8024204	STK11		9.9	serine/threonine kinase 11

Table 2

KEGG PATHWAY analysis of the differentially expressed genes.

KEGG Pathways	Genes
Complement and coagulation cascades ID:04610	SERPINE1, BDKRB1, F2R, C4BPA, C4BPB, F3
Chagas disease (American trypanosomiasis) ID:05142	PLCB4, SERPINE1, CCL5, TLR4, IL1B, PIK3R1
Circadian rhythm–mammal ID:04710	PER3, NPAS2, BHLHE41
Phosphatidylinositol signaling system ID:04070	PLCB4, PIK3C2B, PIK3R1, INPP4B, PLCE1
Hematopoietic cell lineage ID:04640	KITLG, CD36, IL1B, ITGB3, CD33
Arginine and proline metabolism ID:00330	MAOA, ASS1, CPS1, AGMAT
Inositol phosphate metabolism ID:00562	PLCB4, PIK3C2B, INPP4B, PLCE1
Axon guidance ID:04360	SEMA7A, CXCR4, ABLIM3, SEMA3E, SEMA3A, L1CAM