

HPRT1 activates glycolysis to promote proliferation and metastasis of lung adenocarcinoma

Ce Zhao

North China University of Science and Technology Affiliated Hospital

Junqing Gan

North China University of Science and Technology Affiliated Hospital

Chennan Zhao

North China University of Science and Technology Affiliated Hospital

Chaonan Zhu

North China University of Science and Technology Affiliated Hospital

Jianyi Pu

North China University of Science and Technology Affiliated Hospital

Peng Gao

703088818@qq.com

North China University of Science and Technology Affiliated Hospital

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Abstract

Objective: This study investigated whether hypoxanthine phosphoribosyltransferase 1 (HPRT1) regulates malignant biological behavior in lung adenocarcinoma (LUAD) through glycolysis.

Methods: Glycolysis-related genes associated with LUAD prognosis were screened by bioinformatics analyses. HPRT1 expression was examined in LUAD cell lines and normal bronchial epithelial cells. HPRT1 knockdown and overexpression models were established in H1299 and A549 cells, respectively. Cell proliferation, migration and invasion were assessed using CCK-8, colony formation, wound-healing and Transwell assays. Glycolytic activity was evaluated by lactate measurement and Western blotting of hexokinase 2 (HK2) and pyruvate kinase M2 (PKM2), with 2-deoxy-D-glucose (2-DG) used for rescue experiments.

Results: HPRT1 was identified as a glycolysis-related gene with prognostic relevance in LUAD. HPRT1 was highly expressed in H1299 cells and weakly expressed in A549 cells. HPRT1 knockdown inhibited proliferation, migration and invasion, whereas HPRT1 overexpression promoted these malignant phenotypes. HPRT1 also increased lactate production and upregulated HK2 and PKM2. Treatment with 2-DG suppressed glycolysis and reversed the HPRT1-induced enhancement of proliferation, migration and invasion.

Conclusion: HPRT1 promotes malignant biological behavior in LUAD cells by activating glycolysis, suggesting that HPRT1 may be a potential metabolic therapeutic target.

Background

Among respiratory system malignancies, lung cancer remains one of the leading causes of cancer-related mortality worldwide, with persistently high incidence and mortality rates (Denisenko et al. 2018). Owing to the lack of specificity of early symptoms in LUAD, most patients are diagnosed at an advanced stage (IIIB–IV), missing the opportunity for curative surgical resection (Wu et al. 2016). Patients with advanced disease primarily rely on systemic chemotherapy, targeted therapy, or immunotherapy. Although some treatment modalities have achieved progress, a substantial number of patients continue to face therapeutic bottlenecks (Pan et al. 2021), resulting in high overall mortality, a five-year survival rate significantly lower than that of early-stage patients, and an overall poor prognosis (Torres and Puig 2007). Therefore, research on early diagnosis and the identification of novel therapeutic targets for LUAD is of critical importance for improving patient survival.

HPRT1 is a key rate-limiting enzyme in the purine salvage synthesis pathway (Lee et al. 2016), with its core function being the catalysis of hypoxanthine and guanine into IMP and GMP, thereby maintaining purine metabolic homeostasis (Park et al. 2017). It also influences the cell cycle by regulating the supply of nucleic acid synthesis precursors (Monnat et al. 1992; Torres et al. 2017). Recent studies have revealed that HPRT1 is overexpressed in various malignant tumors and is associated with tumor aggressiveness (Ahmadi et al. 2021; Ghafouri-Fard et al. 2022). It may also be involved in the regulation

of resistance to chemotherapeutic agents such as cisplatin, positioning it as a potential therapeutic target (Du et al. 2022). HPRT1 has thus been redefined from a "housekeeping gene" to a tumor-regulatory factor. Given the poor prognosis and limited treatment options for LUAD, investigating the role of HPRT1 in LUAD holds significant clinical value, and assessing its expression level may serve as a potential prognostic biomarker for LUAD patients (Mai et al. 2021).

Glucose catabolism primarily encompasses three major pathways: glycolysis, aerobic oxidation, and the pentose phosphate pathway. Among these, glycolysis is the fundamental process of glucose breakdown, referring to the conversion of glucose into lactate under anaerobic conditions through the catalysis of multiple enzymes, coupled with energy production (Qiao et al. 2024). The uncontrolled proliferation of tumor cells relies on glucose as an energy source, and glycolysis can efficiently generate energy even under hypoxic conditions. Furthermore, studies have shown that certain oncogenic pathways promote tumor progression by regulating tumor glycolysis (de Lima et al. 2015; Cascone et al. 2018).

In this study, we established LUAD cell lines with HPRT1 knockdown and overexpression in vitro, in combination with the glycolytic inhibitor 2-deoxy-D-glucose (2-DG), to investigate the effect of HPRT1 on LUAD cell proliferation, migration, and invasion through the regulation of the glycolytic pathway. This study aims to provide new theoretical insights and directions for the clinical diagnosis and therapeutic strategy development for LUAD.

Methods

1 Cell sources: The normal human lung epithelial cell line BEAS-2B was purchased from Beijing Eallbio Company. The LUAD cell lines NCI-H1299, NCI-H1975, and A549 were obtained from Wuhan Pricella Company, and the LUAD cell line PC-9 was purchased from Shanghai Fuheng Biotechnology Company.

2 Main Reagents: RPMI-1640 medium and fetal bovine serum (FBS) were purchased from Wuhan Pricella Company; F12 medium, DMEM medium, and Opti-MEM medium were obtained from Beijing Eallbio Company; 0.25% trypsin solution and KeygenMax 3000 transfection reagent were purchased from Jiangsu Keygen Biotech Company; penicillin-streptomycin solution, cell cryopreservation medium, and BCA protein assay kit were purchased from Suzhou NewCellMate Company; Matrigel basement membrane matrix was obtained from Corning, USA; CCK-8 kit was purchased from APExBIO, USA; total RNA extraction kit was purchased from Beijing GeneBetter Company; reverse transcription kit and RT-qPCR kit were obtained from Takara, Japan; HPRT1 primer sequences, sh-HPRT1 plasmid vectors (sh-HPRT1-1, sh-HPRT1-2, sh-HPRT1-3), negative control plasmid (sh-NC), oe-HPRT1 plasmid vector, and negative control plasmid (oe-NC) were purchased from Jiangsu Saisuofei Company; GAPDH primer sequences were obtained from Beijing RuiBoXingKe Company; HPRT1 antibody, PKM2 antibody, and Beta Actin antibody were purchased from Wuhan Proteintech Company; HK2 antibody was obtained from Shanghai Aladdin Company; mouse secondary antibody and rabbit secondary antibody were purchased from KPL, USA; SDS-PAGE fast gel preparation kit was purchased from Beijing BioTides Company; ECL chemiluminescence detection kit was obtained from Beijing APPLYGEN Company; BCA

protein assay kit was purchased from Suzhou NewCellMate Company; RIPA lysis buffer was obtained from Shanghai Beyotime Company; 5× SDS-PAGE protein loading buffer was purchased from Wuhan Servicebio Company; PVDF membrane was obtained from Millipore, USA; and the glycolysis inhibitor 2-DG was purchased from Shanghai Yuanye Company.

3 Cell transfection: Digested H1299 and A549 cells were resuspended in antibiotic-free complete medium and seeded into six-well plates at a density of 3×10^5 cells per well. After culturing for 24 hours until reaching 70–90% confluence, the cells were prepared for transfection. Solutions A and B were prepared according to the manufacturer's instructions for the transfection reagent, mixed, and allowed to stand for 15 minutes. The transfection complex was then diluted with antibiotic-free complete medium to a final volume of 2 mL and added dropwise to the cells. The cells were subsequently incubated at 37°C in a 5% CO₂ atmosphere for 24 hours, after which RNA extraction or cellular functional assays were performed.

4 Cell grouping: To investigate the effect of HPRT1 knockdown and overexpression on the biological functions of LUAD cells, H1299 cells were divided into the sh-NC group and the sh-HPRT1 group, and A549 cells were divided into the oe-NC group and the oe-HPRT1 group. To explore whether HPRT1 influences the biological functions of LUAD cells through activation of the glycolytic pathway, transfected A549 cells were treated with the glycolysis inhibitor 2-DG. The A549 cells were then divided into four groups: oe-NC, oe-HPRT1, oe-NC + 2-DG, and oe-HPRT1 + 2-DG.

5 Western blotting for protein expression detection: Total cellular protein was extracted following lysis, centrifugation, and addition of loading buffer, and then stored. The BCA method was used to prepare the working solution, generate a standard curve using protein standards, and dilute the samples. After incubation, the absorbance was measured to calculate protein concentrations. Western blotting was then performed using a 12.5% separating gel and stacking gel for electrophoresis. PVDF membranes were activated with methanol prior to protein transfer. After blocking with non-fat dry milk, the membranes were incubated overnight at 4°C with primary antibodies against HPRT1, PKM2, and HK2, followed by incubation with corresponding secondary antibodies for 1 hour at room temperature. After washing, the protein bands were visualized using ECL reagent.

6 RT-qPCR for RNA expression detection: Total RNA was extracted from transfected cells through lysis, centrifugation, column purification, and elution. Reverse transcription was then performed using the extracted RNA as a template according to the manufacturer's protocol to synthesize cDNA. Subsequently, real-time quantitative PCR was conducted using the appropriate reaction system and cycling conditions. Data were analyzed using the $2^{-\Delta\Delta C_t}$ method, with β -Actin as the internal reference. Each experiment was repeated three times. Primer sequences are listed in Table 1.

Table 1
Primer sequence information

Gene	Sequence (5'→3')
HPRT1	Forward: CCAGTCAACAGGGGACATAAA
	Reverse: CTGACCAAGGAAAGCAAAGTCT
β-actin	Forward: CATGGATGATGATATCGCCGCG
	Reverse: ACATGATCTGGGTCATCTTCTCG

7 CCK-8 Assay for Cell Proliferation: Transfected H1299 and A549 cells were seeded into 96-well plates at a density of 2×10^3 cells per well according to the group assignments described above. The cells were incubated at 37°C in a 5% CO₂ incubator, with three replicate wells per group. At 24, 48, 72, and 96 h after seeding, the old culture medium was aspirated, and 110 µL of CCK-8 working solution (CCK-8 : medium = 1 : 10) was added to each well. The plates were then incubated under the same conditions for an additional 2 h. The optical density (OD) at 450 nm was measured using a microplate reader, and cell growth curves were plotted.

8 Colony Formation Assay: Transfected H1299 and A549 cells in good growth condition were trypsinized, centrifuged, and collected. The cells were seeded into six-well plates at a density of 2×10^3 cells per well. The culture medium was replaced every three days, and the cells were cultured for a total of 14 days. Subsequently, the cells were washed with physiological saline, fixed with methanol for 10 min, and stained with 1% crystal violet for 10 min. Colonies were photographed and counted.

9 Wound Healing Assay: At 24 h post-transfection, H1299 and A549 cells were scratched using the tip of a 200 µL pipette perpendicular to the bottom of the six-well plate. Cell debris was thoroughly removed by washing with PBS, and the cells were then cultured in serum-free medium. Images of the same field of view were captured under an inverted microscope at 0 h and 24 h after scratching. The scratch area was measured using ImageJ software, and the cell migration rate was calculated according to the following formula: Migration rate (%) = [(scratch area at 0 h – scratch area at 24 h) / scratch area at 0 h] × 100%.

10 Transwell Assay: First, 100 µL of serum-free medium containing 2% Matrigel was added to the upper chamber of each Transwell insert, and the inserts were placed in a 37°C, 5% CO₂ incubator for 2 h to allow gelation. Then, 200 µL of a serum-free cell suspension containing approximately 8×10^4 cells was added to the upper chamber, and 600 µL of complete medium containing 20% FBS was added to the lower chamber. The inserts were incubated at 37°C in a 5% CO₂ incubator for 24 h. After incubation, the inserts were removed, fixed with methanol for 15 min at room temperature, and stained with 1% crystal violet for 15 min. The inserts were gently rinsed with tap water, and cells that had not migrated through the membrane were removed with a cotton swab. After mounting with neutral balsam, the membranes were observed and photographed under an inverted light microscope, and the number of migrated cells was counted.

11 Lactate Content Assay: Adherent A549 cells after transfection were collected, trypsinized, and centrifuged. BeyoLysis™ Buffer A was added at the recommended ratio, and the cells were lysed on ice. After centrifugation, the supernatant was collected for subsequent use. WST-8 chromogenic working solution was prepared according to the manufacturer's instructions. L-lactic acid standard was diluted to concentrations within the linear range of the kit, and replicate wells and blank wells were set. Samples, standards, and dilution buffer were added to a 96-well plate, followed by the addition of chromogenic solution to each well with thorough mixing. The plate was incubated for 30 min at room temperature protected from light. The absorbance at 450 nm was measured using a microplate reader. After calibration, a standard curve was plotted to calculate the sample concentration, which was then multiplied by the dilution factor to obtain the final L-lactic acid concentration.

12 Statistical analysis: All data processing and statistical analyses were performed following rigorous standard procedures to ensure accurate and reliable results. Experimental data were analyzed using GraphPad Prism 8 software. Each experiment was independently repeated three times to minimize operational and random errors. After completion of the experiments, data from all samples were pooled for unified analysis. For statistical comparisons, an independent samples t-test was used to compare differences between two independent groups, and one-way analysis of variance (ANOVA) was used for comparisons among three or more independent groups. All data are presented as the mean $\pm$ standard deviation ($\bar{x} \pm s$). A P-value < 0.05 was considered statistically significant.

Results

Bioinformatics analysis identifies HPRT1 as a key gene. In this study, bioinformatics approaches were employed to screen for glycolysis-related genes with prognostic value in lung adenocarcinoma (LUAD). Glycolysis-related genes were retrieved from the GeneCards and GSEA databases, and their intersection yielded 20 candidate genes (Fig. 1A). Through differential expression analysis using the TCGA database and multivariate Cox regression analysis, two prognosis-related genes, HPRT1 and GSTP1, were identified (Figs. 1B–C). ROC curve analysis demonstrated that HPRT1 exhibited superior diagnostic efficacy for LUAD; therefore, HPRT1 was selected as the target gene (Fig. 1D). Prognostic nomogram, calibration curve, and Kaplan–Meier survival curve analyses confirmed that high HPRT1 expression is an independent risk factor for poor prognosis in LUAD (Figs. 1E–G). GO and KEGG enrichment analyses indicated that HPRT1 may promote LUAD progression through the regulation of the glycolytic pathway (Fig. 1H). Collectively, this study elucidates the mechanism of HPRT1 in LUAD, providing a theoretical basis for subsequent experimental investigations and clinical translation.

To validate HPRT1 expression in LUAD cells, Western blotting was performed to detect HPRT1 protein levels in H1299, H1975, PC-9, A549, and normal lung epithelial BEAS-2B cells. The results showed that HPRT1 expression was significantly elevated in H1299, H1975, and PC-9 cells, whereas A549 cells exhibited low HPRT1 expression (Fig. 2A, $P < 0.05$). Given that H1975 cells harbor the EGFR T790M mutation, which may interfere with studies of the glycolytic pathway (Kim et al. 2013), H1299 and A549 cells were selected for the construction of knockdown and overexpression cell models, respectively. In

H1299 cells with high HPRT1 expression, shRNA transfection was performed. RT-qPCR and Western blotting confirmed that sh-HPRT1-2 achieved the highest knockdown efficiency and was therefore selected as the knockdown sequence for subsequent experiments (Fig. 2B, $P < 0.05$). In A549 cells with low HPRT1 expression, an overexpression model was established. RT-qPCR and Western blotting results showed that both mRNA and protein levels of HPRT1 were significantly increased in the oe-HPRT1 group (Fig. 2C, $P < 0.05$), confirming successful construction of the overexpression cell line.

To investigate the effects of HPRT1 knockdown and overexpression on the proliferative capacity of H1299 and A549 cells, CCK-8 and colony formation assays were performed. The CCK-8 assay showed that absorbance values at each time point were significantly decreased in H1299 cells following HPRT1 knockdown, whereas they were increased in A549 cells after HPRT1 overexpression. The colony formation assay revealed that the number of colonies was reduced in H1299 cells and increased in A549 cells compared with their respective control groups (Figs. 2A–B, $P < 0.05$). These findings indicate that HPRT1 knockdown inhibits LUAD cell proliferation, whereas HPRT1 overexpression promotes proliferation. Wound healing assays demonstrated that in H1299 cells, the cell migration rate was $(47.4 \pm 1.96)\%$ in the sh-NC group and $(29.1 \pm 3.58)\%$ in the sh-HPRT1-2 group, representing a significant decrease after HPRT1 knockdown. In A549 cells, the migration rate was $(32.9 \pm 1.31)\%$ in the oe-NC group and $(56.5 \pm 6.82)\%$ in the oe-HPRT1 group, indicating a significant increase following HPRT1 overexpression (Fig. 2C, $P < 0.05$). These results suggest that HPRT1 knockdown suppresses cell migration, whereas HPRT1 overexpression enhances migration. Transwell assays showed that in H1299 cells, the numbers of invasive cells were (532.7 ± 21.5) in the sh-NC group and (227.3 ± 14.84) in the sh-HPRT1-2 group, with a marked reduction after HPRT1 knockdown. In A549 cells, the numbers of invasive cells were (25.7 ± 2.31) in the oe-NC group and (233.7 ± 26.65) in the oe-HPRT1 group, representing a significant increase after HPRT1 overexpression (Fig. 2D, $P < 0.05$). These findings indicate that HPRT1 knockdown inhibits the invasive ability of LUAD cells, whereas HPRT1 overexpression enhances invasion.

To elucidate the regulatory relationship between HPRT1 and glycolysis in LUAD cells, the key glycolytic rate-limiting enzymes hexokinase 2 (HK2) and pyruvate kinase M2 (PKM2) were selected as detection targets. Western blotting was performed to assess the expression changes of these two proteins following HPRT1 knockdown and overexpression. The results showed that HK2 and PKM2 protein expression levels were downregulated in H1299 cells after HPRT1 knockdown, whereas both proteins were significantly upregulated in A549 cells following HPRT1 overexpression, confirming that HPRT1 positively regulates the expression of key glycolytic proteins in LUAD cells (Figs. 4A–B, $P < 0.05$).

To further elucidate the molecular mechanism, the glycolysis inhibitor 2-DG, which is widely used in the field of tumor metabolism, was employed for functional intervention (Lampidis et al. 2006). A549 cells were divided into four experimental groups: empty vector control (oe-NC), HPRT1 overexpression (oe-HPRT1), empty vector control with 2-DG treatment, and HPRT1 overexpression with 2-DG treatment. Western blotting results demonstrated that 2-DG significantly inhibited HK2 and PKM2 protein expression and completely reversed the upregulation of these two proteins mediated by HPRT1

overexpression (Fig. 4C, $P < 0.05$). Meanwhile, lactate level assays confirmed that HPRT1 overexpression increased cellular lactate levels, whereas 2-DG suppressed cellular lactate levels and completely reversed the elevation of lactate levels induced by HPRT1 overexpression (Fig. 4D, $P < 0.05$). These results indicate that HPRT1 overexpression enhances glycolytic efficiency in LUAD cells; treatment with 2-DG effectively suppresses glycolytic efficiency in LUAD cells; and 2-DG effectively reverses the enhanced glycolytic effect resulting from HPRT1 overexpression.

To investigate the regulatory effects of HPRT1 overexpression and the glycolysis inhibitor 2-DG on LUAD cells, A549 cells were selected as the research object to establish HPRT1 overexpression and glycolysis inhibition models. The experimental groups were as follows: oe-NC group, oe-HPRT1 group, oe-NC + 2-DG group, and oe-HPRT1 + 2-DG group.

The CCK-8 assay results showed that after continuous detection at 24, 48, 72, and 96 h, the OD values of cells in the oe-HPRT1 group were increased compared with those in the oe-NC group, indicating that HPRT1 overexpression enhanced the proliferative capacity of A549 cells, and this promoting effect persisted with prolonged culture time. Compared with the oe-NC group, the OD values of cells in the oe-NC + 2-DG group were decreased ($P < 0.05$), demonstrating that 2-DG treatment inhibited the proliferative capacity of A549 cells. Further intergroup comparative analysis revealed that the OD values of cells in the oe-HPRT1 + 2-DG group were significantly lower than those in the oe-HPRT1 group (Fig. 5A, $P < 0.05$), confirming that 2-DG effectively reversed the enhanced proliferative capacity induced by HPRT1 overexpression in A549 cells. The colony formation assay results showed that the numbers of colonies in each group were as follows: (192.0 ± 7.00) in the oe-NC group, (283.0 ± 29.60) in the oe-HPRT1 group, (98.3 ± 10.41) in the oe-NC + 2-DG group, and (154.6 ± 15.53) in the oe-HPRT1 + 2-DG group. Compared with the oe-NC group, the number of colonies in the oe-HPRT1 group was significantly increased ($P < 0.05$), indicating that HPRT1 overexpression enhanced the proliferative capacity of A549 cells. Compared with the oe-NC group, the number of colonies in the oe-NC + 2-DG group was decreased. Further intergroup analysis revealed that the number of colonies in the oe-HPRT1 + 2-DG group was significantly lower than that in the oe-HPRT1 group (Fig. 5B, $P < 0.05$), demonstrating that inhibition of glycolytic pathway activity effectively suppresses the enhanced proliferative capacity of A549 cells induced by HPRT1 overexpression. The wound healing assay results showed that after 24 h of culture, the cell migration rates in each group were as follows: (35.4 ± 0.93)% in the oe-NC group, (66.1 ± 1.58)% in the oe-HPRT1 group, (14.7 ± 1.53)% in the oe-NC + 2-DG group, and (49.1 ± 4.46)% in the oe-HPRT1 + 2-DG group. Intergroup comparison revealed that the wound healing rate was increased in the oe-HPRT1 group compared with the oe-NC group, indicating that HPRT1 overexpression enhanced the migratory capacity of A549 cells. Compared with the oe-NC group, the wound healing rate was decreased in the oe-NC + 2-DG group, confirming that inhibition of the glycolytic pathway effectively suppresses the migratory capacity of A549 cells. Further intergroup analysis showed that the wound healing rate in the oe-HPRT1 + 2-DG group was significantly lower than that in the oe-HPRT1 group (Fig. 5C, $P < 0.05$), suggesting that 2-DG effectively reverses the enhanced migratory capacity of A549 cells induced by HPRT1 overexpression by blocking the glycolytic pathway. The Transwell invasion assay results showed that the numbers of cells invading to the lower chamber in each group were as follows: (151.0 ± 16.52) in

the oe-NC group, (543.0 ± 6.56) in the oe-HPRT1 group, (47.7 ± 10.01) in the oe-NC + 2-DG group, and (159.0 ± 11.00) in the oe-HPRT1 + 2-DG group. Intergroup comparison revealed that the number of invasive cells was increased in the oe-HPRT1 group compared with the oe-NC group, indicating that HPRT1 overexpression enhanced the invasive capacity of A549 cells. Compared with the oe-NC group, the number of invasive cells was decreased in the oe-NC + 2-DG group, indicating that 2-DG treatment inhibited the invasive capacity of A549 cells. Further intergroup comparative analysis showed that the number of invasive cells in the oe-HPRT1 + 2-DG group was significantly lower than that in the oe-HPRT1 group (Fig. 5D, $P < 0.05$), suggesting that HPRT1 promotes the invasive capacity of LUAD cells by activating the glycolytic pathway, whereas 2-DG effectively reverses the enhanced invasive capacity of A549 cells induced by HPRT1 overexpression by blocking the glycolytic pathway.

Discussion

Currently, LUAD has become one of the most common malignancies worldwide and a leading cause of cancer-related death, with its mortality rate ranking among the highest of all malignant tumors (Li et al. 2023a; Leiter et al. 2023). Despite continuous advances in surgical techniques, increasingly sophisticated targeted therapies, and ongoing breakthroughs in immunotherapy, the overall prognosis of LUAD patients has gradually improved. However, owing to local tumor invasion, distant metastasis, impaired immune function, and frequent comorbidities such as chronic obstructive pulmonary disease, pulmonary infection, and cardiac or renal dysfunction, as well as the fact that surgery, radiotherapy, chemotherapy, targeted therapy, and immunotherapy can readily induce life-threatening critical conditions including respiratory failure, severe infection, massive hemorrhage, and multiple organ failure, LUAD patients represent one of the most common groups of critical oncological patients in clinical practice (Huang et al. 2023). Proliferation, migration, and invasion are core features of malignant tumors, enabling tumor cells to directly infiltrate surrounding tissues or spread hematogenously or lymphatically to distant sites and continuously proliferate (Zhang et al. 2023). The precise molecular mechanisms underlying lung cancer remain under extensive investigation. Furthermore, metabolic reprogramming in tumors further promotes cancer cell proliferation, invasion, and distant metastasis (Barta et al. 2019). The main finding of the present study is that HPRT1 is a key regulator of malignant behavior and glycolytic metabolism in LUAD cells, and that the glycolysis inhibitor 2-DG blocks this regulatory effect and reverses the enhanced malignant biological behavior induced by HPRT1 overexpression. These results indicate that high HPRT1 expression is closely associated with rapid tumor invasion and extensive metastasis, whereas 2-DG effectively reverses this effect, potentially offering a novel metabolic targeted therapeutic strategy to improve survival benefit and clinical outcomes for LUAD patients.

Glycolysis, a central pathway in tumor metabolic reprogramming, plays a critical role in LUAD progression. Studies have confirmed that LUAD cells exhibit enhanced glucose uptake capacity and glycolytic activity compared with normal tissue cells, preferentially relying on aerobic glycolysis for energy production even under normoxic conditions—a phenomenon known as the Warburg effect. This metabolic pattern enables tumor cells to rapidly generate ATP to meet the biosynthetic demands

required for proliferation (Alberghina 2023; Liao et al. 2024). In LUAD, aberrant activation of glycolysis-related molecules and signaling pathways has been demonstrated to be closely associated with tumor progression. For example, Li et al. showed that upregulation of glycolysis-related genes enhances metabolic activity in LUAD cells and further promotes tumor cell proliferation (Li et al. 2023b). Thus, aberrantly activated glycolysis not only provides an essential energy source for LUAD cells but also plays an important regulatory role in their malignant progression (Liao et al. 2024; Li et al. 2023b). Targeted inhibition of glycolysis effectively blocks the malignant progression of LUAD, offering an important avenue for improving clinical outcomes in critically ill patients. To screen for glycolysis-related genes with potential prognostic value in LUAD, we selected HPRT1 as the target gene using the GeneCards database, TCGA database, and multivariate Cox regression analysis. Prognostic nomogram and Kaplan–Meier survival curve analyses demonstrated that high HPRT1 expression is associated with worse prognosis in LUAD patients, and GO and KEGG enrichment analyses confirmed that HPRT1 is involved in the regulation of the glycolytic pathway. Traditionally regarded as a housekeeping gene, HPRT1 is primarily involved in the purine salvage synthesis pathway, maintaining purine nucleotide recycling and metabolic homeostasis within cells (Takemura et al. 2023). Under normal physiological conditions, HPRT1 is constitutively and stably expressed at low levels in most human tissues and cells, and its expression is generally not significantly affected by tissue type, developmental stage, or environmental changes (Lu et al. 2024). In recent years, with growing research into tumor metabolism, aberrant expression and potential functions of HPRT1 in cancer have attracted increasing attention. Multiple studies have shown that HPRT1 is significantly upregulated in various malignant tumors and is closely associated with tumor progression, alterations in the immune microenvironment, and patient survival prognosis (Chen et al. 2024). Based on this, we used Western blotting to detect HPRT1 protein expression in LUAD cell lines and BEAS-2B cells. The results showed elevated HPRT1 expression in H1299, H1975, and PC-9 cells, whereas A549 cells exhibited low HPRT1 expression. Given that H1975 cells harbor the EGFR T790M mutation, which can independently activate glycolysis and other pathways and may confound the functional role of HPRT1 (Makinoshima et al. 2014), subsequent functional experiments were performed using H1299 and A549 cells to establish HPRT1 knockdown and overexpression cell lines, respectively, to elucidate the regulatory role of HPRT1 in the biological functions of LUAD cells.

The development and progression of malignant tumors is a complex and systematic pathological process involving multiple genes (Zhang et al. 2024). Under the influence of various intrinsic and extrinsic factors, inactivation of tumor suppressor genes and aberrant activation of proto-oncogenes drive malignant transformation by affecting key biological processes such as cell cycle regulation, apoptosis, and DNA repair, collectively mediating the conversion of normal cells to malignant cells and conferring typical malignant phenotypes, including abnormal proliferation, local invasion, and distant metastasis (Liu et al. 2024; Shin and Cho 2023). Accumulating evidence has demonstrated that HPRT1 plays a key regulatory role in various tumors. For example, Ahmadi et al. reported that HPRT1 is highly expressed in head and neck squamous cell carcinoma, and that its upregulation not only indicates poor prognosis (Ahmadi et al. 2021). Further studies have shown that HPRT1 upregulation not only serves as

a potential prognostic biomarker but also promotes malignant biological behavior of tumor cells by regulating the MMP1/PI3K/Akt signaling pathway and enhances cisplatin resistance in oral squamous cell carcinoma cells (Zhou et al. 2020). In addition, HPRT1 expression varies significantly among different molecular subtypes of breast cancer, with the highest expression levels observed in basal-like and triple-negative breast cancer; HPRT1 knockdown significantly downregulates the expression of tumor-associated genes in breast cancer cells, suggesting that HPRT1 positively regulates malignant progression in breast cancer (Sedano et al. 2020). To further elucidate the regulatory role of HPRT1 in the biological functions of LUAD cells, we established HPRT1 knockdown and overexpression models using transient transfection and demonstrated that HPRT1 promotes proliferation, migration, and invasion of LUAD cells, further supporting its potential role as an oncogene in LUAD development and progression. This finding not only enriches the functional characterization of metabolism-related genes in LUAD but also provides experimental evidence for understanding the functional transition of HPRT1 from a housekeeping gene to a tumor regulator, while offering a potential candidate target for prognostic assessment and targeted therapy of LUAD.

The glycolytic pathway also plays a critical role in regulating LUAD proliferation and metastasis and is one of the key molecular mechanisms involved in LUAD malignant progression (Huang et al. 2024). Glycolysis not only provides sufficient energy and metabolic substrates for rapid tumor cell proliferation but also regulates downstream signaling pathways to enhance tumor cell proliferation and metastatic capacity, thereby driving LUAD malignant progression (Zhao et al. 2024). Glycolysis in tumor cells is characterized by aberrant activation, and its efficient operation depends largely on the coordinated regulation of key glycolytic enzymes. Among these, HK2 and PKM2 are important rate-limiting enzymes of the glycolytic pathway; when their expression levels and activities are significantly enhanced, the overall efficiency of the glycolytic pathway is effectively increased (Xie et al. 2025). The present study showed that HPRT1 overexpression significantly increased the expression levels of key glycolytic proteins, including HK2 and PKM2, in LUAD cells. To further validate this regulatory effect, we introduced 2-DG, a classic glycolysis inhibitor widely used in tumor metabolism research. Because 2-DG lacks a critical hydroxyl group, its metabolites cannot proceed through subsequent steps of the glycolytic pathway, thereby inhibiting glycolysis and reducing ATP production (Singh et al. 2023). The rapid proliferation of LUAD cells requires substantial energy supply; therefore, inhibiting glycolysis with 2-DG effectively suppresses tumor cell proliferation and metastasis (Ediriweera and Jayasena 2023). To further investigate the regulatory effect of HPRT1 overexpression on glycolytic efficiency in LUAD cells and to clarify the interventional effect of 2-DG, lactate level measurements and Western blotting demonstrated that HPRT1 positively promotes glycolytic activity in LUAD cells and enhances malignant biological behaviors, including proliferation, migration, and invasion. Meanwhile, 2-DG effectively reversed the enhancement of glycolytic efficiency induced by HPRT1 overexpression and suppressed the malignant biological behaviors of LUAD cells. Therefore, this study confirms that HPRT1 participates in the malignant progression of LUAD by regulating the expression of key glycolytic enzymes. Furthermore, studies have shown that high PKM2 expression is closely associated with poor prognosis in LUAD patients (Yin et al. 2023), which strongly supports the molecular mechanism proposed in this study that

HPRT1 regulates glycolytic efficiency in LUAD cells. Moreover, the ability of 2-DG to effectively reverse the glycolysis-enhancing effect induced by HPRT1 overexpression is consistent with findings from similar studies using 2-DG combination interventions, suggesting that HPRT1-driven glycolytic abnormalities represent a potential therapeutic target in LUAD.

The findings of this study provide new insights and experimental evidence for the clinical diagnosis, treatment optimization, and prognostic assessment of LUAD. Targeted intervention targeting HPRT1 may help block malignant progression pathways under critical conditions, alleviate complication progression, and ultimately provide a solid theoretical basis for improving clinical efficacy and survival outcomes in LUAD patients. In terms of diagnosis and prognosis assessment, aberrant high expression of HPRT1 has the potential to serve as a molecular marker for LUAD diagnosis and prognosis prediction, offering a novel detection target for early screening, disease monitoring, and prognostic stratification of LUAD. From a therapeutic perspective, a targeted treatment strategy can be developed against the HPRT1-mediated glycolytic pathway, including two main approaches: first, directly inhibiting HPRT1 enzymatic activity to block its activation of glycolysis in LUAD cells, thereby suppressing malignant biological behaviors; and second, combining glycolysis inhibitors (e.g., 2-DG) to enhance metabolic stress levels in tumor cells, induce apoptosis, and improve treatment efficacy. At the mechanistic level, this study preliminarily reveals the molecular mechanism by which HPRT1 regulates malignant progression in LUAD. However, certain limitations should be acknowledged. First, this study was conducted only at the in vitro cellular level; the specific molecular mechanisms by which HPRT1 regulates LUAD require further validation through in vivo animal studies to clarify its true regulatory role within the tumor microenvironment. Second, as this study falls within the realm of basic experimental research, the clinical translational value of the findings requires confirmation through additional clinical studies. Future clinical studies can be designed to detect differences in HPRT1 expression between LUAD tissues and adjacent normal tissues, analyze the correlation between HPRT1 expression levels and clinicopathological characteristics and patient prognosis, and further validate the clinical value of HPRT1 as a prognostic biomarker for LUAD. Elucidating the role of HPRT1 in LUAD progression will facilitate its translation from basic research to clinical application, providing a new theoretical basis and experimental foundation for precision diagnosis and targeted therapy of LUAD.

Declarations

Ethics approval and consent to participate

Not applicable. This study used commercially obtained cell lines and did not involve human participants or animals.

Consent for publication

All authors have approved the publication of this article.

Availability of data and materials

Data will be made available on request.

Competing Interests

The authors have no conflicts of interest to declare.

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Author contributions

Ce Zhao and Junqing Gan contributed to the conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the article and critical revision for important intellectual content. Chennan Zhao, Chaonan Zhu, Jianyi Pu and Peng Gao contributed to the acquisition of data and experimental operation. Peng Gao also contributed to microscopic analyses and data interpretation. All authors read and approved the final version of the manuscript for submission.

References

1. Ahmadi M, Eftekhari Kenzerki M, Akrami SM et al (2021) Overexpression of HPRT1 is associated with poor prognosis in head and neck squamous cell carcinoma. *FEBS Open Bio* 11:2525-2540. <https://doi.org/10.1002/2211-5463.13250>
2. Alberghina L (2023) The Warburg effect explained: integration of enhanced glycolysis with heterogeneous mitochondria to promote cancer cell proliferation. *Int J Mol Sci* 24:15787. <https://doi.org/10.3390/ijms242115787>
3. Barta JA, Powell CA, Wisnivesky JP (2019) Global epidemiology of lung cancer. *Ann Glob Health* 85:8. <https://doi.org/10.5334/aogh.2419>
4. Cascone T, McKenzie JA, Mbofung RM et al (2018) Increased tumor glycolysis characterizes immune resistance to adoptive T cell therapy. *Cell Metab* 27:977-987. <https://doi.org/10.1016/j.cmet.2018.02.024>
5. Chen A, Wang G, Wang D et al (2024) HPRT1: a preliminary investigation on its involvement in nasopharyngeal carcinoma. *Discov Oncol* 15:624. <https://doi.org/10.1007/s12672-024-01506-y>
6. de Lima LP, Seabra SH, Carneiro H et al (2015) Effect of 3-bromopyruvate and atovaquone on infection during in vitro interaction of *Toxoplasma gondii* and LLC-MK2 cells. *Antimicrob Agents Chemother* 59:5239-5249. <https://doi.org/10.1128/AAC.00337-15>

7. Denisenko TV, Budkevich IN, Zhivotovsky B (2018) Cell death-based treatment of lung adenocarcinoma. *Cell Death Dis* 9:117. <https://doi.org/10.1038/s41419-017-0063-y>
8. Du K, Zou J, Wang B et al (2022) A metabolism-related gene prognostic index bridging metabolic signatures and antitumor immune cycling in head and neck squamous cell carcinoma. *Front Immunol* 13:857934. <https://doi.org/10.3389/fimmu.2022.857934>
9. Ediriweera MK, Jayasena S (2023) The role of reprogrammed glucose metabolism in cancer. *Metabolites* 13:345. <https://doi.org/10.3390/metabo13030345>
10. Ghafouri-Fard S, Noie Alamdari A, Noee Alamdari Y et al (2022) Role of PI3K/AKT pathway in squamous cell carcinoma with an especial focus on head and neck cancers. *Cancer Cell Int* 22:254. <https://doi.org/10.1186/s12935-022-02676-x>
11. Huang Q, Fan L, Gong M et al (2024) Metabolic reprogramming in lung cancer and its clinical implication. *Front Pharmacol* 15:1516650. <https://doi.org/10.3389/fphar.2024.1516650>
12. Huang Z, Chen D, Hong Z et al (2023) Estimating the cure proportion of stage IA lung adenocarcinoma: a population-based study. *BMC Pulm Med* 23:417. <https://doi.org/10.1186/s12890-023-02725-9>
13. Kim SM, Yun MR, Hong YK et al (2013) Glycolysis inhibition sensitizes non-small cell lung cancer with T790M mutation to irreversible EGFR inhibitors via translational suppression of Mcl-1 by AMPK activation. *Mol Cancer Ther* 12:2145-2156. <https://doi.org/10.1158/1535-7163.MCT-12-1188>
14. Lampidis TJ, Kurtoglu M, Maher JC et al (2006) Efficacy of 2-halogen substituted D-glucose analogs in blocking glycolysis and killing hypoxic tumor cells. *Cancer Chemother Pharmacol* 58:725-734. <https://doi.org/10.1007/s00280-006-0207-8>
15. Lee PC, Truong B, Vega-Crespo A et al (2016) Restoring ureagenesis in hepatocytes by CRISPR/Cas9-mediated genomic addition to arginase-deficient induced pluripotent stem cells. *Mol Ther Nucleic Acids* 5:e394. <https://doi.org/10.1038/mtna.2016.98>
16. Leiter A, Veluswamy RR, Wisnivesky JP (2023) The global burden of lung cancer: current status and future trends. *Nat Rev Clin Oncol* 20:624-639. <https://doi.org/10.1038/s41571-023-00798-3>
17. Li C, Lei S, Ding L et al (2023a) Global burden and trends of lung cancer incidence and mortality. *Chin Med J (Engl)* 136:1583-1590. <https://doi.org/10.1097/CM9.0000000000002529>
18. Li H, Guo H, Huang Q et al (2023b) Circular RNA P4HB promotes glycolysis and tumor progression by binding with PKM2 in lung adenocarcinoma. *Respir Res* 24:252. <https://doi.org/10.1186/s12931-023-02563-7>
19. Liao M, Yao D, Wu L et al (2024) Targeting the Warburg effect: a revisited perspective from molecular mechanisms to traditional and innovative therapeutic strategies in cancer. *Acta Pharm Sin B* 14:953-1008. <https://doi.org/10.1016/j.apsb.2023.12.003>
20. Liu Y, Su Z, Tavana O et al (2024) Understanding the complexity of p53 in a new era of tumor suppression. *Cancer Cell* 42:946-967. <https://doi.org/10.1016/j.ccell.2024.04.009>
21. Lu Y, Chen R, Zhang H et al (2024) Prognostic significance and immunological role of HPRT1 in human cancers. *Biomol Biomed* 24:262-291. <https://doi.org/10.17305/bb.2023.9775>

22. Mai Z, Chen H, Huang M et al (2021) A robust metabolic enzyme-based prognostic signature for head and neck squamous cell carcinoma. *Front Oncol* 11:770241. <https://doi.org/10.3389/fonc.2021.770241>
23. Makinoshima H, Takita M, Matsumoto S et al (2014) Epidermal growth factor receptor (EGFR) signaling regulates global metabolic pathways in EGFR-mutated lung adenocarcinoma. *J Biol Chem* 289:20813-20823. <https://doi.org/10.1074/jbc.M114.575464>
24. Monnat RJ Jr, Chiaverotti TA, Hackmann AF et al (1992) Molecular structure and genetic stability of human hypoxanthine phosphoribosyltransferase (HPRT) gene duplications. *Genomics* 13:788-796. [https://doi.org/10.1016/0888-7543\(92\)90154-K](https://doi.org/10.1016/0888-7543(92)90154-K)
25. Pan J, Huang Z, Xu Y (2021) m5C-related lncRNAs predict overall survival of patients and regulate the tumor immune microenvironment in lung adenocarcinoma. *Front Cell Dev Biol* 9:671821. <https://doi.org/10.3389/fcell.2021.671821>
26. Park Y, Pacitto A, Bayliss T et al (2017) Essential but not vulnerable: indazole sulfonamides targeting inosine monophosphate dehydrogenase as potential leads against *Mycobacterium tuberculosis*. *ACS Infect Dis* 3:18-33. <https://doi.org/10.1021/acsinfecdis.6b00103>
27. Qiao Q, Hu S, Wang X (2024) The regulatory roles and clinical significance of glycolysis in tumor. *Cancer Commun (Lond)* 44:761-786. <https://doi.org/10.1002/cac2.12549>
28. Sedano MJ, Ramos EI, Choudhari R et al (2020) Hypoxanthine phosphoribosyl transferase 1 is upregulated, predicts clinical outcome and controls gene expression in breast cancer. *Cancers (Basel)* 12:1522. <https://doi.org/10.3390/cancers12061522>
29. Shin D, Cho KH (2023) Critical transition and reversion of tumorigenesis. *Exp Mol Med* 55:692-705. <https://doi.org/10.1038/s12276-023-00969-3>
30. Singh R, Gupta V, Kumar A et al (2023) 2-Deoxy-D-glucose: a novel pharmacological agent for killing hypoxic tumor cells, oxygen dependence-lowering in Covid-19, and other pharmacological activities. *Adv Pharmacol Pharm Sci* 2023:9993386. <https://doi.org/10.1155/2023/9993386>
31. Takemura H, Choi JH, Fushimi K et al (2023) Role of hypoxanthine-guanine phosphoribosyltransferase in the metabolism of fairy chemicals in rice. *Org Biomol Chem* 21:2556-2561. <https://doi.org/10.1039/D3OB00026E>
32. Torres RJ, Puig JG (2007) Hypoxanthine-guanine phosphoribosyltransferase (HPRT) deficiency: Lesch-Nyhan syndrome. *Orphanet J Rare Dis* 2:48. <https://doi.org/10.1186/1750-1172-2-48>
33. Torres RJ, Puente S, Menendez A et al (2017) Unapparent hypoxanthine-guanine phosphoribosyltransferase deficiency. *Clin Chim Acta* 472:136-138. <https://doi.org/10.1016/j.cca.2017.08.002>
34. Wu H, Zhong Q, Zhong R et al (2016) Preparation and antitumor evaluation of self-assembling oleanolic acid-loaded Pluronic P105/d-alpha-tocopheryl polyethylene glycol succinate mixed micelles for non-small-cell lung cancer treatment. *Int J Nanomedicine* 11:6337-6352. <https://doi.org/10.2147/IJN.S119839>

35. Xie H, Yu J, Ou Z et al (2025) Emerging roles of ncRNAs regulating PKM2 in cancer progression. *Curr Pharm Biotechnol* 26:1814-1824. <https://doi.org/10.2174/0113892010311953240628084818>
36. Yin L, Shi J, Zhang J et al (2023) PKM2 is a potential prognostic biomarker and related to immune infiltration in lung cancer. *Sci Rep* 13:22243. <https://doi.org/10.1038/s41598-023-49558-4>
37. Zhang S, Xiao X, Yi Y et al (2024) Tumor initiation and early tumorigenesis: molecular mechanisms and interventional targets. *Signal Transduct Target Ther* 9:149. <https://doi.org/10.1038/s41392-024-01848-7>
38. Zhang Y, Vaccarella S, Morgan E et al (2023) Global variations in lung cancer incidence by histological subtype in 2020: a population-based study. *Lancet Oncol* 24:1206-1218. [https://doi.org/10.1016/S1470-2045\(23\)00444-8](https://doi.org/10.1016/S1470-2045(23)00444-8)
39. Zhao J, Jin D, Huang M et al (2024) Glycolysis in the tumor microenvironment: a driver of cancer progression and a promising therapeutic target. *Front Cell Dev Biol* 12:1416472. <https://doi.org/10.3389/fcell.2024.1416472>
40. Zhou YM, Yao YL, Liu W et al (2020) MicroRNA-134 inhibits tumor stem cell migration and invasion in oral squamous cell carcinomas via downregulation of PI3K-Akt signaling pathway by inhibiting LAMC2 expression. *Cancer Biomark* 29:51-67. <https://doi.org/10.3233/CBM-191362>

Figures

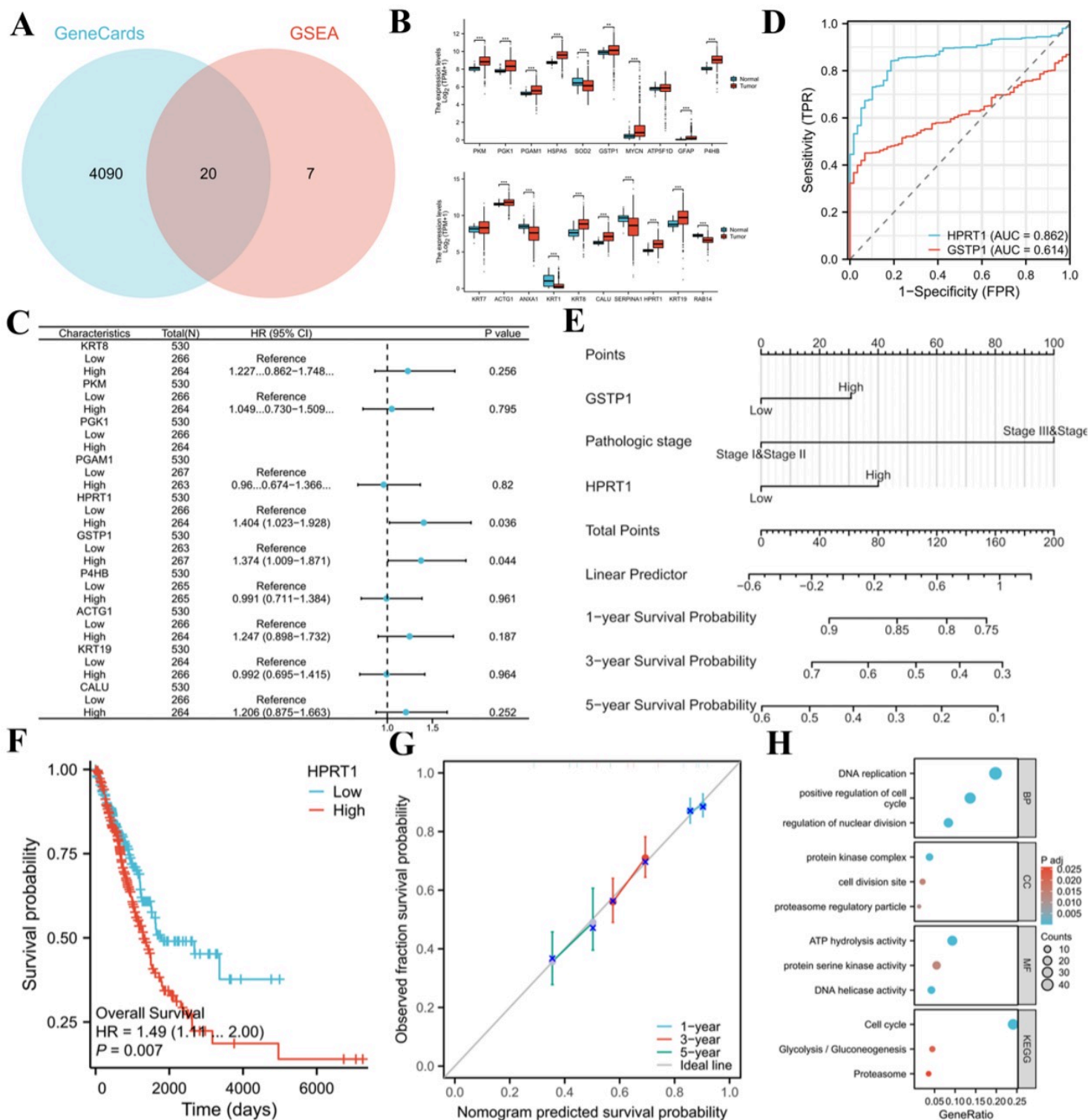


Figure 1

Bioinformatics analysis identifies HPRT1 as a key gene in LUAD (A) Glycolysis-related genes retrieved from database searches. (B) Differential expression analysis of the 20 glycolysis-related genes. (C) Multivariate Cox regression analysis of glycolysis-related genes. (D) Diagnostic value of HPRT1 for predicting LUAD. (E-G) Patients with high HPRT1 expression exhibit a poorer prognosis. (H) HPRT1 is involved in the regulation of the glycolytic pathway. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$

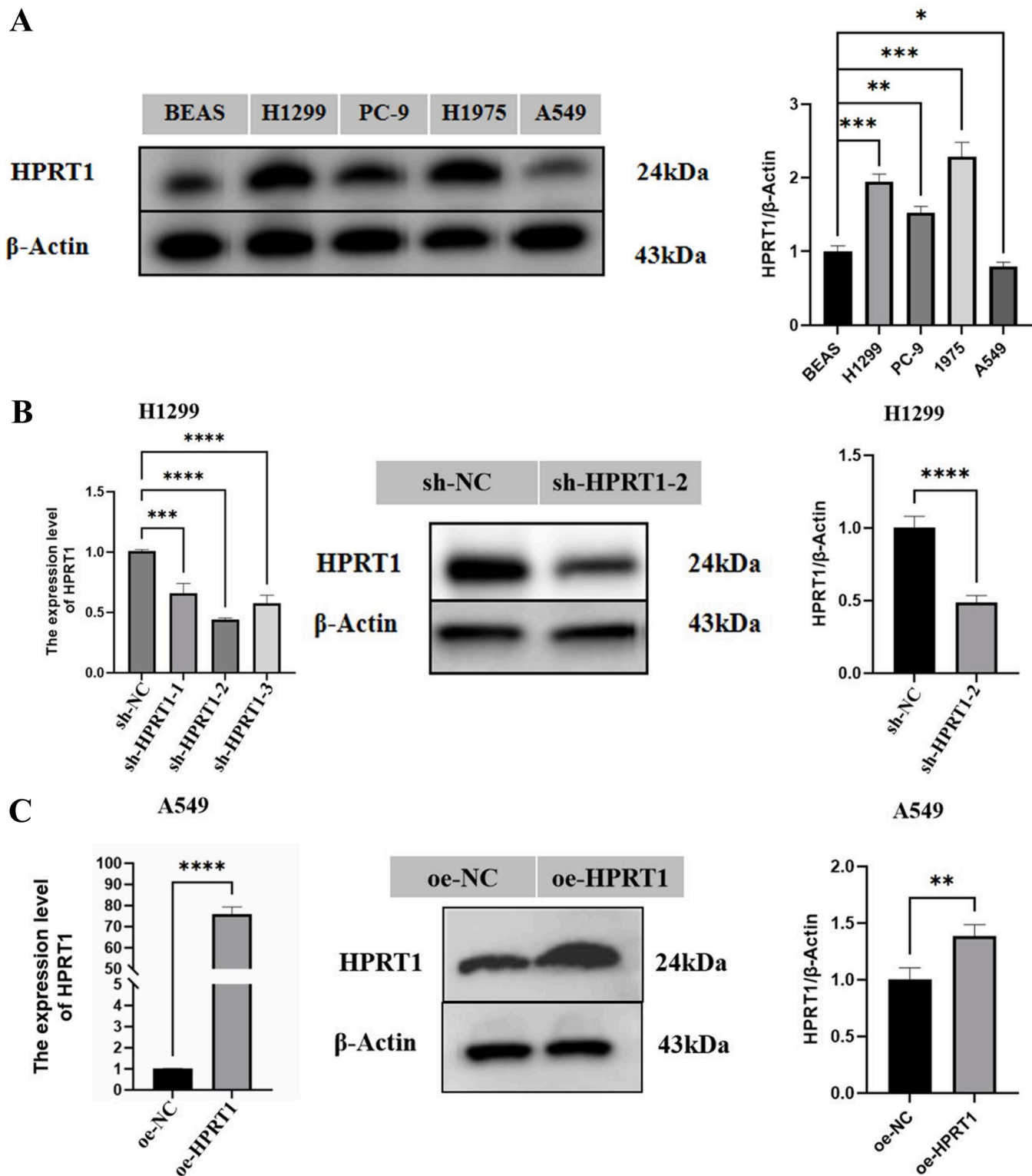


Figure 2

HPRT1 protein expression in LUAD cells and validation of knockdown and overexpression efficiency (A) HPRT1 protein expression levels in LUAD cells. (B) Validation of HPRT1 knockdown efficiency by RT-qPCR and Western blotting. (C) Validation of HPRT1 overexpression efficiency by RT-qPCR and Western blotting. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$

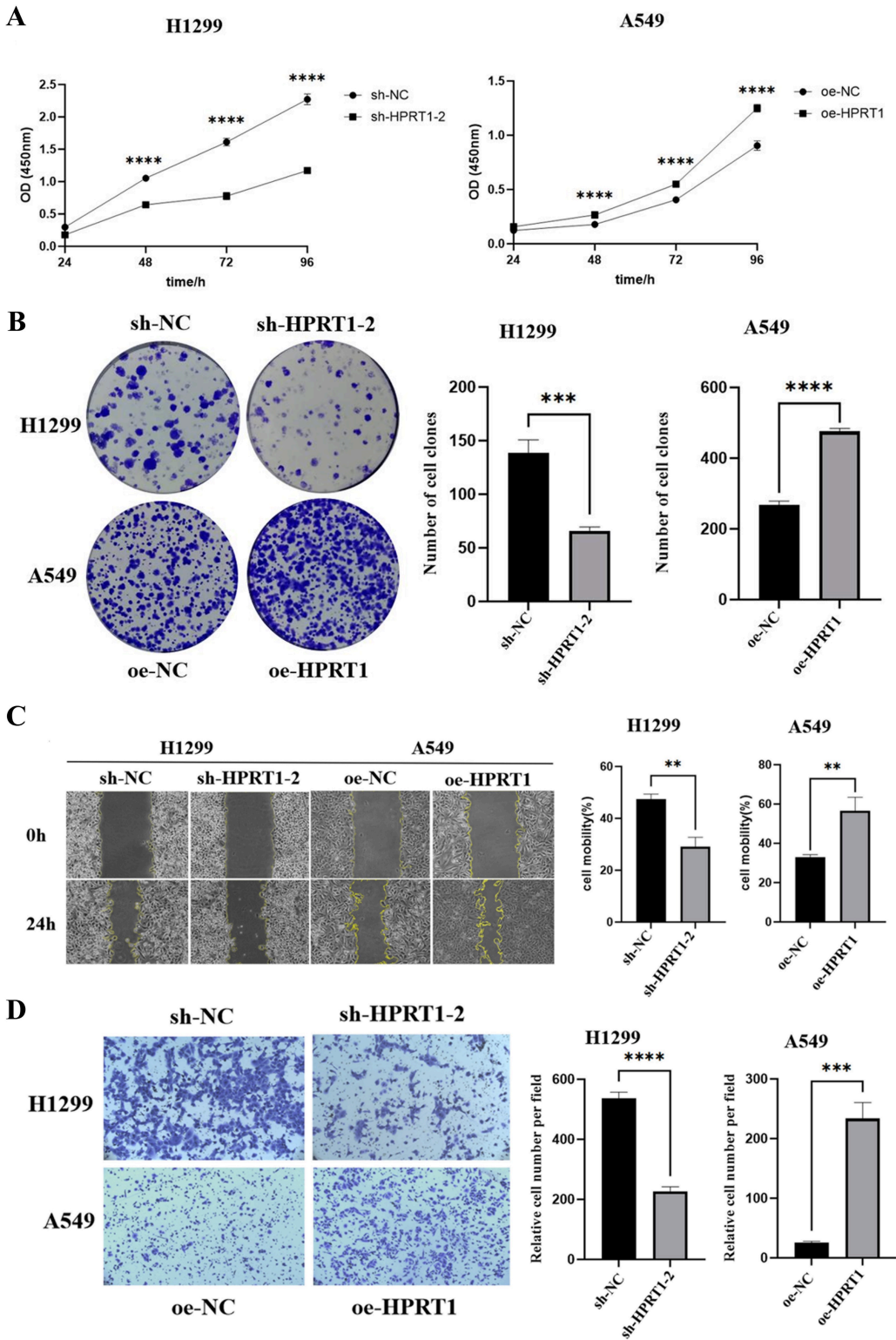


Figure 3

HPRT1 promotes the proliferation, migration, and invasion of LUAD cells (A–B) HPRT1 promotes the proliferative ability of LUAD cells. (C) HPRT1 promotes the migratory ability of LUAD cells. (D) Transwell invasion assay showing that HPRT1 promotes the invasive capacity of LUAD cells. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$

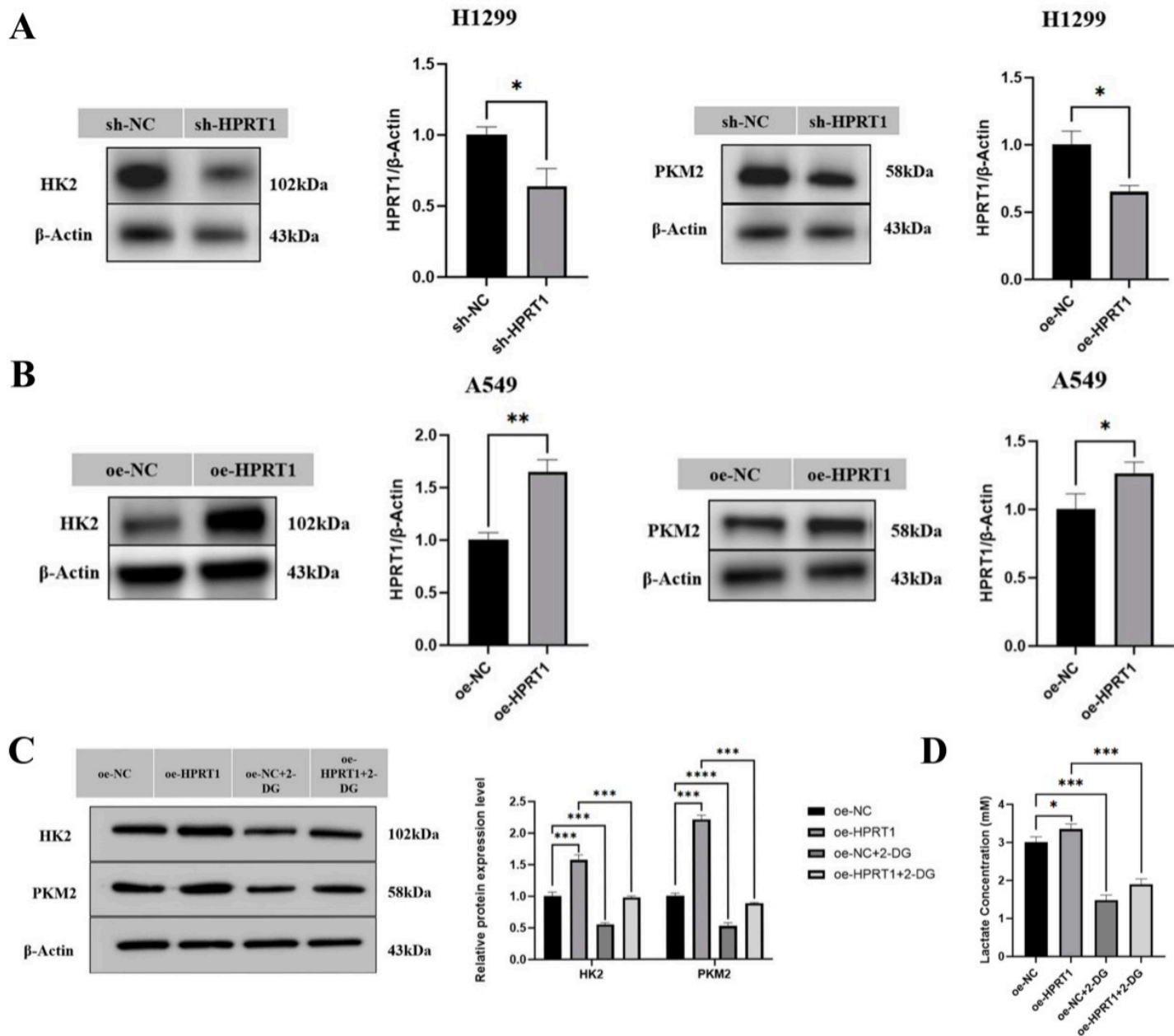


Figure 4

HPRT1 regulates HK2 and PKM2 expression and lactate production in LUAD cells (A) Knockdown of HPRT1 inhibits the protein expression of HK2 and PKM2. (B) Overexpression of HPRT1 promotes the protein expression of HK2 and PKM2. (C) Protein expression levels of HK2 and PKM2 are upregulated following HPRT1 overexpression, whereas they are downregulated after treatment with 2-DG. (D) HPRT1 promotes lactate production in LUAD cells, and treatment with 2-DG inhibits lactate production. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$

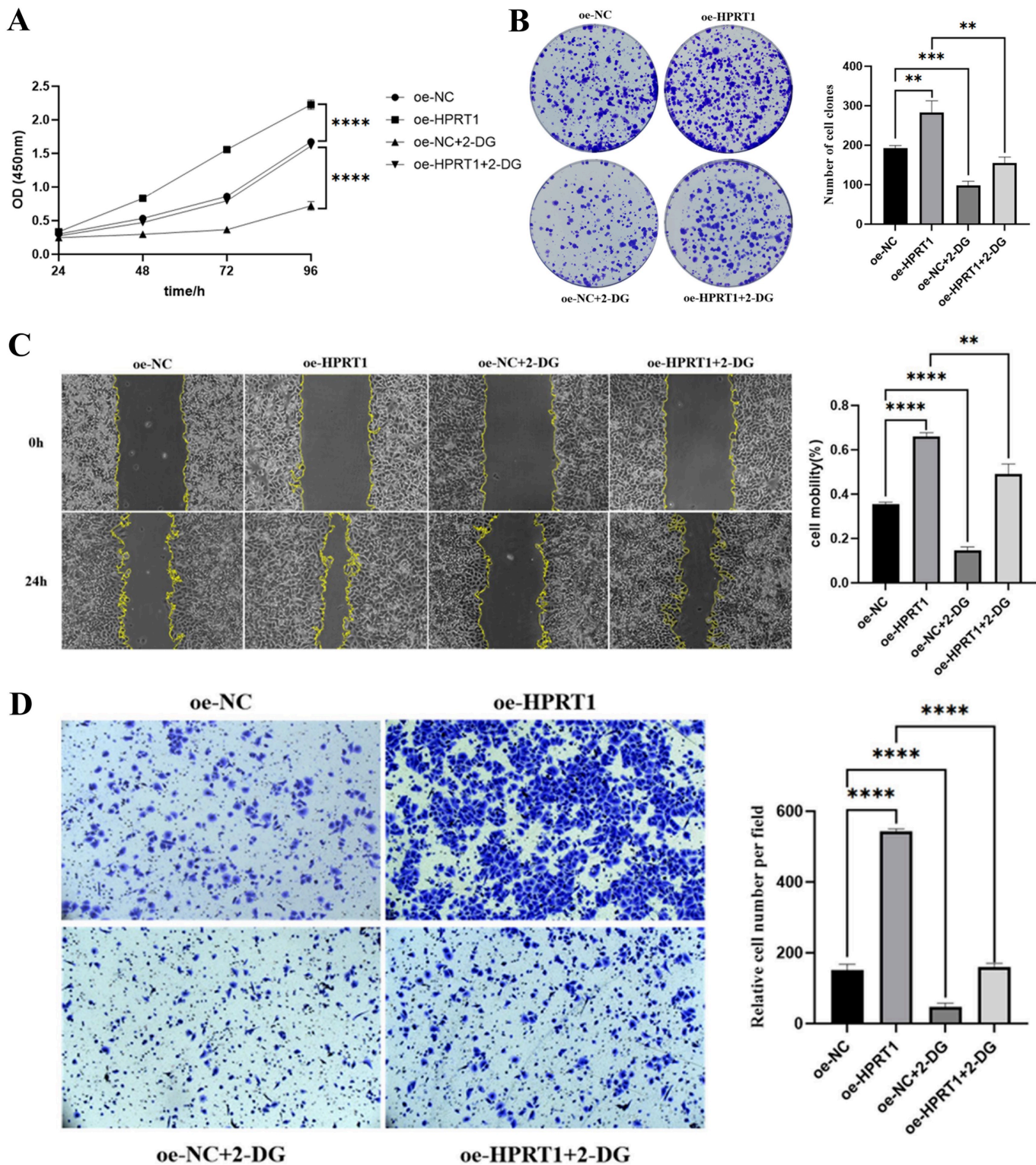


Figure 5

HPRT1 promotes the proliferation, migration, and invasiveness of LUAD cells by activating glycolysis (A–B) CCK-8 assay and plate colony formation assay demonstrated that HPRT1-induced glycolysis enhances LUAD cell proliferation. (C) Cell scratch assay confirmed its enhancement of LUAD cell migration. (D) Transwell experiment demonstrated that HPRT1 activation of glycolysis enhances the invasive capacity of LUAD cells. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$

