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Article

BIRC3–HSP90B1 Interaction Inhibits Non-Small Cell Lung Cancer Progression through the Extracellular Signal-Regulated Kinase Pathway

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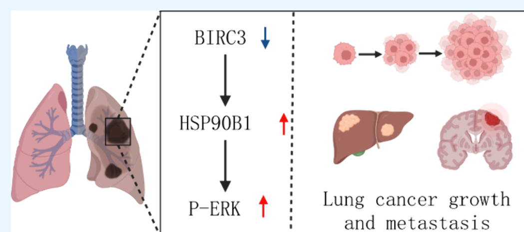
ABSTRACT: The long-term prognosis of nonsmall cell lung cancer (NSCLC) remains unsatisfactory, which is a major challenge in lung cancer treatment. BIRC3 is an inhibitor of apoptosis (IAP) protein that contributes to tumor regulation. However, the underlying regulatory mechanisms of BIRC3 in NSCLC remains unknown. We initiated an analysis of BIRC3 expression data in NSCLC tumors and adjacent tissues using the TCGA and GEO databases and examined the variations in prognosis. Further, we conducted overexpression (OE) and knockdown (KD) studies on BIRC3 to evaluate its effects on NSCLC cell proliferation, migration, and invasion. Additionally, through utilization of a nude mouse model, the regulatory effects of BIRC3 on NSCLC were verified in vivo. Co-immunoprecipitation (Co-IP) assay served to pinpoint the proteins with which BIRC3 interacts. The results indicated that BIRC3 is down-regulated in NSCLC tissues and that patients with high BIRC3 expression demonstrate a better prognosis. BIRC3 is a tumor suppressor, inhibiting the proliferation and metastasis of NSCLC. Co-IP results revealed that BIRC3 interacts with HSP90B1, leading to a decrease in HSP90B1 expression and subsequent negative regulation of the ERK signaling pathway. BIRC3 may serve as a prognostic biomarker for NSCLC. It directly interacts with HSP90B1 to negatively regulate the ERK signaling pathway, thereby hindering the progression of NSCLC.



1. INTRODUCTION

Lung cancer remains the leading cause of cancer-related deaths. Roughly 350 individuals succumb to lung cancer daily in the United States alone, as per data reported in A Cancer Journal for Clinicians.[1] NSCLC is the most prevalent histological type, encompassing approximately 85% of reported cases.[2] Despite advancements in targeted therapy and immunotherapy yielding varied levels of progress, the NSCLC prognosis remains grim. Data from the American Cancer Society reveal a 5-year survival rate of 28% for NSCLC.

Lung cancer has been associated primarily with mutated oncogenes and down-regulated tumor suppressor genes in previous studies.[3] These genes have been earmarked as potential diagnostic and treatment biomarkers for lung cancer, making the exploration and understanding of the roles and mechanisms of NSCLC-related biomarkers crucial for successful NSCLC treatment.

Inhibitor of apoptosis (IAP) protein BIRC3 plays an instrumental role in tumor cell death, inflammation activation, carcinogenesis, and proliferation and metastasis.[4] There is evidence to suggest a relationship between BIRC3 and the clinicopathological characteristics and prognosis of colon cancer, ovarian cancer, and chronic lymphocytic leukemia.[5−7] Consequently, BIRC3 is contemplated as a possible therapeutic target for various cancers. However, how BIRC3 regulates lung cancer remains undefined. This study aims to decipher the regulatory mechanisms of BIRC3 with respect to NSCLC.

Our study evaluated the role BIRC3 plays in regulating NSCLC cell progression. Additionally, we aimed to identify the proteins directly affected by BIRC3, as well as its underlying mechanism of action on NSCLC.

2. METHODS

2.1. Cell Culture. A549 and H1299 cell lines were purchased from the Shanghai Academy of Sciences cell bank and cultured in DMEM (HyClone) with 10% FBS.

2.2. Lentivirus, shRNAs, and siRNAs Transfection. Lentiviruses for the overexpression and knockdown of BIRC3 were supplied by GeneChem. Post-transfection, cells were cultured in media with puromycin for a week. HSP90B1 short-hairpin RNA (shRNA) was obtained from Hema. The efficiency of the target gene's overexpression and knockdown was

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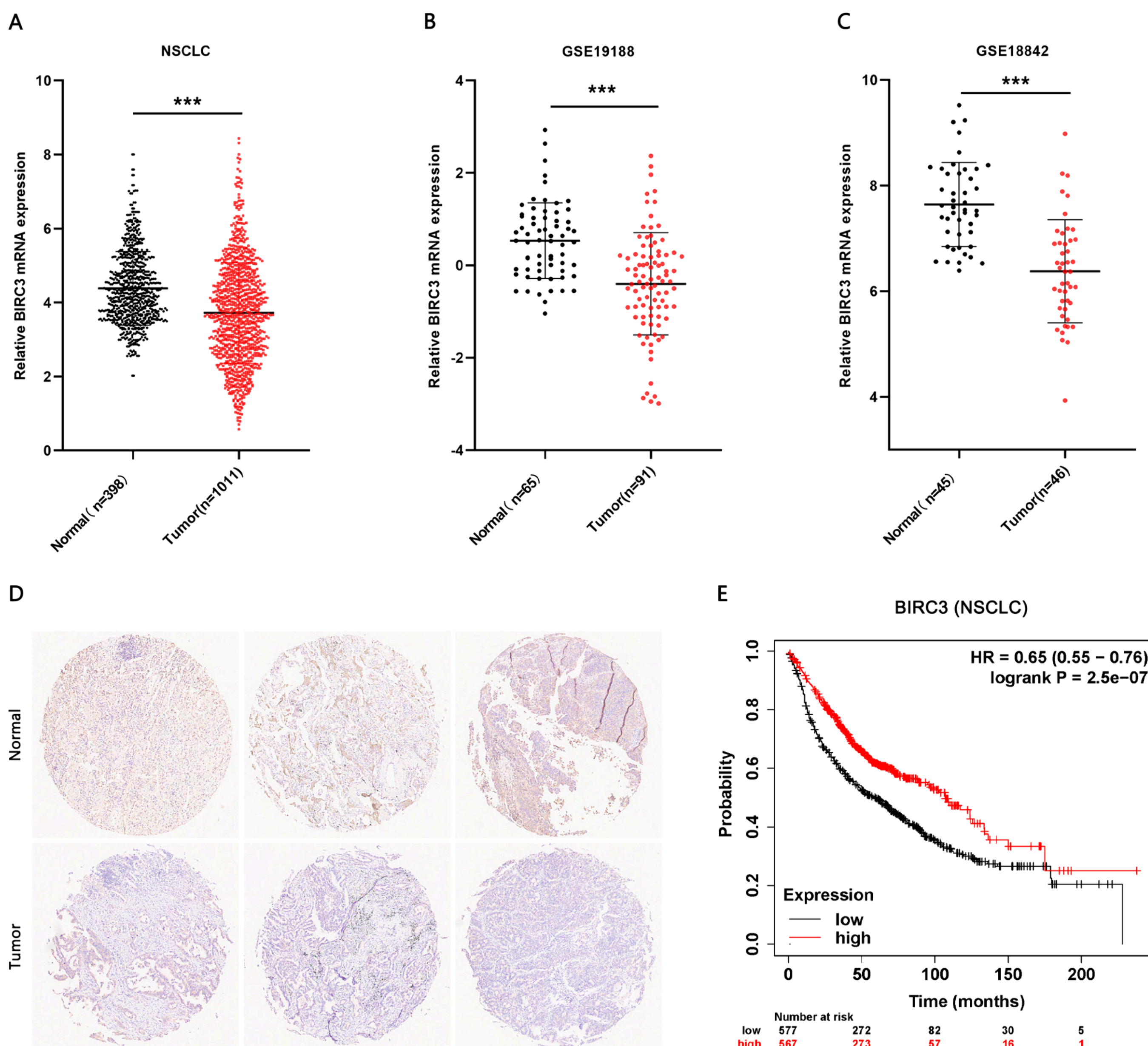

Figure 1. BIRC3 is associated with survival in patients with NSCLC. (A−C) Analyzing BIRC3 level in NSCLC tissues and normal tissues was conducted using data from TCGA, GTEX, and GEO databases. (D) Immunohistochemistry staining for the level of BIRC3 in normal and tumor lung tissue; scale bars = 500 μm. (E) Examining the correlation between BIRC3 and survival through Kaplan−Meier analysis from Kaplan−Meier Plotter yielded a significant result of $P = 2.5 \times 10^{-7}$, determined using a log-rank test.

confirmed via quantitative PCR (qPCR) and Western blot analysis (WB).

2.3. CCK-8 Assay. 2×10^3 cells were seeded into a 96-well plate. Following cell adhesion, CCK-8 enhance solution (Beyotime) was added in a 96-well plate for 1 h. Microplate readers (Thermo, USA) were used to determine the absorbance at 450 nm. Replications of all of the experiments were performed in triplicate.

2.4. Colony Formation Assay. 1000 cells were seeded in six-well plate using DMEM medium enhanced with 2% FBS. Post a week of cultivation, cells were fixed with 4% paraformaldehyde for a span of 10 min, staining using 0.1% crystal violet. Light microscopy was utilized to capture images of the colonies, and the subsequent analysis was conducted based on these images.

2.5. Transwell Assay. The migration and invasion assays were conducted using a transwell plate with (invasion) or without (migration) Matrigel. A total of 2.5×10^5 cells were placed in the upper chamber with serum-free medium, while 600 μL of medium with 40% FBS was placed in a 24 well plate. Place H1299 and A549 cells were placed in the incubator for 24 h. Subsequently, the cells that have not been penetrated were removed and fixed with 4% paraformaldehyde, staining with crystal violet for 15 min. Images of these cells were captured under a microscope manufactured by Leica, Germany. Five randomly chosen microscopic fields were taken into consideration per well, and the mean value was ascertained.

2.6. Western Blot Assay and Antibodies. H1299 and A549 were rinsed with PBS. These cells were subsequently lysed with lysis buffer (Beyotime), enduring such conditions for 30 min at 4 °C. Following the lysis, samples were centrifuged at

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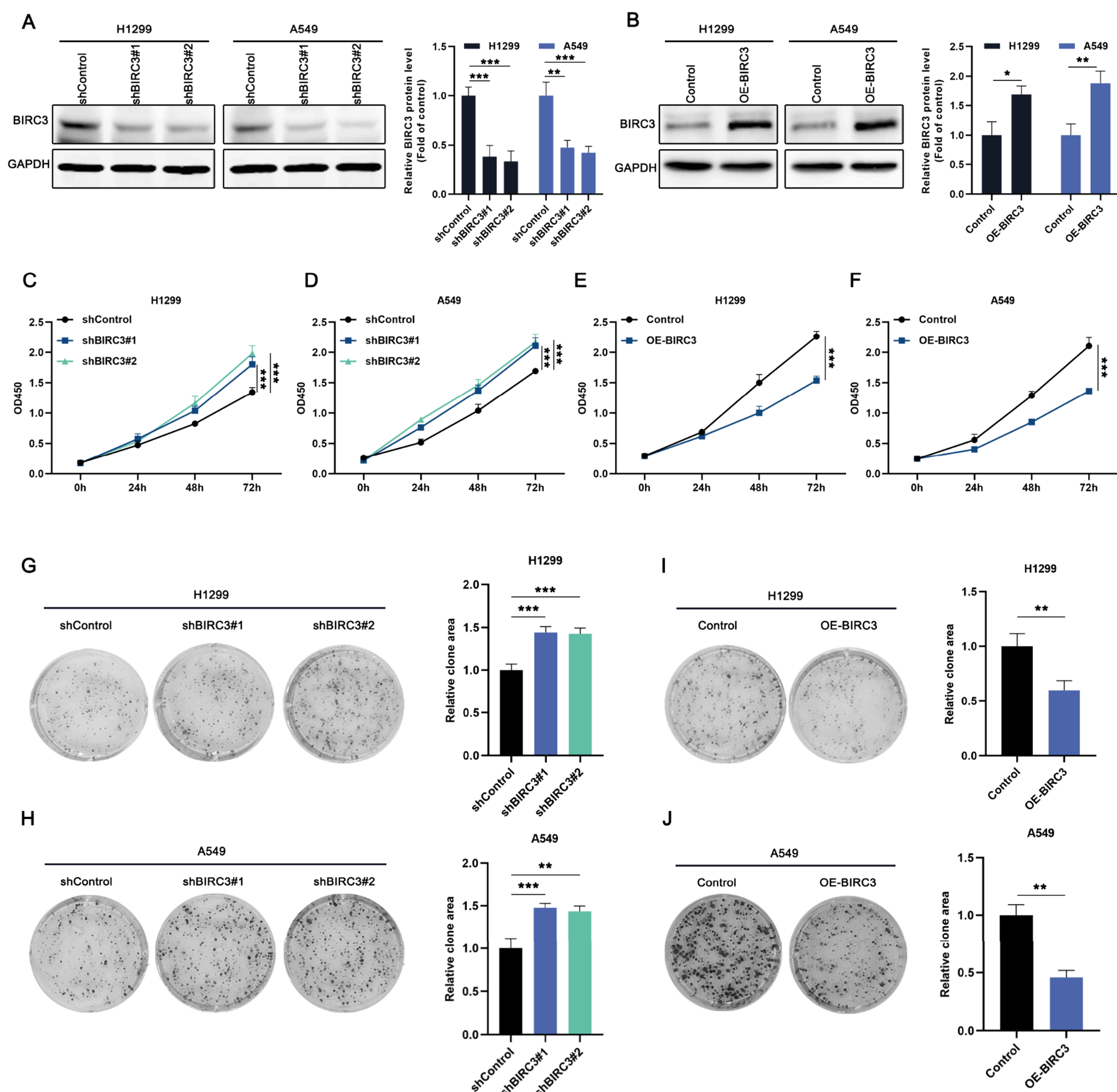

Figure 2. BIRC3 knockdown promotes the proliferation of A549 and H1299 cells. (A) WB was used to detect the level of BIRC3 after shBIRC3 treatment. (B) WB was used to detect the level of BIRC3 after overexpression treatment. (C−F) Utilizing CCK-8 assays, the impact of BIRC3 KD or OE on cancer cell growth was determined. (G−J) The effects of BIRC3 KD or OE on cancer cell growth were evaluated using clone assays.

high velocity, facilitating protein separation. The protein concentration within these samples was detected via a BCA assay. Each protein sample, consisting of 25 μg, was denatured, followed by undergoing SDS−PAGE. They were then shifted onto polyvinylidene fluoride membranes (Millipore) and blocked, then subjected to incubation with cIAP2 antibodies at 4 °C for 12 h, which was incubated with HRP-conjugated antibodies. The proteins were henceforth visualized using a chemiluminescence detection kit (Beyotime). Antibodies: anti-cIAP2 (ab32059; 1:10,000; Abcam), anti-HSP90B1 (ab23812; 1:1,000; Abcam), anti-ERK1/2 (ab184699; 1:5,000; Abcam), and anti- PERK (ab229912; 1:1,000; Abcam).

2.7. qRT-PCR. RNA was isolated by using the TRIzol reagent. A TaKaRa Step One Plus Real-Time PCR system (Applied Biosystems) was used for qRT-PCR using SYBR Premix Ex Taq (TaKaRa).

2.8. Co-Immunoprecipitation (Co-IP) Assay. This assay was performed using the Classic IP/Co-IP Kit (Thermo). Cells were lysed in IP lysis buffer. Then the solution was incubated with 1 μg of IgG or anti-BIRC3 antibody at 4 °C for 24 h. After the immunocomplexes were washed, they were eluted through boiling in SDS. The samples were then analyzed by Western Blot.

2.9. In Vivo Experiments in Mouse Models. Ten BALB/c mice (4 weeks old) were placed in an SPF environment and

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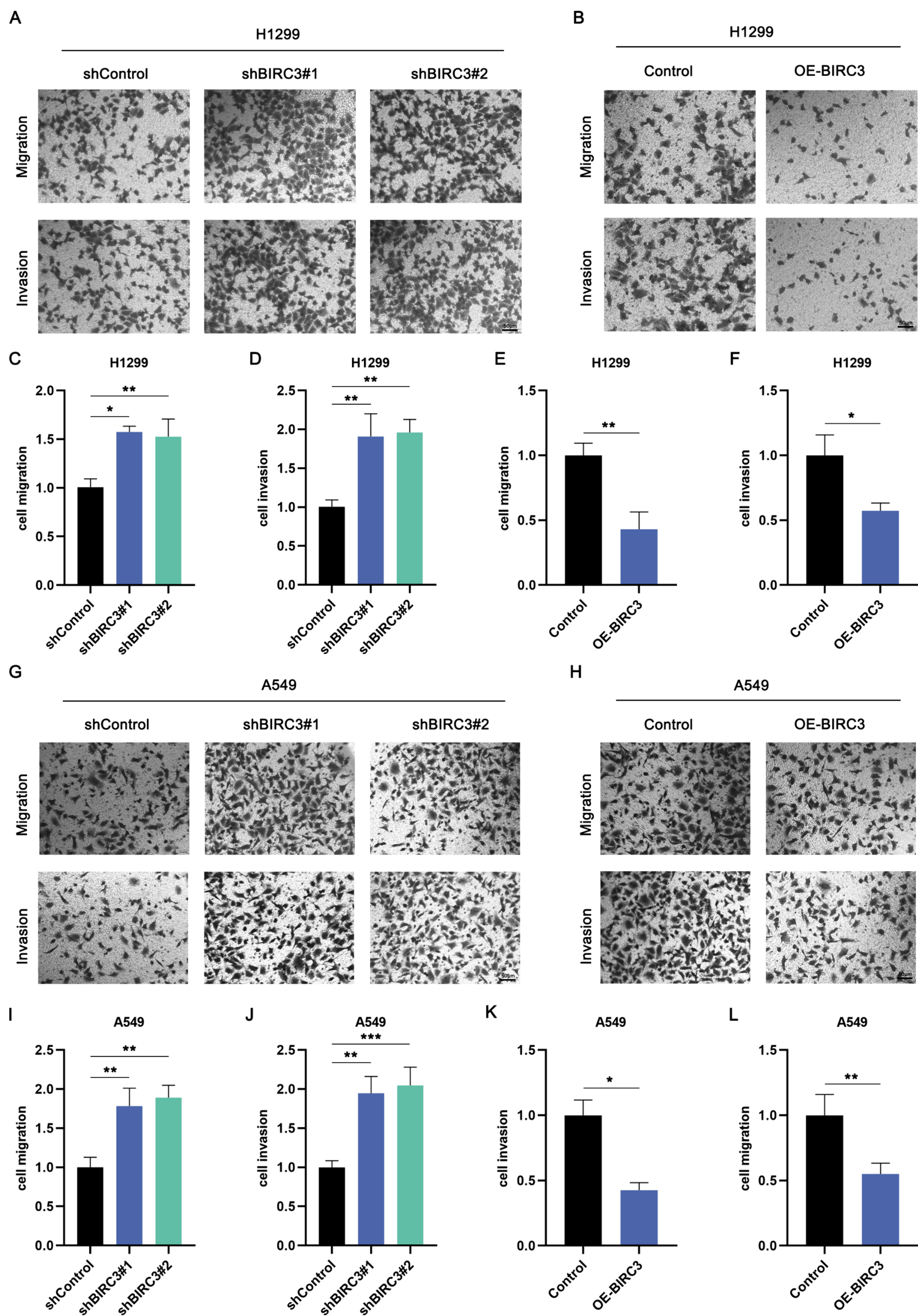

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Figure 3. continued

Figure 3. BIRC3 knockdown promotes the invasion and migration of A549 and H1299 cells. (A−F) Transwell assay for H1299 cells with BIRC3 knockdown or overexpression ($n = 3$), bars = 50 μm. (G−L) Transwell assay for A549 cells with BIRC3 knockdown or overexpression ($n = 3$), bars = 50 μm.

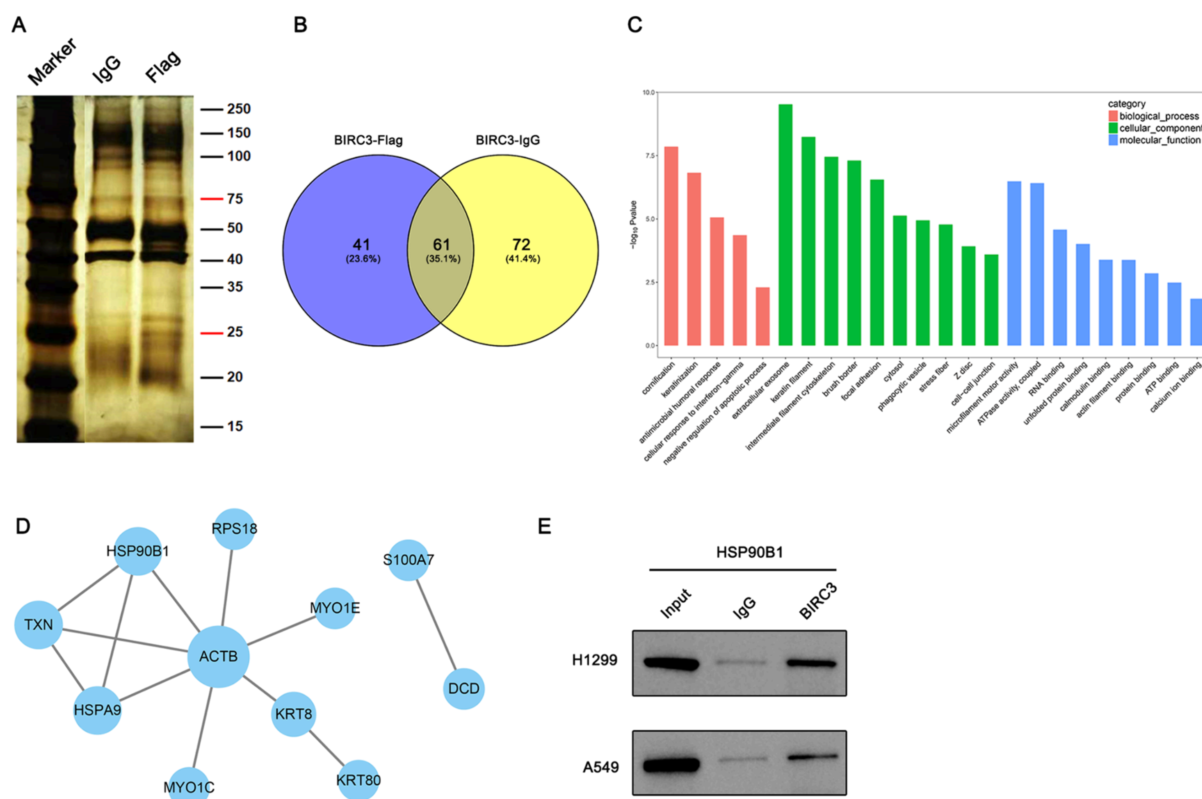

Figure 4. BIRC3 interacts with HSP90B1. (A) Silver staining of IP products. (B) Venn diagram analysis of significant proteins. (C) GO enrichment analysis of significant proteins. (D) PPI network of significant proteins. (E) IP-Western blot of BIRC3 in cancer cells.

divided into two groups randomly ($n = 5$/group). In the BIRC3 knockdown group and the control group, 5×10^6 cells of each shBIRC3 H1299 and shControl H1299 were injected for subcutaneous tumor formation, respectively. Mice were then monitored over the next 4−6 weeks. Tumor volume = length × width^2/2. Ultimately, we administered an overdose of pentobarbital (150 mg/kg, ip) to mice until cardiac and respiratory arrest. The tumors were weighed. The protocols were according to the regulations of the IACUC and with the approval of the Xuzhou medical University.

2.10. Immunohistochemical Staining (IHC). Normal lung tissue and tumor tissue were obtained from Shanghai Outdo Biotech Co, Ltd. (Shanghai, China). IHC was conducted using the All-in-One Kit for Immunohistochemical Staining of Tissues (Invitrogen, USA). Staining for Anti-BIRC3 (Abcam, ab32059) was carried out at a dilution of 1/600.

2.11. Statistical Analysis. Data analysis using GrapPad Prism 5.02 software. Mean ± SD values were utilized to present the results, ANOVA test was used to compare more than 2 means, and paired t-test was used to compare paired data. A P value <0.05 was defined as statistically significant (symbolic meaning: NS $P > 0.05$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

3. RESULTS

3.1. BIRC3 Expression Decreases in NSCLC Tissues. To ascertain the clinical relevance of BIRC3 in NSCLC, BIRC3 expression was examined in TCGA database and GEO data set (GSE19188 and GSE18842). Analysis results indicated considerably less BIRC3 expression in NSCLC tissues as compared to adjacent tissues (Figure 1A−C). This was further confirmed by immunohistochemical staining (Figure 1D). Survival data from the Kaplan−Meier Plotter Web site revealed that NSCLC patients exhibiting high BIRC3 expression (Figure 1E) had a more favorable prognosis. Based on the research findings, BIRC3 could be a viable target for lung cancer therapy.

3.2. BIRC3 Inhibits the Proliferation, Migration and Invasion of NSCLC. The expression levels of BIRC3 in BEAS-2B, A549, H1975, H1299, H23, H292, H226, and PC-9 cell lines were assessed using qRT-PCR and WB analysis (Figure S1A,B). Furthermore, experiments involving shRNA-mediated knockdown and overexpression of BIRC3 were conducted in H1299 and A549 cells (Figure 2A,B).

To explore the influence of BIRC3 on tumor cell proliferation, logarithmic growth phase cells were subjected to a CCK-8 assay, wherein OD values were measured at 0, 24, 48, and 72-h intervals. With respect to the control group, both H1299 and A549 cell lines exhibiting BIRC3 knockdown displayed heightened proliferation capabilities (Figure 2C,D). Conversely,

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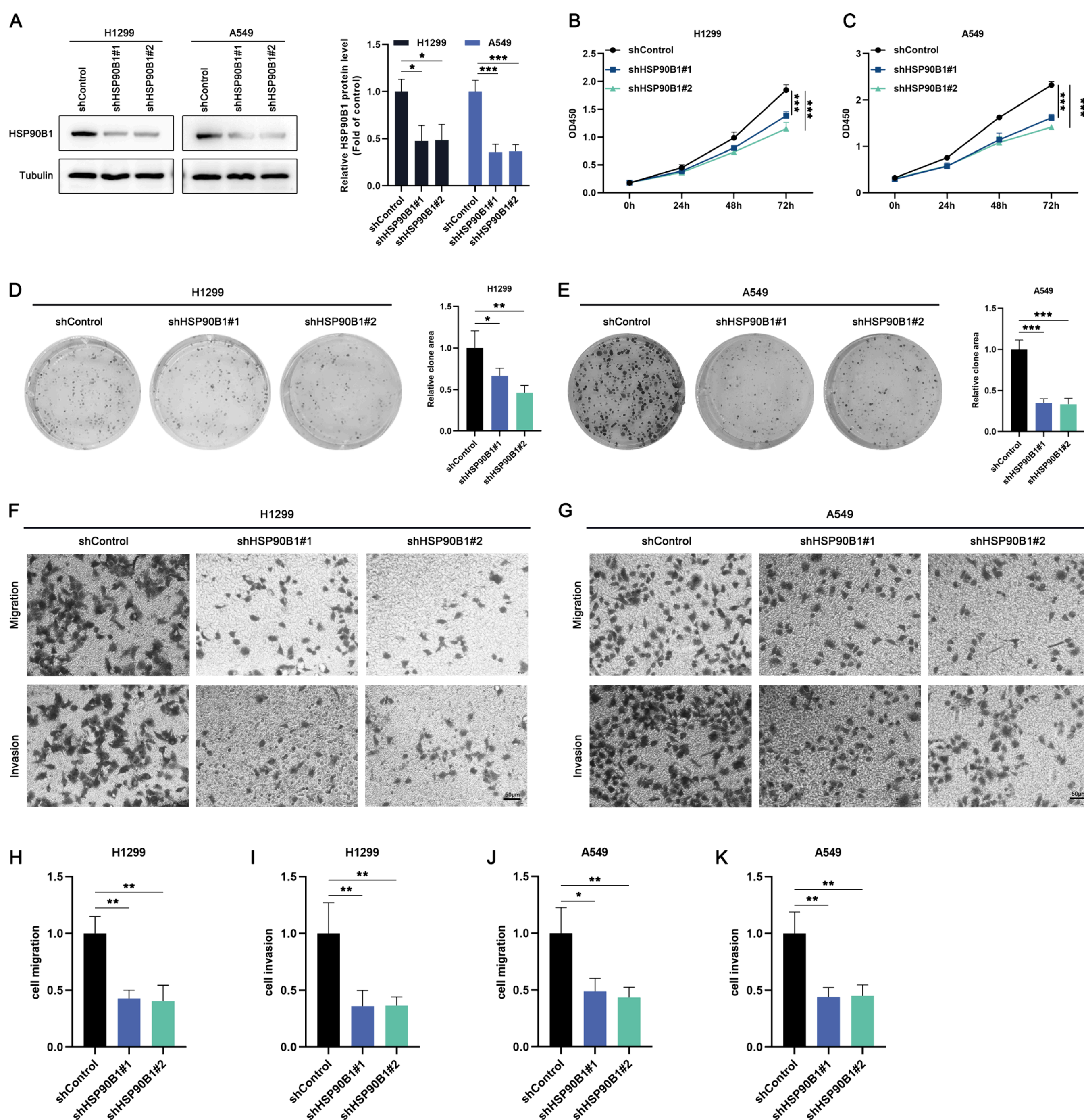

Figure 5. HSP90B1 knockdown reduces the proliferation, migration and invasion of lung cancer cells. (A) Assessment of HSP90B1 level by WB in A549 and H1299 cells with HSP90B1 knockdown. (B, C) Evaluation of the impact of HSP90B1 knockdown on cell growth in cancer cells using CCK-8 assays. (D, E) Investigation of the influence of HSP90B1 knockdown on cancer cell growth through clone assays. (F−K) Transwell assay for cancer cells with HSP90B1 knockdown (n = 3), bars = 50 μm.

H1299 and A549 cells overexpressing BIRC3 showed inhibited proliferation abilities (Figure 2E,F). Further, a cell colony formation assay echoed these results; more colonies emerged in the BIRC3 knockdown group, while fewer colonies were observed in the overexpression cohort (Figure 2G−J). This segment confirmed BIRC3's role in negatively regulating the proliferation of NSCLC cells.

Subsequently, a transwell assay was employed to validate BIRC3′s influence on tumor cell migration and invasion. When compared to the control, the BIRC3-knockdown group within H1299 showed enhanced migrational and invasional capabilities, while the overexpression group displayed significantly diminished migration and invasion in H1299 cells (Figure 3A−F). Analogously, A549 cell line experiments also demonstrated comparable results (Figure 3G−L), thus reinforcing the argument that BIRC3 imparts an inhibitory effect on NSCLC migration and invasion.

3.3. BIRC3 Interacts with HSP90B1. To identify potential interaction protein targets of BIRC3, IP-MS analysis with the A549 cell line was conducted. This result shows that 102

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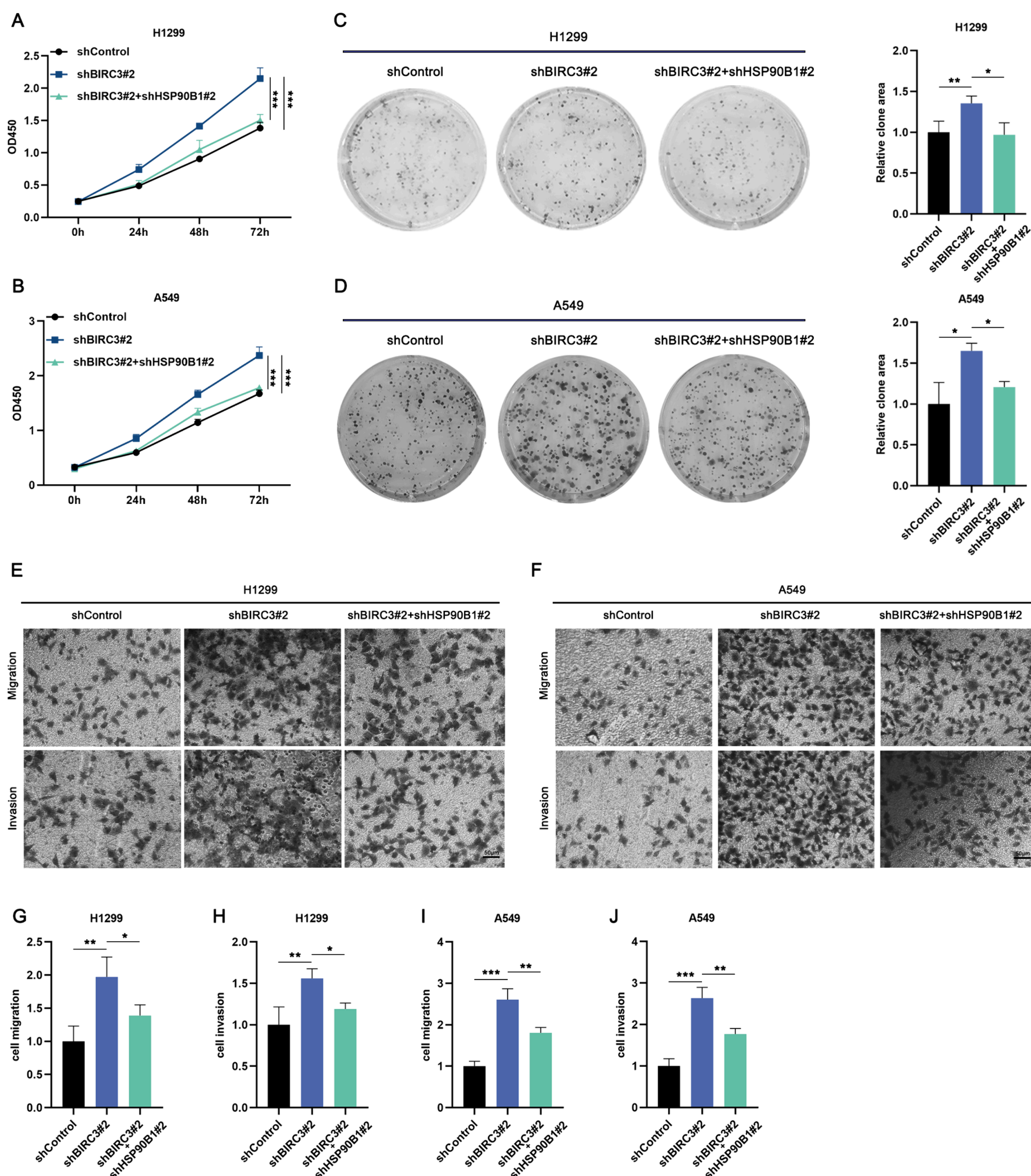

Figure 6. Hsp90B1 knockdown prevents increased proliferation and metastasis caused by BIRC3 knockdown. (A, B) The proliferation of cancer cells was detected by CCK-8 assays. (C, D) Proliferation of cancer cells were evaluated through clone assays. (E–J) Invasion and migration of cancer cells were assessed using transwell assay, bars = 50 μm.

proteins were pulled down by BIRC3. Among them, 61 proteins were also pulled down by IgG. Ultimately, 41 proteins were identified that may interact with BIRC3 (Figure 4A,B). Additionally, we performed gene ontology (GO) term enrichment analysis on 41 proteins and found that "focal adhesion," "unfolded protein binding", and "protein binding" were among the enriched GO categories (Figure 4C). Next, we analyze 41 genes via PPI String, we obtained four genes which had at least three links. These genes included HSP90B1, HSPA9, TXN, and ACTB (Figure 4D). Co-IP analysis was performed for the IP/MS results to be validated. The results indicate that BIRC3

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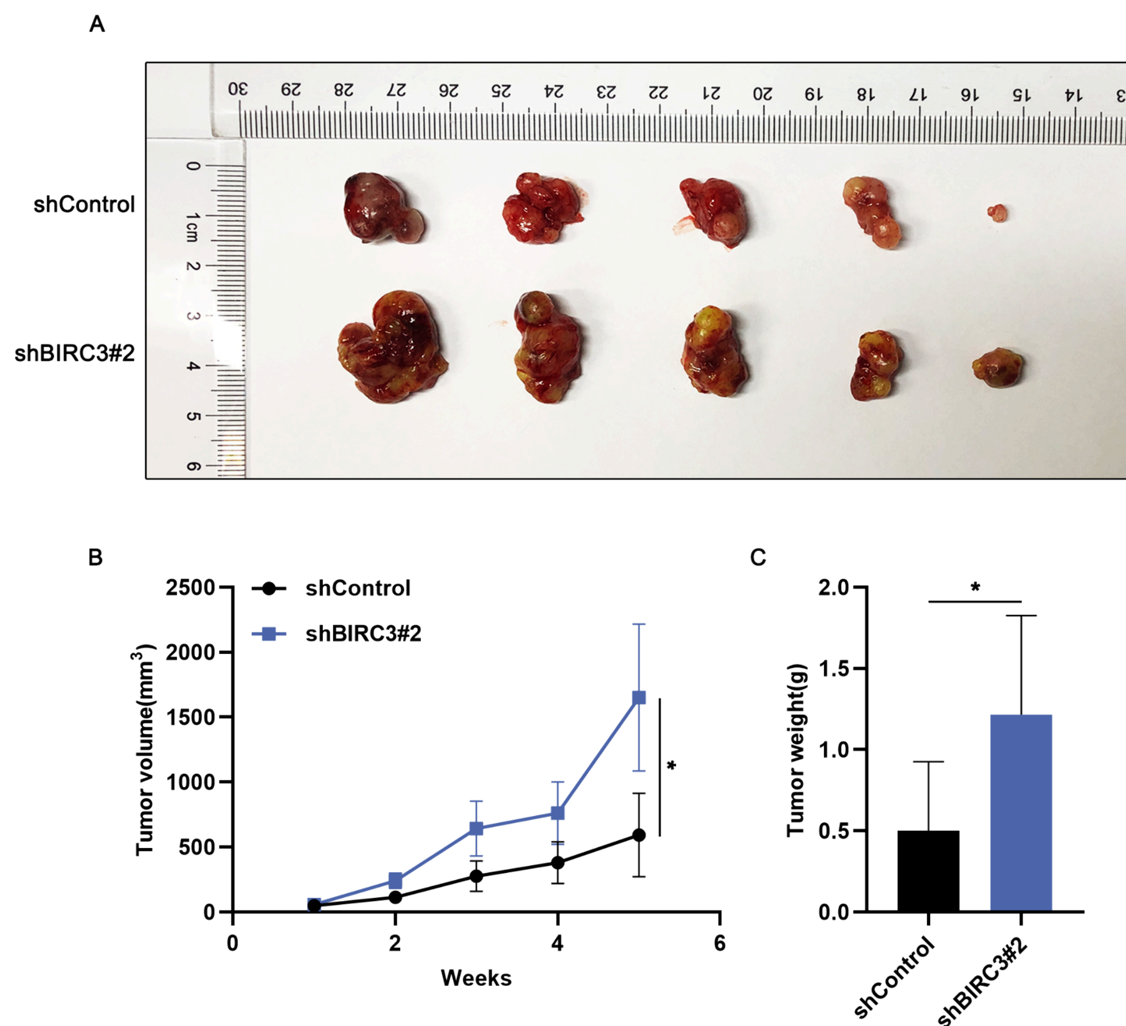

Figure 7. BIRC3 knockdown inhibits lung tumor growth in vivo. (A−C) Images of tumor removed from mice following 28 days as well as quantitative assessment of both tumor volume and weight ($n = 5$).

interacts with HSP90B1 (Figure 4E) rather than with other proteins (data not shown).

Furthermore, to confirm HSP90B1's regulatory influence on NSCLC cell function, HSP90B1 expression was attenuated in H1299 and A549 cells (Figure 5A). Findings revealed that cell proliferation within the HSP90B1 knockdown group was markedly impeded (Figure 5B,C). Correspondingly, a colony formation assay replicated these outcomes (Figure 5D,E). Additionally, transwell outcomes indicated a substantial reduction in migration and invasion capabilities after HSP90B1 knockdown (Figure 5F−K).

Furthermore, we verify that BIRC3 regulates NSCLC function by interacting with HSP90B1. H1299 and A549 cell lines were used to knock down the expression of BIRC3 alone or BIRC3 and HSP simultaneously. Compared to the knockdown of BIRC3 alone group, the proliferation, migration, and invasion of cells were inhibited in the group with simultaneous knockdown of HSP and BIRC3 (Figure 6). The results of this part verified that BIRC3 suppressed the functional phenotype of NSCLC by inhibiting HSP90B1 protein expression.

3.5. BIRC3 KD Promotes NSCLC Cell Proliferation In Vivo. In order to further evaluate BIRC3 for in vivo tumorigenesis regulation function, we use shBIRC3 H1299 and shControl H1299 in tumor experiments in nude mice. The tumor growth curve showed that the tumor proliferation of the shBIRC3 group was significantly more active than control group (Figure 7A,B). After 4 weeks, the mice were killed and the tumors were weighed and recorded. The average weight of the tumors in the shBIRC3 H1299 group is significantly heavier than those of the control group (Figure 7C).

3.6. BIRC3 Interacts with HSP90B1 to Regulate the ERK1/2 Pathway and Inhibit the Progression of NSCLC. Several studies have indicated that an aberrant elevation in the phosphorylation levels of the ERK1/2 pathway plays a pivotal role in lung cancer.[8,9] The activation of the ERK pathway serves a fundamental role in promoting lung cancer cell proliferation and metastasis through cell cycle progression stimulation and apoptosis suppression. This pathway operates by regulating genes involved in cell cycle orchestration, DNA replication, and antiapoptotic processes.[8−12] Any disruption or dysregulation of the ERK pathway can result in uncontrolled cellular proliferation and the advancement of tumor formation. Here, Western blot analysis indicated that BIRC3 knockdown significantly upregulated the phospho-ERK1/2 level. While, knockdown of HSP90B1 significantly reduced phospho-ERK1/2 level (Figure 8A,B). In our study, we observed that BIRC3 knockdown resulted in a decrease in HSP90B1 expression. However, when we knocked down HSP90B1, there was no significant effect on the BIRC3 expression. These findings provide evidence that BIRC3 plays a regulatory role in the expression of HSP90B1.

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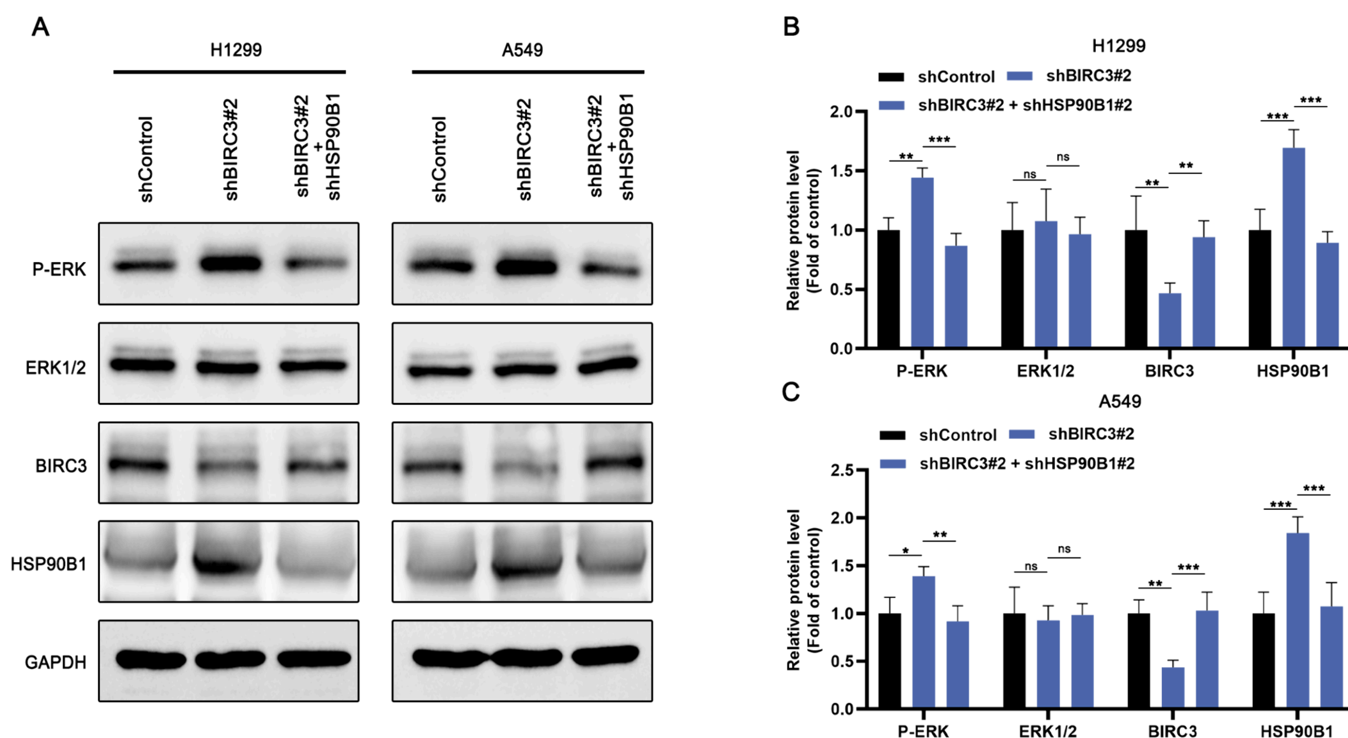

Figure 8. BIRC3 interacts with HSP90B1 to regulate ERK1/2 pathway. (A–C) WB analysis for the level of P-ERK and ERK1/2 in cancer cells with BIRC3 knockdown or HSP90B1 knockdown.

These results indicated that BIRC3 inhibited lung cancer progression through the ERK1/2 pathway.

4. DISCUSSION

The long-term survival for patients with NSCLC is still unsatisfactory due to the high recurrence and metastasis.[13] Genetic alterations in oncogenes and tumor suppressors significantly contribute to tumor onset and progression, ultimately influencing treatment efficacy and disease progression.[14] Further understanding of lung cancer mechanisms holds potential for the development of more effective therapeutic strategies.

IAP encompass a class of proteins involved in apoptosis regulation, with cIAP2 (also known as BIRC3), being a potential therapeutic target in various cancers.[4,15] Recurrent leukemia progresses more rapidly with low BIRC3 expression, a gene that serves as a suppressor of the nonclassical NF-kB pathway, crucial for chronic lymphocytic leukemia.[16] Additionally, BIRC3 inactivation can spearhead resistance to chemoimmunotherapy in chronic lymphocytic leukemia patients.[5] In ovarian cancer, BIRC3 participates in the EMT process of ovarian cancer by binding to Snail, promoting cell proliferation.[6] Conversely, within hepatocellular carcinoma (HCC), BIRC3 stimulates tumor proliferation and metastasis, rendering it a potential prognostic marker and therapeutic target for HCC.[17]

Given the diverse roles BIRC3 plays in different cancers, it is paramount to establish whether BIRC3 functions as a proto-oncogene or a tumor suppressor. Our findings propose BIRC3 was proposed as a tumor-suppression gene in NSCLC. Initial analyses utilized the TCGA database, revealing reduced BIRC3 expression in cancerous and adjacent tissue. This reduction correlated to improved prognosis in patients exhibiting high BIRC3 expression. In vitro studies reflect that depleting BIRC3 enhances NSCLC cell proliferation, migration, and invasion, while overexpressing BIRC3 dampens this stimulatory action on NSCLC. This modulating effect was corroborated by animal studies.

HSP90B1, a member of the heat shock protein 90 family, plays a crucial role in tumor progression. Existing evidence suggests HSP90B1 overexpresses in most tumors, leading to poor survival for patients with various cancers.[18] In pursuit of identifying BIRC3 interactive proteins, we implemented both Co-IP and MS experiments. These implied that BIRC3 directly interacts with HSP90B1, negatively regulating HSP90B1 expression, thus, inhibiting NSCLC cell proliferation, migration, and invasion.

The ERK signaling pathway has multiple roles in tumors, including proliferation and metastasis.[11,19] Hyperactivation of the ERK often occurs in lung cancer patients.[20] Excessive activation of the ERK pathway promotes the malignant transformation of lung epithelial cells.[21] Our findings indicate BIRC3 mediates the regulatory function on tumor cells via negative regulation of ERK phosphorylation, as a significant increase in P-ERK expression was observed following BIRC3 knockdown. Moreover, HSP90B1, a molecular chaperone of ATP, regulates the phosphorylation of ERK and the stability of oncoproteins in the pathway.[22,23] HSP90B1 depletion reversed the regulatory consequences of BIRC3 knockdown on P-ERK. Such findings suggest that BIRC3 regulates ERK signaling via interactions with HSP90B1.

5. CONCLUSIONS

In conclusion, we found that BIRC3 can act as a tumor suppressor in NSCLC by interacting with HSP90B1 and negatively regulating the phosphorylation of ERK to regulate tumor cellular function. This study provides new insights into the regulatory mechanism of BIRC3 in NSCLC.

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ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c10274.

Determination of the basal expressions of BIRC3 (Figure S1); proteins identified by BIRC FLAG (Table S1); proteins identified by IGG (Table S2); proteins of BIRC3 FLAG minus IGG (Table S3) (PDF)

Accession Codes

BIRC3, Q13489; HSP90B1, P14625; ERK1/2, P27361

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Author Contributions

‡F.S., Y.W., and Q.Z. contributed equally to this work. X.W. proposed the project, while F.S., Q.Z., and L.L. conducted experiments using cell lines and mice. Y.W. assisted in creating lung cancer models. F.S. and Q.Z. were responsible for drafting the manuscript. F.S. took a picture of Figure 7. Each contributor played a part in experimental design and data interpretation.

Notes

The authors declare no competing financial interest.

The experimental protocols and care of the mice were approved by the Laboratory Animal Ethics Committee at Xuzhou Cancer Hospital, in accordance with the guidelines for ethical examination of experimental animals.

Every author has consented to the publication of this manuscript.

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REFERENCES

(1) Siegel, R. L.; Miller, K. D.; Wagle, N. S.; et al. Cancer statistics, 2023[J]. *CA Cancer J. Clin* **2023**, *73* (1), 17−48.

(2) Bade, B. C.; Dela, C. C. Lung Cancer 2020: Epidemiology, Etiology, and Prevention. *Clin Chest Med.* **2020**, *41* (1), 1−24.

(3) Villalobos, P.; Wistuba, I. I. Lung Cancer Biomarkers[J]. *Hematol Oncol Clin North Am.* **2017**, *31* (1), 13−29.

(4) Frazzi, R. BIRC3 and BIRC5: multi-faceted inhibitors in cancer. *Cell Biosci.* **2021**, *11* (1), 8.

(5) Diop, F.; Moia, R.; Favini, C.; et al. Biological and clinical implications of BIRC3 mutations in chronic lymphocytic leukemia[J]. *Haematologica* **2020**, *105* (2), 448−456.

(6) Rho, S. B.; Byun, H. J.; Kim, B. R.; et al. Snail Promotes Cancer Cell Proliferation via Its Interaction with the BIRC3[J]. *Biomol Ther (Seoul)* **2022**, *30* (4), 380−388.

(7) Zhang, Z.; Pan, Y.; Zhao, Y.; et al. RIPK2 promotes the progression of colon cancer by regulating BIRC3-mediated ubiquitination of IKBKG[J]. *Exp. Cell Res.* **2023**, *429* (1), No. 113644.

(8) Heigener, D. F.; Gandara, D. R.; Reck, M. Targeting of MEK in lung cancer therapeutics[J]. *Lancet Respir Med.* **2015**, *3* (4), 319−327.

(9) Han, J.; Liu, Y.; Yang, S.; et al. MEK inhibitors for the treatment of non-small cell lung cancer. *J. Hematol. Oncol.* **2021**, *14* (1), 1.

(10) Shaul, Y. D.; Seger, R. The MEK/ERK cascade: From signaling specificity to diverse functions[J]. Biochimica et Biophysica Acta (BBA) - Molecular. *Cell Research* **2007**, *1773* (8), 1213−1226.

(11) Roskoski, R. J. ERK1/2 MAP kinases: structure, function, and regulation[J]. *Pharmacol. Res.* **2012**, *66* (2), 105−143.

(12) Boucherat, O.; Nadeau, V.; Berube-Simard, F. A.; et al. Crucial requirement of ERK/MAPK signaling in respiratory tract development[J]. *Development* **2014**, *141* (16), 3197−3211.

(13) Torre, L. A.; Bray, F.; Siegel, R. L.; et al. Global cancer statistics, 2012[J]. *CA Cancer J. Clin* **2015**, *65* (2), 87−108.

(14) Neal, J. W.; Sledge, G. W. Decade in review-targeted therapy: successes, toxicities and challenges in solid tumours[J]. *Nat. Rev. Clin Oncol* **2014**, *11* (11), 627−628.

(15) Bai, L.; Smith, D. C.; Wang, S. Small-molecule SMAC mimetics as new cancer therapeutics[J]. *Pharmacology & Therapeutics* **2014**, *144* (1), 82−95.

(16) Asslaber, D.; Wacht, N.; Leisch, M.; et al. BIRC3 Expression Predicts CLL Progression and Defines Treatment Sensitivity via Enhanced NF-kappaB Nuclear Translocation[J]. *Clin. Cancer Res.* **2019**, *25* (6), 1901−1912.

(17) Fu, P. Y.; Hu, B.; Ma, X. L.; et al. New insight into BIRC3: A novel prognostic indicator and a potential therapeutic target for liver cancer[J]. *J. Cell Biochem* **2019**, *120* (4), 6035−6045.

(18) Wang, Y.; Wang, X. A Pan-Cancer Analysis of Heat-Shock Protein 90 Beta1(HSP90B1) in Human Tumours. *Biomolecules* **2022**, *12* (10), 1377 DOI: 10.3390/biom12101377.

(19) Carneiro, B. A.; El-Deiry, W. S. Targeting apoptosis in cancer therapy[J]. *Nat. Rev. Clin Oncol* **2020**, *17* (7), 395−417.

(20) Zhong, C.; Chen, C.; Yao, F.; et al. ZNF251 promotes the progression of lung cancer by activating ERK signaling[J]. *Cancer Sci.* **2020**, *111* (9), 3236−3244.

(21) Saad, M. I.; Alhayyani, S.; McLeod, L.; et al. ADAM17 selectively activates the IL-6 trans-signaling/ERK MAPK axis in KRAS-addicted lung cancer. *EMBO Mol. Med.* **2019**, *11* (4), No. e9976, DOI: 10.15252/emmm.201809976.

(22) Chatterjee, S.; Bhattacharya, S.; Socinski, M. A.; et al. HSP90 inhibitors in lung cancer: promise still unfulfilled. *Clin Adv. Hematol Oncol* **2016**, *14* (5), 346−356.

(23) Niu, M.; Zhang, B.; Li, L.; et al. Targeting HSP90 Inhibits Proliferation and Induces Apoptosis Through AKT1/ERK Pathway in Lung Cancer. *Front. Pharmacol.* **2021**, *12*, No. 724192.

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