BAI1-associated protein 2-like 2 is a potential biomarker in lung cancer

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Abstract. Lung cancer is the leading cause of cancer-related death worldwide. The underlying molecular mechanisms that trigger this disease remain largely unknown. The I-BAR family is involved in regulating cell membrane formation and some members, such as BAIAP2L1, IRSp53 and MIM have been shown to participate in tumorigenic progression. However, the role of BAI1-associated protein 2-like 2 (BAIAP2L2) in cancer development is unclear. In the present study, we determined that BAIAP2L2 was upregulated in lung adenocarcinoma tissues and various lung cancer cell lines. *In vitro*, BAIAP2L2 silencing resulted in decreased viability and colony formation capacity of both A549 and H1299 cells. By contrast, BAIAP2L2 overexpression promoted the proliferation and growth of 95D cells. These results indicated that BAIAP2L2 was essential for lung cancer cell proliferation and growth. We also found that BAIAP2L2 knockdown increased the apoptosis of A549 and H1299 cells. At the molecular level, BAIAP2L2 knockdown led to dysregulation of numerous genes, among which the Estrogen-mediated S-phase Entry pathway was significantly suppressed. Collectively, our findings revealed BAIAP2L2 as a novel biomarker and potential therapeutic target for lung cancer.

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Introduction

Lung cancer is a severe disease that causes extensive death worldwide (1). Once diagnosed, patients have a poor prognosis and there are no effective treatments. The overall 5-year survival rate of lung cancer patients is <14%, less than breast and colon cancer (2). In the last decade, pathological molecular mechanisms have been widely established in lung cancer, such as epidermal growth factor receptor (EGFR) (3,4) and fibroblast growth factor receptor (FGFR) (5,6) signaling. Based on these targets, rational treatments have been developed, among which tyrosine kinase inhibitors are well-known (7). Although the patients harboring EGFR mutations are more sensitive to tyrosine kinase inhibitor treatment, frequent resistance renders its clinical applications difficult for this disease (8). Thus, it is urgent to develop novel efficient drug targets for lung cancer.

BAI1-associated protein 2-like 2 (BAIAP2L2), also known as Pinkbar, is located on chromosome 22q13.1 (9). Along with BAIAP2L1 (insulin receptor tyrosine kinase substrate; also known as IRTKS), IRSp53, MIM (missing in metastasis; also known as MTSS1) and ABBA (actin-bundling protein with BAIAP2 homology; also known as MTSS1L), BAIAP2L2 belongs to the Inverse Bin-Amphiphysin-Rvs (I-BAR) subfamily (10-12). The I-BAR domain interacts with signaling pathways to induce the formation of actin-based membrane protrusions (13,14). For example, Tir has been shown to interact with the I-BAR domain of BAIAP2L1 to regulate actin pedestal formation (15). Likewise, it was also revealed that Rif colocalizes with BAIAP2L2 that is involved in edge ruffling at cell-cell contacts (16).

The I-BAR family has been revealed to participate in various tumorigenic progression. Interaction of IRSp53 and Eps8 is essential to cancer cell motility or invasiveness (17). Mice lacking MIM spontaneously develop tumors characterized by diffusing large B lymphoma at a late age (18). BAIAP2L1 was revealed to be upregulated and a potential biomarker in ovarian cancers (19). Fusion protein of FGF receptor 3 (FGFR3)-BAIAP2L1 also contributed to bladder cancer (20). These findings support the critical role of I-BAR family members in carcinogenesis. However, little evidence has reported the role of BAIAP2L2 in cancer development, including lung cancer.

In the present study, BAIAP2L2 was upregulated in lung adenocarcinoma tissues and lung cancer cells. Based on knockdown and overexpressing strategies, we revealed that BAIAP2L2 was essential to the proliferation and colony formation of lung cancer cells. Mechanistically, BAIAP2L2 silencing led to enhanced apoptosis in A549 and H1299 cells. At the molecular level, thousands of genes were dysregulated after BAIAP2L2 knockdown. Pathway enrichment analysis further indicated that Estrogen-mediated S-phase Entry pathway was significantly inhibited. In addition, CCNA2, CCND1 and CDK6 were downregulated and CDKN1B was upregulated in A549 cells with silenced BAIAP2L2. These findings revealed BAIAP2L2 as a potential biomarker and therapeutic target for lung adenocarcinoma.

Materials and methods

Cell culture. Lung epithelial cells BEAS-2B and human lung cancer cells 95D, H1975, H1299 and A549 were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA) and cultured in RPMI-1640 medium (HyClone; GE Healthcare Life Sciences, Logan, UT, USA). The culture medium was supplemented with 10% fetal bovine serum and 1% penicillin and streptomycin solution (both from Corning Inc., Corning, NY, USA). All the cells were maintained at 37°C with 5% CO_2.

Microarray of human lung adenocarcinoma and adjacent normal samples. The HLug-Ade030PG-01 microarrays of lung adenocarcinoma and adjacent normal samples were obtained from Shanghai Outdo Biotech Co., Ltd. (Shanghai, China). This array contained a total of 15 samples with 11 stage II, 4 stage III lung adenocarcinoma samples and 15 adjacent normal samples. A core represented a separate case and each sample was fixed in formalin. Thick 5-μm slices coated with paraffin were subjected to immunohistochemical staining of BAIAP2L2.

Immunohistochemistry (IHC). Microarray of human lung adenocarcinoma and adjacent normal sample were subjected to immunohistochemistry staining of BAIAP2L2. Briefly, 5 μm sections of tissues were deparaffinized in xylene and rehydrated in descending alcohol series. Antigen-retrieval was performed by incubating 0.01 M boiled citrate buffer in a microwave for 20 min. After cooling to room temperature, slides were rehydrated in double distilled H_2O for 10 min. The slides were then blocked with 10% goat serum (cat. no. C0265; Beyotime Institute of Biotechnology, Beijing, China) at room temperature for 30 min, followed by incubating with primary anti-BAIAP2L2 antibody (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany; cat. no. HPA003043, dilution 1:50) at 4°C overnight. Then, the slides were washed with TBS and incubating with the secondary antibodies (dilution 1:100; cat. no. SPN-9001; OriGene Technologies, Inc., Rockville, MD, USA) at room temperature for 2 h. After washing with TBS, the sections were stained by Vulcan Fast Red Chromogen kit 2 for 15 min at room temperature (Biocare, Shanghai, China). The extent and intensity of BAIAP2L2 immunostaining were taken into consideration. The intensity of extent of BAIAP2L2 expression was graded as follows: Negative, 0;

weak, 1; moderate, 2; and strong, 3. The extent of staining was grouped according to the percentage of high-staining cells in the cancer nest: Negative, 0; 1-25, 1; 26-50, 2; 51-75, 3; and 76-100%, 4. The final quantitation of each staining was obtained by multiplying the two scores. Immunoreactivity was assessed independently by two expert pathologists blinded to all clinical data.

BAIAP2L2 knockdown in A549 and H1299 cells. A lentiviral system containing pGCSIL-GFP (stably expressed shRNA fused with a GFP marker), pHelper1.0 (gag/pol element) and Helper2.0 (VSVG element) were used to silence BAIAP2L2 in A549 and H1299 cells. The vectors were transfected into 293T cells using Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA). The lentiviral supernatants were collected 48 h after transfection, filtered through 0.45-μm filters, and then subjected to infection of A549 and H1299 cells. Knockdown efficiency was determined by qRT-PCR and western blot analysis of BAIAP2L2.

BAIAP2L2 overexpression in 95D cells. The coding sequence of BAIAP2L2 was inserted into pBABE-puro vector. Targeted vectors or control vectors accompanied with PIK packaging vectors were co-transfected into 293T cells using Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc.). Forty-eight hours later, viral supernatants were collected and filtered through 0.45-μm filters. Then, they were subjected to infection of 95D cells. Puromycin was used to select stable cell lines.

Western blotting. Total proteins were isolated from indicated cells using lysis buffer [2 g SDS, 1.55 g DTT, 6 ml Tris (1 M, pH 6.8), 10 ml glycerol and ddH_2O up to 100 ml]. Cell lysates were boiled at 98°C for 10 min, and then centrifuged at 16,000 x g for 5 min. Briefly, the lysates were separated on 10 or 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride membranes (PVDF; EMD Millipore, Billerica, MA, USA). The PVDF membranes were then blocked in 5% skim milk at room temperature for 60 min and incubated with primary antibodies at 4°C overnight. Antibodies against BAIAP2L2 for western blotting were purchased from Abcam (Cambridge, UK) (dilution 1:1,000; cat. no. ab135897). Antibodies against CCNA2 (dilution 1:1,000; cat. no. 4656), CDK6 (dilution 1:1,000; cat. no. 3136) and CDKN1B (dilution 1:1,000; cat. no. 3698) were purchased from Cell Signaling Technology (Danvers, MA, USA). Antibodies against CCND1 (dilution 1:200; cat. no. ab16663) were purchased from Abcam. Antibodies against GAPDH (dilution 1:2,000; cat. no. sc-32233) and all the secondary antibodies (goat anti-mouse IgG, dilution 1:5,000; cat. no. sc-2005; goat anti-rabbit IgG, dilution 1:5,000; cat. no. sc-2004) were obtained from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA). Following incubation with the secondary antibodies for 2 h at room temperature, the membranes were visualized using the ECL Plus kit (GE Healthcare, Chicago, IL, USA).

RNA isolation and quantitative real-time PCR. shCtrl and shBAIAP2L2 A549 and H1299 cells were lysed in TRIzol reagent and subjected to total RNA isolation using Ultrapure

RNA Kit (CWBIO, Beijing, China). Total RNA (0.8 μg) was subjected to reverse transcription using ReverTra Ace® qPCR RT Master Mix with gDNA Remover (Toyobo Life Science, Osaka, Japan). Quantitative real-time PCR was analyzed using TransStart Top Green qPCR SuperMix (TransGene Biotech Co., Ltd., Beijing, China) on an iQ5 Real-Time PCR detection system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). GAPDH served as an internal control. The primer sequences were as follows: BAIAP2L2 forward, GTCCCACCATCA CCAATGACC and reverse, TCTGCTGCTGTTGCTGCT GTC; GAPDH forward, 5'-TGACTTCAACAGCGACAC CCA-3' and reverse, 5'-CACCCTGTTGCTGTAGCCAAA-3'.

Colony formation assay. A total of 2,000 Ctrl or BAIAP2L2 overexpressed 95D cells were seeded in 10-cm plates, and shCtrl or shBAIAP2L2 A549 and H1299 cells (800 cells/well) were seeded in 6-well plates. After being cultured at 37°C for 12-16 days, the cell colonies were fixed with methanol for 20 min and stained with 0.5% crystal violet solution for 15 min. The colony numbers (>50 cells/colony) were quantified using a fluorescence microscope (Olympus Corp., Tokyo, Japan).

Cell growth screening. Multiparametric high-content screening (HCS) assay was used to determine cell viability. In brief, indicated cells were cultured in 96-well plates for 5 days. The cell density was assessed using a fluorescence-imaging microscope each day from day 1 to day 5. The density and distribution of the fluorescence determined the cell viability of shCtrl and shBAIAP2L2 A549 and H1299 cells. Quantitative results were analyzed using the ArrayScan™ HCS software (Cellomics, Inc., Pittsburgh, PA, USA).

MTT assay. shCtrl and shBAIAP2L2 A549 and H1299 cells were seeded at a density of 3,000 cells/well in 96-well plates. After 1, 2, 3, 4 or 5 days, the cells were washed with phosphate-buffered saline (PBS) and incubated with 3-(4,5-dimethyl-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg/ml) for 3 h at 37°C. Then, cell viability was detected at 490 nm of spectrophotometric absorbance.

Caspase-3/7 activity assessment. Caspase-Glo® reagent (Promega Corporation, Madison, WI, USA) was prepared according to the manufacturer's instructions. After seeding a total of 10,000 cells/well into 96-well plates, 100 μl Caspase-Glo® reagent was added into the plates. Then, the plates were rotated at 9.99-25 x g for 0.5 h and maintained at room temperature for 1.5 h. The activity was detected by a microplate reader.

Apoptosis assay. Apoptosis of shCtrl and shBAIAP2L2 A549 and H1299 cells was examined using Annexin V-APC apoptosis detection kit (eBioscience; Thermo Fisher Scientific, Inc.), following the manufacturer's instructions. Briefly, after washing with PBS, the cells were resuspended with 100 μl staining buffer and incubated with 5 μl Annexin V-APC at room temperature for 15 min, and subsequently subjected to flow cytometric detection of apoptosis.

Microarray assay. Total RNA from A549 cells was extracted using TRIzol reagent (Invitrogen; Thermo Fisher

Scientific, Inc.). NanoDrop 2000 (Thermo Fisher Scientific, Inc., Wilmington, DE, USA) and Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA) were used to detect the RNA quantity and quality. Affymetrix Human GeneChip PrimeView (Affymetrix; Thermo Fisher Scientific, Inc.) was used for microarray processing to determine gene expression profiles according to the manufacturer's instructions. Significantly different genes between A549 cells treated with shCtrl and shBAIAP2L2 were identified depending on the follow criteria: P<0.05 and the absolute fold change >1.5. Pathway enrichment analysis was performed for all significantly different genes based on Ingenuity Pathway Analysis v9.0 (IPA; www.ingenuity.com; Ingenuity Systems, Redwood City, CA, USA).

Statistical analysis. The statistical analysis was performed using GraphPad Prism 6.0 (GraphPad Software, Inc., La Jolla, CA, USA). The data were presented as the mean ± SEM of at least 3 independent repeats. Paired Student's t-tests were used to analyze the differences of the IHC scores between the adenocarcinoma and adjacent tissues. Unpaired Student's t-tests were used to analyze the difference between two other groups. Differences among groups were determined by one-way analysis of variance (ANOVA) with repeated measures, followed by the Bonferroni post hoc test. P<0.05 was considered to indicate a statistically significant difference.

Results

BAIAP2L2 is upregulated in lung adenocarcinoma and lung cancer cells. To explore the clinical relevance of BAIAP2L2 in lung cancer, we collected 15 pairs of lung adenocarcinoma specimens and detected the expression of BAIAP2L2 using immunohistochemical assay. We revealed that BAIAP2L2 was overexpressed in the tumor areas compared to adjacent normal tissues (Fig. 1A and B). In addition, we analyzed the expression of BAIAP2L2 in normal lung epithelial cells and lung cancer cells. The qRT-PCR results revealed that the mRNA level of BAIAP2L2 was higher in lung cancer cells compared to BEAS-2B cells (Fig. 1C). These results indicated that BAIAP2L2 was a potential biomarker for lung cancer and that it may be essential for lung cancer progression.

BAIAP2L2 is critical for lung cancer cell proliferation and growth. We next determined the role of BAIAP2L2 in lung cancer. BAIAP2L2 was silenced in A549 cells using lentivirus-mediated knockdown strategy (Fig. 2). Then, we analyzed the proliferation of shCtrl and shBAIAP2L2 in A549 cells from day 1 to day 5 through HCS. We found that A549 cells expressing shCtrl lentivirus proliferated robustly from day 1 to day 5 (Fig. 3A). However, BAIAP2L2 knockdown completely suppressed the proliferation of A549 cells (Fig. 3A). Cell count analysis was consistent with the results (Fig. 3B). Likewise, MTT detection also demonstrated that blockage of BAIAP2L2 largely inhibited the proliferation of A549 cells (Fig. 3C). In addition, we silenced BAIAP2L2 in another lung cancer cell line, H1299. qRT-PCR and western blot results revealed that BAIAP2L2 was knocked down in H1299 cells (Fig. 2). Consistently, blockage of BAIAP2L2 significantly decreased the viability of H1299 cells as indicated
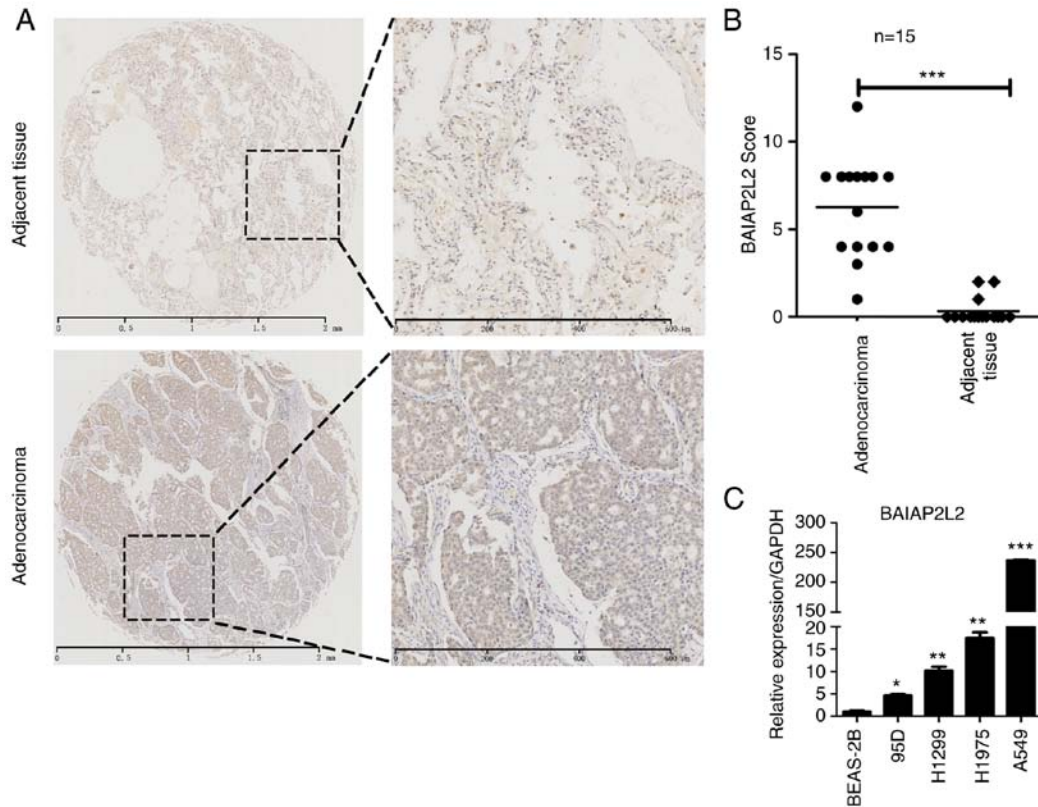
Figure 1. BAIAP2L2 expression is increased in lung adenocarcinoma tissues and lung cancer cells. (A and B) Immunohistochemical results of BAIAP2L2 in lung adenocarcinoma and adjacent normal tissues. (A) Representative images of immunohistochemical staining of BAIAP2L2. (B) Relative BAIAP2L2 expression was revealed as the BAIAP2L2 score (n=15). ***P<0.001. (C) The expression of BAIAP2L2 was determined by qRT-PCR in 4 lung cancer cell lines 95D, H1299, H1975, A549 and one normal lung embryonic fibrosis cell line (BEAS-2B). BAIAP2L2, BAI1-associated protein 2-like 2. *P<0.05, **P<0.01 and ***P<0.001.
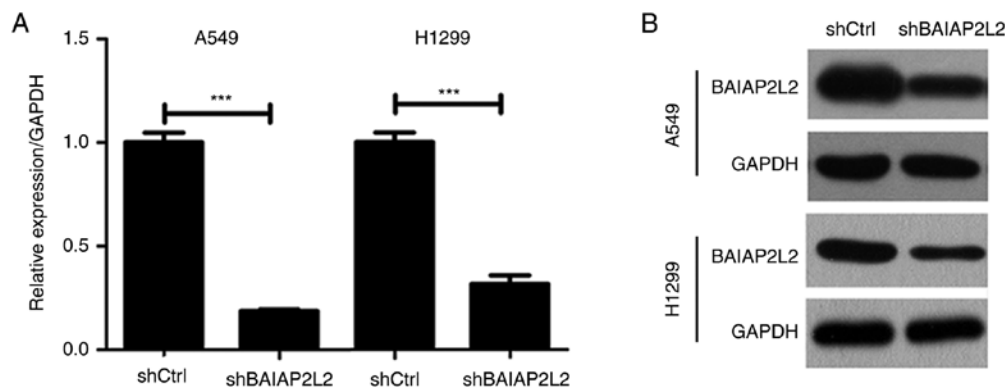

Figure 2. BAIAP2L2 is efficiently silenced in A549 and H1299 cells using lentivirus-mediated knockdown strategy. (A) qRT-PCR and (B) western blot results of BAIAP2L2 in A549 and H1299 cells expressing shCtrl or shBAIAP2L2 lentivirus. ***P<0.001. BAIAP2L2, BAI1-associated protein 2-like 2.

by the suppressed cell proliferation rate (Fig. 3D). Moreover, we detected a reduced number of colonies in shBAIAP2L2 A549 or H1299 cells compared with shCtrl A549 or H1299 cells (Fig. 4).

To ascertain our results, we overexpressed BAIAP2L2 in 95D cells. It was revealed that BAIAP2L2 ectopic expression accelerated the proliferation and growth of 95D cells (Fig. 5). Thus, we concluded that BAIAP2L2 is critical for lung cancer cell viability.

BAIAP2L2 knockdown leads to increased apoptosis. Suppressed cell proliferation and growth may be a consequence of increased cell death. Apoptosis is a common cell death type and plays an important role in cancer development. Therefore, we investigated whether apoptosis participated in BAIAP2L2-mediated lung cancer cell proliferation and growth. To address this question, Annexin V-APC was used to detect the apoptosis of A549 and H1299 cells expressing shCtrl or shBAIAP2L2 lentivirus. Our results revealed that BAIAP2L2 silencing increased the apoptosis of A549 and H1299 cells (Fig. 6A and C). Quantitative results revealed that >2-fold enhanced apoptosis was found in A549 and H1299 cells after BAIAP2L2 knockdown (Fig. 6B and D). Furthermore, we also detected increased caspase-3/7 activity
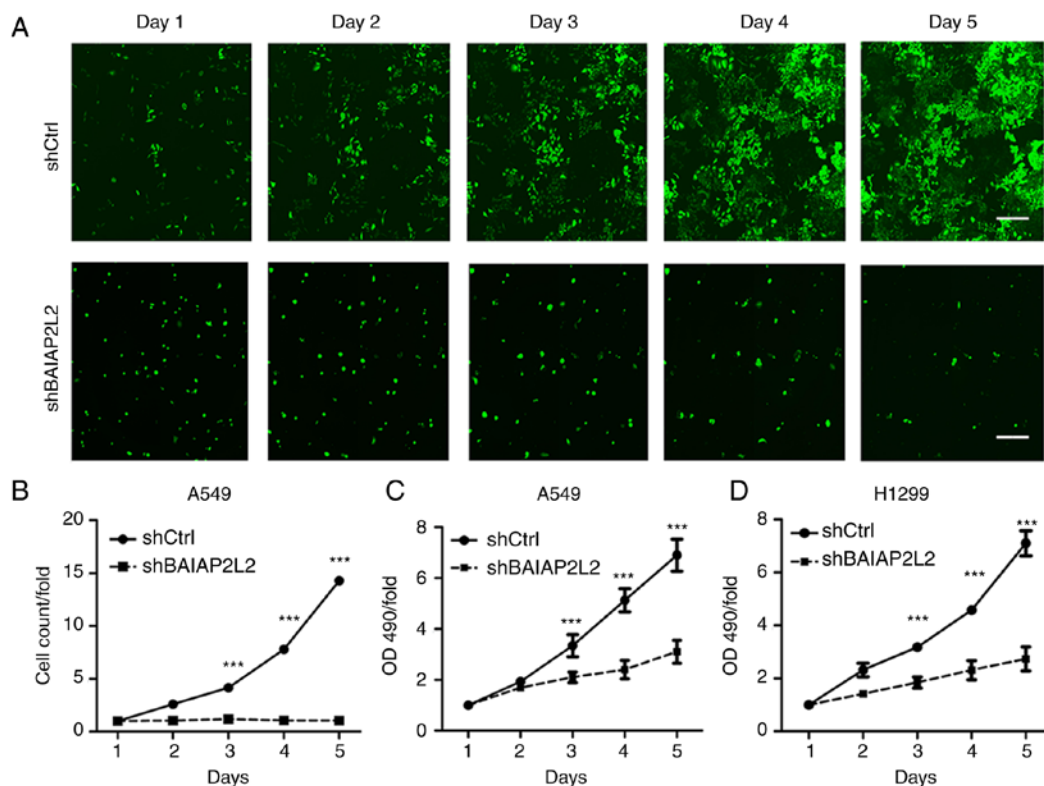
Figure 3. BAIAP2L2 knockdown suppresses the proliferation rate of A549 and H1299 cells. (A and B) Cell viability of shCtrl or sBAIAP2L2 A549 cells was determined by multiparametric high-content screening (HCS) from day 1 to day 5. (A) Representative images of HCS. (B) Quantification of HCS. ***P<0.001. The green fluorescence represented viable cells. Scale bar, 100 μm. (C and D) MTT analysis of shCtrl and shBAIAP2L2 A549 (C) or H1299 (D) cells. ***P<0.001. BAIAP2L2, BAI1-associated protein 2-like 2; HCS, high-content screening.
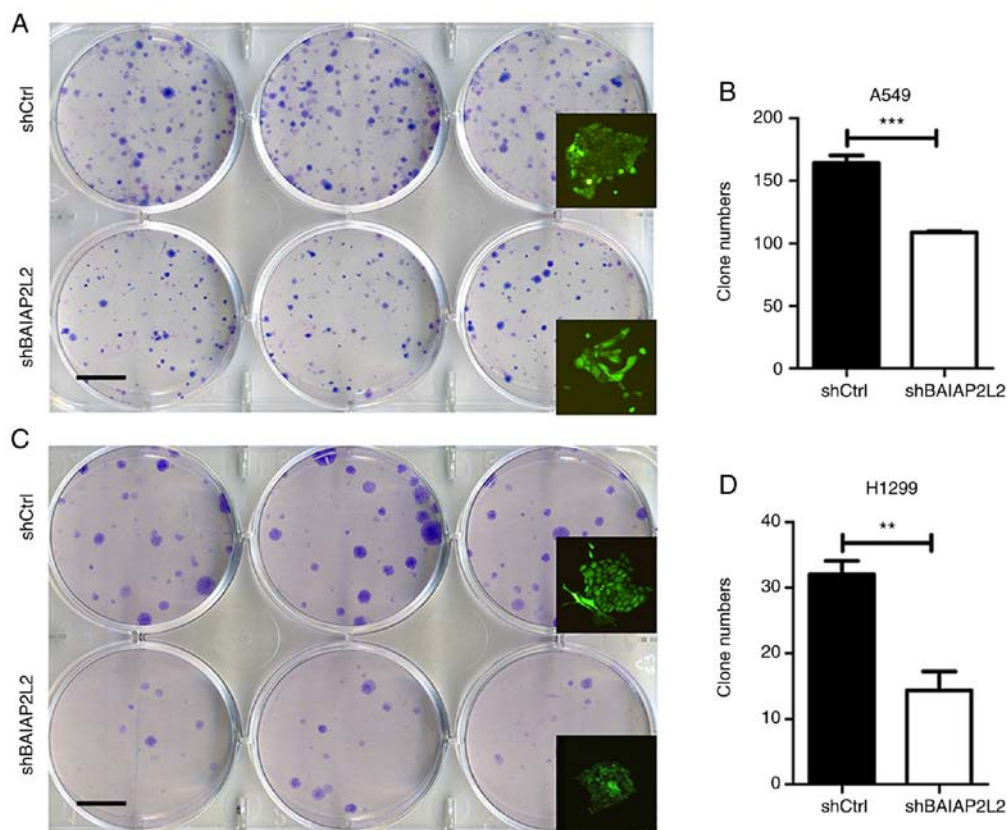

Figure 4. BAIAP2L2 knockdown suppresses the colony formation of A549 and H1299 cells. (A and B) Colony formation of shCtrl and shBAIAP2L2 A549 cells. (A) Representative images of colonies. (B) Quantitative results of the number of colonies. ***P<0.001. Scale bar, 1 cm. (C and D) Colony formation of shCtrl and shBAIAP2L2 H1299 cells. (C) Representative images of colonies. (D) Quantitative results of the number of colonies. **P<0.01. Scale bar, 1 cm. BAIAP2L2, BAI1-associated protein 2-like 2.
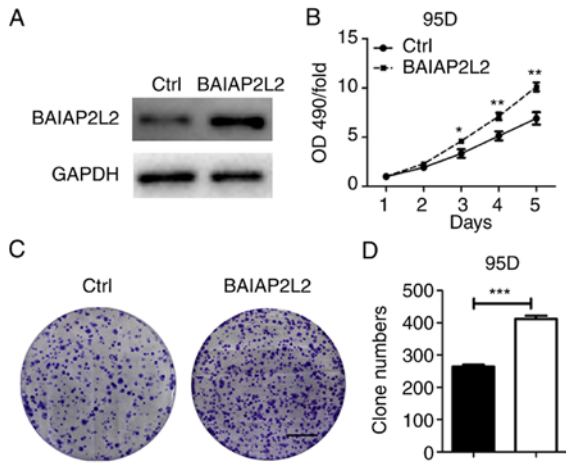
Figure 5. BAIAP2L2 overexpression increases the proliferation and colony formation of 95D cells. (A) Western blotting of Ctrl and BAIAP2L2-overexpressed 95D cells. (B) MTT analysis of cells described in A. *P<0.05, **P<0.01. (C and D) Colony formation of cells described in A. (C) Representative images of colonies. Scale bar, 2 cm. (D) Quantitative results of the number of colonies. ***P<0.001. BAIAP2L2, BAI1-associated protein 2-like 2.

in shBAIAP2L2 A549 cells compared with shCtrl A549 cells (Fig. 6E). Likewise, shBAIAP2L2 H1299 cells exhibited increased apoptosis and caspase-3/7 activity (Fig. 6F). These results indicated that BAIAP2L2 knockdown triggered lung cancer cell apoptosis, mainly by enhancing caspase-3/7 activity. Collectively, our results revealed that BAIAP2L2 silencing may suppress cell proliferation and growth at least partly by increasing apoptosis.

Depletion of BAIAP2L2 results in inhibition of Estrogen-mediated S-phase Entry pathway and dysregulation of numerous genes. By revealing that BAIAP2L2 is critical for apoptosis, proliferation and growth of lung cancer cells, we further explored the potential molecular mechanism. Firstly, GeneChip was performed to determine dysregulated genes in A549 cells after BAIAP2L2 knockdown. We found that 1,240 genes were upregulated and 1,157 genes were downregulated in BAIAP2L2 silenced A549 cells (Fig. 7A). By pathway enrichment analysis, we revealed that the Estrogen-mediated S-phase Entry pathway was significantly
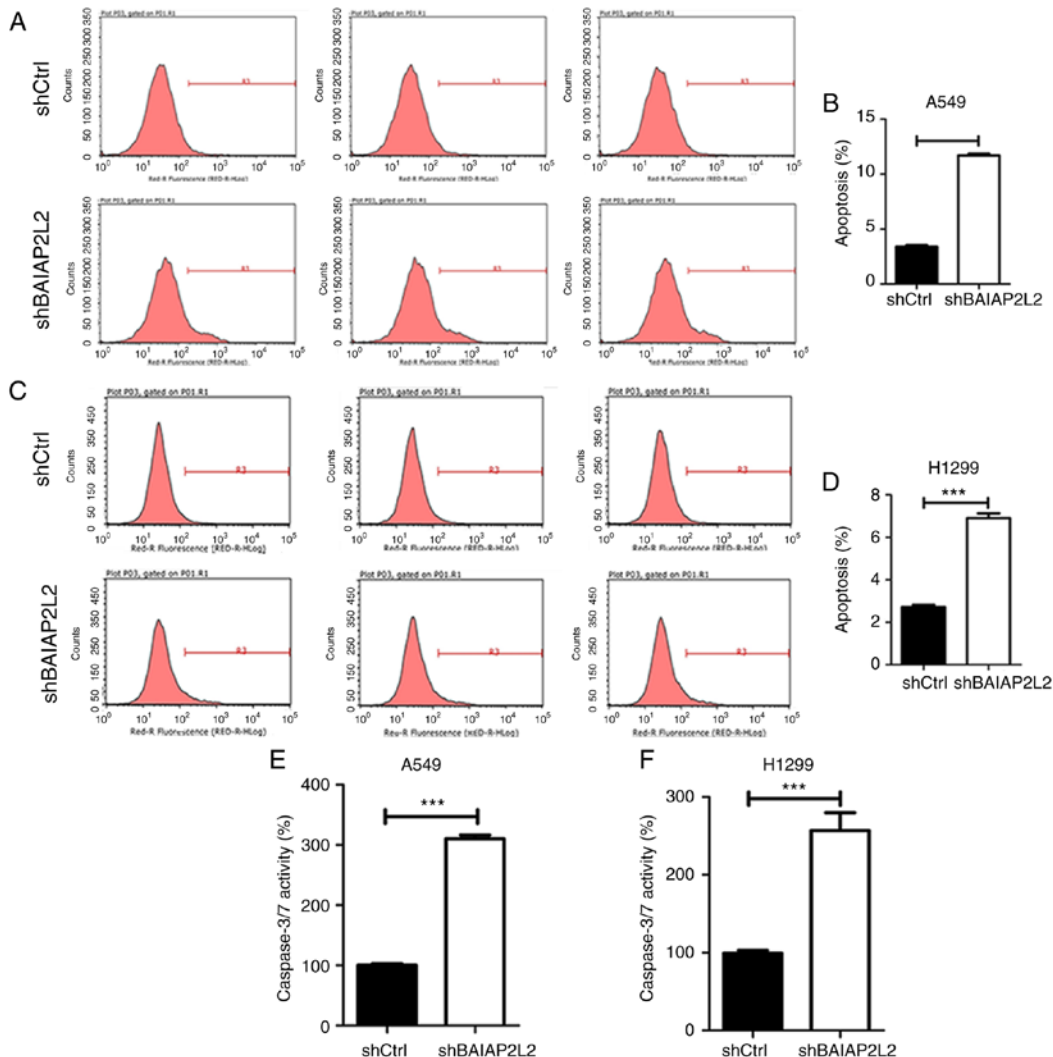

Figure 6. BAIAP2L2 knockdown induces apoptosis of A549 and H1299 cells. (A and B) Apoptosis analysis of shBAIAP2L2 or shCtrl A549 cells. (A) Annexin V-APC staining and flow cytometric analysis of apoptosis. (B) Quantitative results of apoptosis. ***P<0.001. (C and D) Apoptosis analysis of shBAIAP2L2 or shCtrl H1299 cells. (C) Annexin V-APC staining and flow cytometric analysis of apoptosis. (D) Quantitative results of apoptosis. ***P<0.001. (E and F) Caspase-3/7 activity was increased in shBAIAP2L2 (E) A549 and (F) H1299 cells. ***P<0.001. BAIAP2L2, BAI1-associated protein 2-like 2.
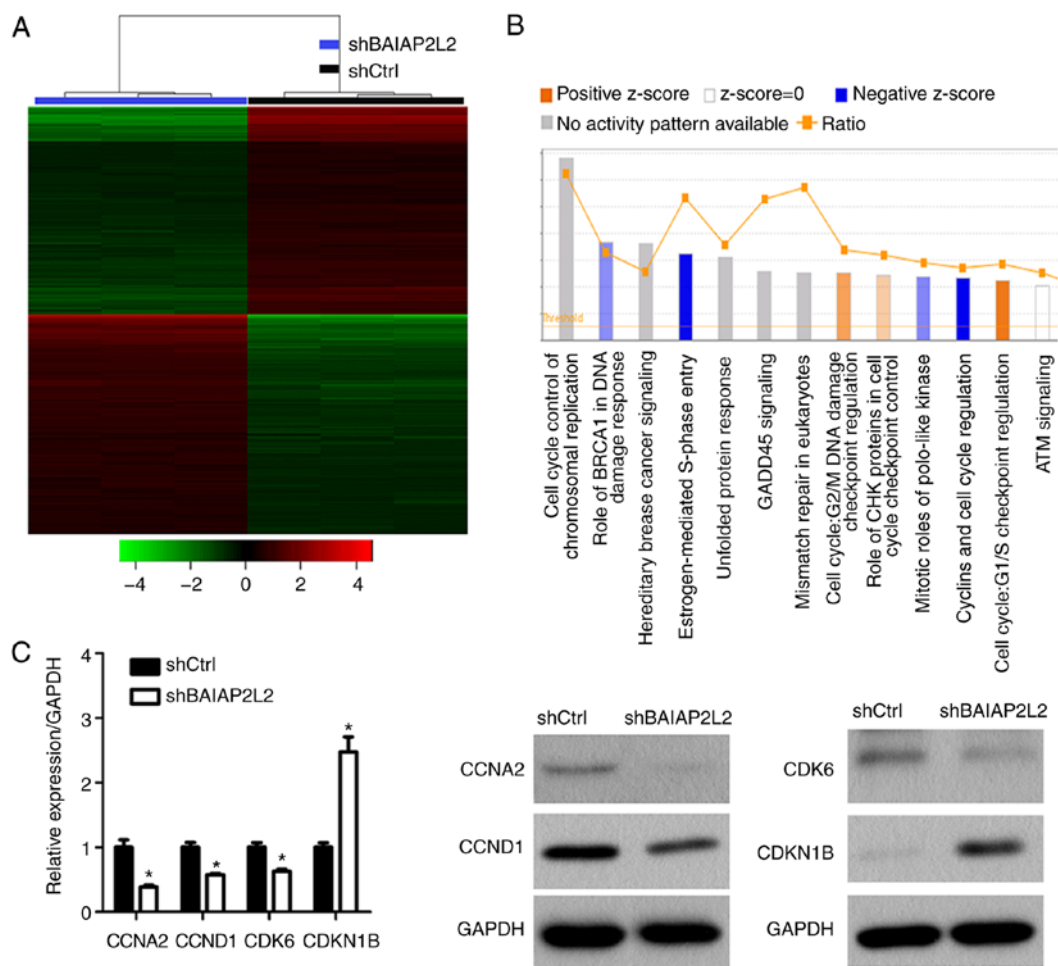
Figure 7. Multiple signaling pathways and proteins are regulated by BAIAP2L2 knockdown. (A) Gene-Chip results revealed that 1,240 genes were upregulated and 1,157 genes were downregulated after BAIAP2L2 knockdown. (Criteria P<0.05, absolute fold change >2). (B) Pathway enrichment analysis was determined by IPA software. (C) qRT-PCR and western blot results revealed that CCNA2, CCND1 and CDK6 were downregulated and CDKN1B was upregulated in shBAIAP2L2 A549 cells. BAIAP2L2, BAI1-associated protein 2-like 2. *P<0.05.

inhibited after BAIAP2L2 knockdown (Fig. 7B). Cyclins and cell cycle regulation signaling was suppressed (Fig. 7B). However, the predictive analysis revealed that the G1/S checkpoint regulation pathway involved in the cell cycle was upregulated (Fig. 7B). Additionally, BAIAP2L2 knockdown upregulated the G2/M DNA damage checkpoint regulation pathway to a lesser extent (Fig. 7B). Next, we confirmed our predictive analysis using qRT-PCR and western blot assays. The results revealed that CCNA2, CCND1, CDK6 were downregulated and CDKN1B was upregulated in shBAIAP2L2 A549 cells (Fig. 7C).

Discussion

Lung cancer is continuously a threat to the health of people due to its high lethality. During the last decade, crucial signaling pathways triggering this disease have been identified, including epidermal growth factor receptor (21), vascular endothelial growth factor (22) and insulin-like growth factor-1 network (23). Subsequently, newly targeted drugs against lung cancer have been developed. However, the outcomes of these targeted agents are less than satisfactory. Therefore, there is constant need to explore novel critical molecular mechanisms

involved in lung cancer progression. In the present study, BAIAP2L2 was revealed as a novel oncogene for lung adenocarcinoma. Upregulation of BAIAP2L2 was found in lung adenocarcinoma specimens. Knockdown of BAIAP2L2 reduced the proliferation and growth of lung cancer cells.

BAIAP2L2 is identified as an I-BAR family member that plays an important role in regulating membrane protrusions (13). Although some studies have demonstrated that BAIAP2L1, another I-BAR family member, is a promising biomarker for ovarian cancer (19), the precise function of BAIAP2L2 in cancer development, particularly in lung cancer, remains largely unknown. This study provided the first evidence that BAIAP2L2 was a proto-oncogene for lung cancer. Since it was highly expressed in lung adenocarcinoma tissues and lung cancer cells, we attempted to explore the role of BAIAP2L2 through depletion of this gene in lung cancer cells A549 and H1299. Our results revealed that silencing of BAIAP2L2 markedly blunted the proliferation of A549 and H1299 cells. In addition, colony formation results also supported the oncogenic function of BAIAP2L2 since reduction of BAIAP2L2 significantly suppressed the colony formation of A549 and H1299 cells. Collectively, it was concluded that BAIAP2L2 was critical for lung cancer proliferation and growth.

It has been demonstrated that BAIAP2L1 prevented the apoptosis of ovarian cancer cells (19). Additionally, BAIAP2L2 has also been reported to activate EGFR/ERK signaling and promote cell proliferation of hepatocellular carcinoma (24). Our mechanistic study revealed that BAIAP2L2 silencing promoted the apoptosis of lung cancer cells. Caspase-3/7 activity was enhanced in A549 and H1299 cells after BAIAP2L2 knockdown. These results demonstrated the crucial role of BAIAP2L2 in apoptosis. Furthermore, our pathway enrichment analysis indicated that cell cycle regulation pathways were dysregulated after BAIAP2L2 knockdown. Western blotting results revealed that CCNA2, CCND1 and CDK6 were downregulated and CDKN1B was upregulated in shBAIAP2L2 A549 cells. These findings indicated that BAIAP2L2 may be critical for cell cycle progression. It is well-known that estrogen has different functions in cancer development. Estrogen prevents hepatocellular carcinoma (25) and is shown to promote lung cancer (26). In this study, pathway enrichment analysis revealed that the Estrogen-mediated S-phase Entry pathway was significantly inhibited after BAIAP2L2 knockdown in A549 cells. Thus, further study must be performed to elucidate how this signaling is regulated by BAIAP2L2 and the functional role of this pathway in lung cancer development.

In summary, the study demonstrated for the first time, to the best of our knowledge, that BAIAP2L2 was upregulated in lung adenocarcinoma and functioned as an oncogene in lung cancer. BAIAP2L2 was essential for lung cancer cell survival since depletion of BAIAP2L2 led to increased apoptosis, decreased cell proliferation and growth. Mechanistically, caspase-3/7 activity was increased in shBAIAP2L2 lung cancer cells. In addition, cell cycle progression may also be blunted since dysregulation of cell cycle-related proteins was also found in shBAIAP2L2 lung cancer cells. Nevertheless, further study should be performed to investigate the involvement of estrogen in BAIAP2L2-mediated lung cancer development since the Estrogen-mediated S-phase Entry pathway was markedly suppressed after BAIAP2L2 knockdown. Therefore, BAIAP2L2 was revealed to be a potential biomarker and promising therapeutic target for lung cancer.

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Availability of data and materials

The datasets used during the present study are available from the corresponding author upon reasonable request.

Authors' contributions

XF conceived the study, carried out the experimental design and the data interpretation, prepared and revised the manuscript. LX performed most of the experiments, wrote, reviewed and edited the manuscript. HD and QZ performed the immunohistochemical assay and packaging of lentivirus. CW and LY performed the colony formation and the qPCR assays. GT performed the western blot assay and the statistical analysis. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

The present study was approved by the Research Ethics Committee of the Affiliated Hospital of Inner Mongolia Medical University.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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