YAP Promotes Malignant Progression of Lkb1-Deficient Lung Adenocarcinoma through Downstream Regulation of Survivin

Wenjing Zhang1, Yijun Gao1, Fuming Li1, Xinyuan Tong1, Yan Ren1, Xiangkun Han1, Shun Yao1, Fei Long2, Zhongzhou Yang3, Hengyu Fan4, Lei Zhang1, and Hongbin Ji1

Abstract

The serine/threonine kinase LKB1 is a well-characterized tumor suppressor that governs diverse cellular processes, including growth, polarity, and metabolism. Somatic-inactivating mutations in LKB1 are observed in about 15% to 30% of non–small cell lung cancers (NSCLC). LKB1 inactivation confers lung adenocarcinomas (ADC) with malignant features that remain refractory to therapeutic intervention. YAP activation has been linked to LKB1 deficiency, but the role of YAP in lung ADC formation and progression is uncertain. In this study, we showed that ectopic expression of YAP in type II alveolar epithelial cells led to hyperplasia in mouse lungs. YAP overexpression in the KrasG12D lung cancer mouse model accelerated lung ADC progression. Conversely, YAP deletion dramatically delayed the progression of lung ADC in LKB1-deficient KrasG12D mice. Mechanistic studies identified the antiapoptotic oncoprotein survivin as the downstream mediator of YAP responsible for promoting malignant progression of LKB1-deficient lung ADC. Collectively, our findings identify YAP as an important contributor to lung cancer progression, rationalizing YAP inhibition in the context of LKB1 deficiency as a therapeutic strategy to treat lung ADC.

Introduction

Lung cancer is the leading cause of cancer-related death worldwide (1). Non–small cell lung cancer (NSCLC), accounting for approximately 85% of all cases, is a major subgroup of lung cancer, which is often diagnosed at advanced stage and holds poor prognosis (2). Adenocarcinoma (ADC) is one of the common histologic subtypes of NSCLC (3). Although the pathogenesis of ADC is partially understood and new therapeutic options have substantially increased the survival of patients with ADC over the last decade, it still remains as largely incurable (4). Thus, further mechanistic insights into lung ADC carcinogenesis are urgently needed to pave the way for the development of effective therapeutic strategies.

The serine/threonine kinase gene LKB1 (also known as STK11), which is originally characterized as a tumor suppressor responsible for Peutz–Jeghers syndrome, has been implicated in coordinating a wide range of fundamental cellular processes, including polarity, metabolism, and growth, through its downstream kinases (5). LKB1 is somatically mutated in 15% to 30% of NSCLC, making it one of the most prevalently mutated tumor suppressors in human lung cancer (6). Studies of human NSCLC specimens and genetically engineered mouse models (GEMM) have revealed that LKB1 inactivation confers tumors with distinct biologic features. For instance, LKB1 mutations were more frequently observed in patients with poorly differentiated ADC (7). A lung cancer mouse model harboring simultaneous activation of Kras and biallelic deletion of Lkb1 exhibits short latency, increased tumor burden and frequent metastasis. Those Lkb1 deficiency tumors also have an expanded histologic spectrum (e.g., adenocarcinoma and squamous cell carcinoma; ref. 8). Our recent work demonstrate that Lkb1-deficient lung ADC can progressively transdifferentiates into squamous cell carcinoma (SCC), via a pathologically mixed Ad-SCC intermediate (9), indicating the high plasticity of this specific subset of lung cancer. Interestingly, we recently found that the ADC to SCC transdifferentiation represents a novel mechanism for the development of resistance to certain preclinical drugs, including phenformin, targeting cancer metabolism (10). Thus, this mouse model represents a clinically relevant NSCLC subtype, which shows malignant phenotype without effective therapeutic regimens (8). Our previous work have revealed that targeting LOX and NEDD9 by LOX enzymatic inhibitor or Nedd9 RNAi achieved partial inhibition on Lkb1-deficient tumor progression (11, 12). Considering the limited inhibitory efficacy on LOX and NEDD9, we propose other...
molecules may play pivotal roles in mediating tumor progression evoked by Lkb1 loss.

Recent work has shown that YAP, the major executor of the Hippo pathway, is activated in Lkb1-mutant tumors (13). The Hippo pathway is an emerging kinase cascade governing organ size through balancing cell proliferation and apoptosis (14). In mammals, the Hippo pathway is composed of upstream kinase cassette, including MST1/2, LAIS1/2, and two primary downstream effectors, YAP and TAZ. When the Hippo pathway is activated, the kinases MST1 and MST2 phosphorylate and activate the LAST1/2 kinases, which in turn phosphorylate YAP and TAZ, leading to the cytoplasmic retention of downstream effectors (15). Accumulating evidence suggests that dysregulation of the Hippo pathway plays a crucial role in cancer development (16). The upstream components of the Hippo pathway are reported to be frequently inactivated by mutations or epigenetic silencing, whereas the downstream effectors YAP and TAZ are often hyperactivated in a wide spectrum of human cancers (17). Further functional studies have characterized YAP as an oncogenic driver in multiple cancers, including liver and skin cancers (18, 19). YAP is also highly expressed in human lung ADC and is associated with poor clinical outcomes (20). Although overexpression of YAP promotes proliferation and invasion in ADC cell lines, the role of YAP in de novo lung cancer formation and progression in the context of Lkb1-deficient ADC still remains elusive.

Here, we show that lineage-specific overexpression of YAP in type II alveolar epithelial cells leads to hyperplasia in mouse lungs. We further present functional evidence that YAP is an important mediator of Lkb1-deficient ADC progression through genetic studies in mice. Mechanistic studies identified survivin as a downstream mediator of YAP in Lkb1-deficient ADC malignant progression.

Materials and Methods

Mouse cohorts and treatment

All mice were housed in a specific pathogen-free environment at the Shanghai Institute of Biochemistry and Cell Biology and treated in strict accordance with protocols approved by the Institutional Animal Care and Use Committee of the Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. KrasG12D/Lkb1+/−, and Yap+/− mice were originally generously provided by Dr. T. Jacks (Koch Institute for Integrative Cancer Research, Cambridge, MA), Dr. R. Depinho (MD Anderson Cancer Center, Houston, TX), and Dr. D. Pan (Johns Hopkins University School of Medicine, Baltimore, MD), respectively. Lung cancer mouse models with KrasG12D/Lkb1+/− mice were generated as previously described (8). To make the SP-C-YAP transgenic mice, a DNA fragment containing the YAP-coding region with the SV40 poly A was ligated to the 3.7-kb region of the human SP-C promoter (21) and was microinjected into C57 blastocysts. PCR strategy was used to screen mice bearing the SP-C-YAP fragment. Among the three lines positive for SP-C-YAP, we chose two with relatively higher YAP expression for further crossing with KrasG12D mice to generate the SP-C-YAP, KrasG12D mouse cohort.

Adeno-Cre infections were carried out as previously described (9). Briefly, SP-C-YAP, KrasG12D, and Yap+/−/KrasG12D/Lkb1+/− (YKL) mice were treated with 2 × 106 PFU Adeno-Cre via nasal inhalation and analyzed after 24 and 11 weeks of treatments, respectively.

Xenograft assay

KrasG12D/Lkb1+/− ADC primary cells were established as previously described (9). Indicated groups of cells were subcutaneously transplanted into either side of nude mice (n = 5). Tumor volume was measured every other day. Mice were sacrificed 3 weeks later for tumor weight and pathologic analysis.

Histopathologic analysis

All mice were euthanized for gross inspection and histopathologic examination. Xenograft tumors and mice lungs were isolated and fixed in 4% formalin. Lung lobes were embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E). Histologic examinations were performed by the pathologist. Tumor number was counted under microscope, and tumor area was quantified using ImageJ software by measuring pixel units.

Immunohistochemical analyses

Immunohistochemistry was performed as described previously (22). Paraffin-embedded lung tissues and xenograft tumors were incubated with the following antibodies: anti-Yap (Cell Signaling Technology; 1:500 dilution), anti-Ki67 (Leica; 1:500 dilution), and anti-cleaved caspase-3 (Cell Signaling Technology; 1:500 dilution). The proliferation rate was evaluated by counting Ki67-positive nuclear staining at high-power field for more than 30 fields for each group. The apoptosis was assessed by the analysis of cleaved caspase-3 immunostaining. The survivin immunostaining was reviewed and scored blindly into three criteria as low, medium, and high expression. Representative pictures of each grade were shown in Supplementary Fig. S4D.

MTT assay

KrasG12D/Lkb1−/− ADC primary cells of indicated groups were seeded in a 96-well plate, and the viability of cells was assessed by the MTT assay daily for 5 days. Briefly, 20 µL of MTT working solution (5 mg/mL) was added into each well and incubated at 37°C for 4 hours. The supernatants were then removed, and the resultant MTT formazan was dissolved in 100 µL of DMSO. The absorbance was measured at the wavelengths of 570 and 630 nm.

RT-PCR and quantitative real-time PCR

Total RNA was extracted and retro-transcribed into first-strand complementary DNA as previously described (23). cDNAs were subjected to quantitative real-time PCR with gene-specific primers on the 7500 Fast Real-Time PCR System using SYBR-Green Master PCR mix.

Sequences of real-time PCR primers are listed as follows:

Ctgf-forward: AGCACCTGGAGAACTGTGT
Ctgf-reverse: GCTGTCTTGGAAGACATCAC
Cyrl61-forward: GATGCCATCCCTCGGATGTGATTG
Cyrl61-reverse: CTCGCAGACACCAAATGAGG
Axl-forward: ATGGCCGACATTGCCAGTG
Axl-reverse: CGTCGAGCTCCCTCCCTGGAT
Arg-forward: GGGGACACTGACITCACAGAG
Arg-reverse: TCTTGCGGCTTAATCCCTAGTIC
Fgf1-forward: CTCTCCGGCTCACTGCTGG
Fgf1-reverse: ACACGATCCCGTCTTCAGTGA
Survivin-forward: GAGGCTCTGGCTCATCACCAG
Survivin-reverse: ATGCTTCTCTATGGTTTCG

Downloaded from cancerres.aacrjournals.org on May 28, 2017. © 2015 American Association for Cancer Research.
β-Actin-forward: TGAGCGCAAGTACTCTGTGTGGAT
β-Actin-reverse: ACTCATCGTACTCCTGCTTGCTGA
Human/mouse YAP-forward: AAGCTGCCCGACTCCTTCTTC-
                       AAG
Human/mouse YAP-reverse: TGAGCTCGAACATGCTGTGG-
                         AGTCAG.

Immunoblotting
Cells were lysed in lysis buffer and subjected to Western blot analysis with the following primary antibodies: anti-Yap (Cell Signaling Technology, 1:500 dilution), anti-survivin (Cell Signaling Technology, 1:500 dilution), and anti-actin (Sigma, 1:5,000 dilution).

Plasmids
To construct the survivin expression plasmid, the coding sequence of survivin was amplified with primers: forward:

  GCTCTAGAGCCACCATGGGATACCCCTACGACGTCCCCGACTAGCCCATGGGAGCTCCG
  sense: 5'-CCGGGAAGACTACCCGTCAGTCAACTCGAGTTG-ACTGAGCGGTAGTCTTTTGT-3';
  antisense: 5'-AATTCAAAAACAGACTACCCGTCAGTCAACTCGAGTTG-ACTGAGCGGAGTC-3'.

To generate the Yap and survivin knockdown construct, the following oligonucleotides were cloned into pLKO.1 at the AgeI/EcoRI sites:

- shYap-1:
  sense: 5'-CCGGGAAGCGCTGAGTTCCGAAATCCTCGAGGATTTCGGAACTCAGCGCTTCTTTTTG-3';
  antisense: 5'-AATTCAAAAAGAAGCGCTGAGTTCCGAAATCCTCGAGGATTTCGGAACTCAGCGCTTC-3'.

- shYap-2:
  sense: 5'-CCGGACTTGGAGGCGCTCTTCAATGCTCGAGCAT-TGAAGAGCGCCTCCAAGTTTTTTG-3';
  antisense: 5'-AATTCAAAAAACTTGGAGGCGCTCTTCAATGCTCGAGCAT-TGAAGAGCGCCTCCAAGTTTTTTG-3'.

- shSurvivin-1:
  sense: 5'-CCGGGAAGAACTAACCGTCAGTGAACTCGAGTTC-ACTGACGGTTAGTTCTTCTTTTTG-3';
  antisense: 5'-AATTCAAAAACAGACTACCCGTCAGTCAACTCGAGTTG-ACTGAGCGGAGTC-3'.

- shSurvivin-2:
  sense: 5'-CCGGAAAAGACTACCCGTCAGTCAACTCGAGTTG-ACTGAGCGGAGTC-3';
  antisense: 5'-AATTCAAAAACAGACTACCCGTCAGTCAACTCGAGTTG-ACTGAGCGGAGTC-3'.

Statistical analyses
Statistical analyses were performed by the Student t test (two-tailed) using Prism GraphPad software. Differences with P < 0.05 were considered statistically significant. Data were represented as mean ± SEM.

Results
Overexpression of YAP in type II alveolar epithelial cells induces hyperplasia in mouse lungs
To understand the role of YAP in lung tumor initiation, we took advantage of a transgenic mouse model with ectopic YAP expression, specifically in type II alveolar epithelial cells. Microscopic analyses revealed that all of mice (YAP Tg#43, 8/8, and YAP Tg#31, 5/5) with constitutive YAP expression harbored hyperplasia in the lung (Fig. 1A; Supplementary Fig. S1A). Detailed molecular analyses showed that those hyperplastic lesions exhibited strong YAP staining and high Ki67-positive rate (Fig. 1B), indicating that ectopic expression of YAP confers lung epithelial cells with high proliferating potential and YAP is sufficient to drive hyperplasia in the lung.

Ectopic expression of YAP promotes de novo lung cancer progression in the KrasG12D mouse model
We next asked if YAP affects lung ADC progression driven by oncogenic Kras in vivo. To address this, we crossed the SP-C-YAP transgenic mice with KrasG12D mice to obtain SP-C-YAP, KrasG12D mice. Mice were given with Adeno-Cre by nasal inhalation and sacrificed for gross inspection and pathologic studies 24 weeks after viral administration (Fig. 2A). Ectopic YAP expression, which was confirmed by immunostaining analyses (Fig. 2B), did not cause a significant change in the total tumor number (Supplementary Fig. S2A). As compared with the control group, SP-C-YAP, KrasG12D mice showed a dramatic increase in tumor burden as measured by tumor size (Fig. 2C and D; Supplementary Fig. S2B). Ectopic YAP expression increased the number of large tumors (≥1 mm³), indicating that YAP promotes the lung tumor progression driven

Figure 1.
Ectopic expression of YAP in type II alveolar epithelial cells leads to lung hyperplasia. A, images of H&E staining in wide-type (WT) or SP-C-YAP transgenic mouse lungs. B, representative images with YAP and Ki67 immunostaining on serial sections of WT or SP-C-YAP transgenic mouse lungs. Scale bar, 50 μm.
by oncogenic KRAS (Fig. 2E). Consistently, a higher proliferation rate evidenced by Ki67 staining was observed in lung tumors from SP-C-YAP, Kras\textsuperscript{G12D} mice, suggesting that YAP overexpression exhibited a promotive effect on tumor cell growth (Fig. 2F).

Homozgyous deletion of Yap inhibits de novo lung cancer progression elicited by Lkb1 loss

To further investigate if YAP is necessary for lung ADC progression, we used a well-characterized NSCLC mouse model initiated by mutated Kras and Lkb1 loss (Kras\textsuperscript{G12D}/Lkb1\textsuperscript{+/−}, KL in short). In line with recent studies (13), ADC derived from KL mice exhibits high YAP expression and focused nuclear localization compared with those from Kras\textsuperscript{G12D} mice (Supplementary Fig. S3A). As YAP is hyperactivated in KL mice, we then crossed Yap\textsuperscript{+/−}/Lkb1\textsuperscript{+/−} mice with Kras\textsuperscript{G12D}/Lkb1\textsuperscript{+/−} mice to generate Yap\textsuperscript{−/−}/Kras\textsuperscript{G12D}/Lkb1\textsuperscript{L−/−} (YKL) mice. KL and YKL mice were treated with Adeno-cre and analyzed 11 weeks after viral infection (Fig. 3A). Biallelic deletion of Yap, which was validated by Yap immunostaining (Fig. 3B), did not affect the total tumor number (Supplementary Fig. S3B). However, the tumor size in YKL mice was significantly reduced compared with the control group (Fig. 3C and D), and the number of large tumors (>1 mm²) was significantly reduced in the YKL group (Fig. 3E). Detailed pathologic analyses revealed that the majority of the lesions from the YKL model were atypical adenomatous hyperplasia (AAH) or adenoma, and the SCC were rarely detected (Supplementary Fig. S3C), indicating that Yap deletion significantly inhibited lung cancer progression in KL mice. Tumors from YKL mice displayed a moderate decrease of Ki67-positive cells and a significant increase of cleaved caspase-3–positive cells (Fig. 3F and G), suggesting the essential role of Yap in maintaining ADC cell survival.

Survivin acts as a downstream mediator of YAP in promoting malignant progression of LKB1-deficient lung ADC

To study the potential mechanisms involved in the promotive role of YAP in LKB1-deficient ADC malignant progression, we knocked down the Yap level in primary Kras\textsuperscript{G12D}/Lkb1\textsuperscript{−/−} adenocarcinoma cells. Survivin, a member of the inhibitor of apoptosis (24), was most significantly downregulated among the examined Yap target genes (Fig. 4A). This was further
YAP Mediates Progression of Lkb1-Deficient Lung Adenocarcinoma

confirmed at the protein level (Fig. 4B). Consistent with these findings, lung tumors from SP-C-YAP, KrasG12D mice showed an increased survivin level in comparison with those from KrasG12D mice (Fig. 4C), whereas lung tumors from YKL mice exhibited a marked decrease of survivin compared with those from KL mice (Fig. 4D), providing a potential link between Yap and survivin in regulating lung tumorigenesis.

To further prove the functional link between Yap and survivin, MTT and xenograft assays were performed in mouse KrasG12D/Lkb1+/− primary cells. Our data clearly showed that survivin knockdown significantly inhibited cell proliferation in vitro and tumor growth in vivo (Fig. 4E–4H; Supplementary Fig. S4B). Similar inhibition was observed when Yap was knocked down (Fig. 4I–K; Supplementary Fig. S4C). Consistently, decreased cell proliferation was observed when either survivin or Yap was knocked down (Fig. 4H and L). Importantly, survivin overexpression rescued the inhibitory function of Yap knockdown upon cell proliferation in vitro, tumor growth, and cell proliferation in vivo (Fig. 4I–L; Supplementary Fig. S4C). Together, these data support the notion that survivin serves as a downstream mediator of YAP in Lkb1-deficient ADC malignant progression.

Discussion

In this study, we investigate the role of YAP in lung ADC formation and progression utilizing de novo lung cancer mouse models and demonstrate that those Lkb1-mutant ADC are addicted to YAP activation. Ectopic expression of YAP in lung type II alveolar epithelial cells results in hyperplasia. Overexpression of YAP in KrasG12D mice significantly promotes de novo lung ADC progression, whereas homozygous deletion of Yap in KrasG12D/Lkb1−/− mice dramatically inhibits the malignant progression. YAP is a well-documented oncogene in a wide range of cancer types. Studies in mice have shown that activation of YAP leads to striking tissue overgrowth and tumor formation in multiple organs. For instance, overexpression of YAP in the liver drives liver enlargement as well as liver tumor formation (18). Activation of YAP triggers the expansion of epithelial stem cell compartment and the formation of SCC-like tumors in mouse skin (19). Activation of YAP is potently prometastatic in breast cancer and melanoma cells (25). However, increasing evidence indicates the suppressive role of YAP in several cancer types or under certain conditions. For example, knockdown of YAP in breast cancer cell lines suppresses cell anoikis and enhances the cell migration and invasion ability (26). YAP is described to interact with and enhance p73-dependent apoptosis in response to DNA damage (27). Thus, the function of YAP in tumorigenesis may be context dependent.

A recent study from Lau and colleagues (28) has shown that activation of Yap in combination with mutant Kras results in an increase in tumor number and higher tumor grade (28). Using SP-C-YAP transgenic mice, we have shown that
YAP overexpression does not cause a significant change in the total tumor number. This could be due to different genetic modification systems as well as the potentially differential YAP expression level. Consistent with the findings of Lau and colleagues (28), we found that ectopic expression of YAP did result in a dramatic increase in tumor burden, indicative of accelerated lung tumor progression. In addition, taking advantage of the primary cell line derived from Kras/p53 lung ADC, Lau and colleagues have found that knockdown of Yap/Taz attenuates the metastasis potential of cells (28). Instead of studying cancer metastasis, our work focuses on the role of Yap in tumor formation using the KrasG12D/Lkb1−/−ADC primary cells with or without Yap knockdown. Here, we determined a functional link between Yap and survivin as a crucial mediator of ASC formation and malignant progression despite different genetic approaches.

Notably, we identify survivin as a crucial mediator of YAP in the malignant progression of KrasG12D/Lkb1−/− mice. A, real-time PCR quantification of Yap target genes in the KrasG12D/Lkb1−/− ADC primary cells with or without Yap knockdown. B, Western blot detection of survivin protein level in the KrasG12D/Lkb1−/− ADC primary cells with or without Yap knockdown. C, representative photos and statistical analyses of survivin immunostaining in lung tumors from KrasG12D or SP-C-YAP#31, KrasG12D mice. D, representative photos and statistical analyses of survivin immunostaining in lung tumors from KrasG12D/Lkb1−/− (KL) or Yap−/−/KrasG12D/Lkb1−/− (YKL) mice. E, the proliferation of the KrasG12D/Lkb1−/− ADC primary cells with or without survivin knockdown was measured by the MTT assay. F, tumor formation of the KrasG12D/Lkb1−/− ADC primary cells with or without survivin knockdown (n = 5). G, tumor weight of the KrasG12D/Lkb1−/− ADC primary cells with or without survivin knockdown (n = 5). H, representative photos of Ki67 immunostaining and statistical analyses of the Ki67-positive index in xenograft tumors of the KrasG12D/Lkb1−/− ADC primary cells with or without survivin knockdown. I, the proliferation of the KrasG12D/Lkb1−/− ADC primary cells with Yap knockdown and/or survivin overexpression was measured by the MTT assay. J, tumor formation of the KrasG12D/Lkb1−/− ADC primary cells with Yap knockdown and/or survivin overexpression (n = 5). K, tumor weight of the KrasG12D/Lkb1−/− ADC primary cells with Yap knockdown and/or survivin overexpression (n = 5). L, representative photos of Ki67 immunostaining and statistical analyses of the Ki67-positive index in xenograft tumors of the KrasG12D/Lkb1−/− ADC primary cells with Yap knockdown and/or survivin overexpression. Scale bar, 50 μm. Data, means ± SEM, *P < 0.05; **P < 0.01; ***P < 0.005.
survivin in the context of Lkb1-deficient lung ADC by utilizing a Kras\(^{G12D}\)/Lkb1\(^{-}\)/C0 adenocarcinoma primary cell line. Knockdown of Yap decreased the mRNA and protein level of survivin. Reexpression of survivin rescued the suppressed cell proliferation and impaired tumor growth upon Yap knockdown. Thus, our findings not only confirm the regulatory relationship between Yap and survivin, but also provide functional evidence that survivin serves as an executor of Yap in promoting malignant progression.

LKB1 exerts its tumor suppressive effect via regulating cell growth, reprogramming cell metabolism, and modulating cell polarity through distinct downstream kinases (5). Emerging experimental evidence that survivin serves as an executor of Yap in the context of Lkb1-deficient lung ADC. Meanwhile, Mohseni and colleagues (13) also demonstrated that deletion of Lkb1 in the liver suppresses hepatomegaly and hepatocyte hyperplasia induced by acute deletion of Lkb1. Therefore, our data not only clarify the functional importance of Yap in the lung cancer progression evoked by Lkb1 loss, but also corroborate the notion that the functional relationship between LKB1 and Yap is concordant among organs.

LKB1 is one of the frequently mutated tumor suppressors in human NSCLC. Loss of LKB1 contributes to more aggressive phenotype with limited therapeutic options (8, 9, 33, 34). Recently, increasing efforts have been made to identify a powerful therapeutic strategy for Lkb1-deficient lung cancer. Combined inhibition of PI3K–mTOR, MEK, and SRC family kinases resulted in significant tumor regression in Kras\(^{G12D}\)/Lkb1\(^{-}\)/C0 lung tumors (35). Shackelford and colleagues (36) have identified phenformin as a cancer metabolism–based therapeutic drug to target Lkb1-deficient tumors. Based on our genetic studies in mice, we propose that targeting Yap may serve as a potential therapeutic approach for the LKB1-deficient lung tumor. Moreover, considering the prevalence of LKB1 mutations in multiple cancer types, our work may also provide significant implication for the treatment of other LKB1-deficient cancers.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: W. Zhang, Y. Gao, H. Ji
Development of methodology: W. Zhang, Y. Gao, Z. Yang
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): W. Zhang, Y. Gao, F. Li, H. Fan, I. Zhang
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): W. Zhang, Y. Gao, F. Li
Writing, review, and/or revision of the manuscript: W. Zhang, Y. Gao, H. Ji
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): W. Zhang, F. Li, X. Tong, Y. Ren, X. Han, S. Yao, F. Long, Z. Yang, L. Zhang, H. Ji
Study supervision: H. Ji

Acknowledgments
The authors thank Drs. T. Jacks, R. Depinho, K. Wong, and D Pan for providing the Kras\(^{G12D}\)/Lkb1\(^{−}\)/C0 and Yap\(^{−}\)/ mice. Drs. Lijian Hui and Xinyuan Liu for sharing materials; and Dr. K.-L. Guan for helpful discussion and technical support.

Grant Support
This work was supported by the National Basic Research Program of China (973 Program 2012CB910800 and 2012CB945001), the National Natural Science Foundation of China (81430066, 81402276, 81402371, 81401898, 81402498, 81101583, 81372509, 31370747, and 81325015), the Science and Technology Commission of Shanghai Municipality (12JC1409800), the ‘Cross and Cooperation in Science and Technology Innovation Team’ program, Shanghai Institutes for Biological Sciences (2013KIP303, 2013KIP102), the Science and Technology Commission of Shanghai Municipality (12JC1409800 and 15D1504000), the China Postdoctoral Science Foundation (2011M50826), The Second Military Medical University Youth Startup Funds (2013QN10), and Youth Research Projects of Shanghai Municipal Bureau of Health (2013YV210). The authors gratefully acknowledge the support of Sanofi-Aventis Shanghai Institutes for Biological Sciences (SA-SIBS) scholarship program and K.C. Wong education foundation scholarship.

Received November 22, 2014; revised June 17, 2015; accepted August 5, 2015, published OnlineFirst November 9, 2015.
YAP Promotes Malignant Progression of Lkb1-Deficient Lung Adenocarcinoma through Downstream Regulation of Survivin

Wenjing Zhang, Yijun Gao, Fuming Li, et al.

Cancer Res Published OnlineFirst September 11, 2015.

Updated version Access the most recent version of this article at:
doi:10.1158/0008-5472.CAN-14-3396

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.