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Hypoxanthine guanine phosphoribosyltransferase 1, a target of miR-125b-5p, promotes cell proliferation and invasion in head and neck squamous cell carcinoma

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ABSTRACT

The mechanism of hypoxanthine-guanine phosphoribosyltransferase 1 (HPRT1) upregulation and its function in head and neck squamous cell carcinoma (HNSCC) remains obscure. Herein, the expression and function of HPRT1 and the mechanism underlying its upregulation in HNSCC were explored. Firstly, the expression of HPRT1 and its prognostic values were simultaneously validated using bioinformatic analysis and quantitative real-time PCR (qRT-PCR), and immunohistochemistry staining with local HNSCC samples. The effects of HPRT1 knockdown on proliferation and invasion of HNSCC cells were detected using cell counting kit-8 (CCK-8), plate clone formation, Transwell invasion, nude mouse xenograft model assays. Moreover, the miRNA targeting HPRT1 was validated using dual-luciferase report assay, qRT-PCR and Western blot analysis. The functions of miRNA targeting HPRT1 and its dependence on HPRT1 were further investigated in HNSCC. The results indicated that HPRT1 was highly expressed in HNSCC tissues and cells, which positively correlated with advanced tumor progression and predicted poor prognosis in patients with HNSCC. HPRT1 knockdown markedly inhibited proliferation and invasion of HNSCC cells both *in vitro* and *in vivo*. MiR-125b-5p, which was downregulated and positively correlated with a favorable outcome for patients, directly targeted and downregulated HPRT1 expression, and subsequently suppressed cell proliferation and invasion in HNSCC. Collectively, the present study demonstrates that HPRT1 upregulation, at least partially caused by miR-125b-5p downregulation, could promote the malignant progression of HNSCC, highlighting the potential application of the miR-125b-5p/HPRT1 axis as a novel indicator and target in the diagnosis and treatment of HNSCC.

1. Introduction

Head and neck squamous cell carcinoma (HNSCC), originating from the mucosal epithelium in the oral cavity, pharynx and larynx, is the most common type of head and neck cancers and ranks as the sixth most common cancer worldwide [1]. The incidence of HNSCC

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is rising annually owing to increased exposure to carcinogens, such as tobacco and alcohol consumption, environmental pollutants, and virus infection, which randomly induce cumulative genetic alterations, subsequently activate or inactivate oncogenic or tumor-suppressive genes, and eventually lead to malignant transformation [1,2]. Thus, identifying tumor-related genes and uncovering their functions and underlying mechanisms could provide novel therapeutic targets and strategies to improve clinical efficacy.

Hypoxanthine guanine phosphoribosyltransferase 1 (HPRT1) is a rate-limiting enzyme in the DNA salvage pathway, whose activity is critical to maintaining the purine pool and promoting cell cycle progression through the regulation of guanine and inosine production [3,4]. Although it is broadly regarded as a housekeeping gene, recent studies have demonstrated the differential expression level of HPRT1 between malignant and normal tissues, implying that it may play functional roles in tumorigenesis and is unreliable as an endogenous control in cancer-related studies [5–8]. For example, HPRT1 was evaluated in breast cancer tissues and positively correlated with poor distant metastasis-free survival, possibly through activating oncogenic pathways [7]. Besides, upregulation of HPRT1, which predicts poor prognosis, has been confirmed in other cancers, such as prostate cancer and colorectal cancer [9,10]. A recent study using online RNA-sequencing data found that HPRT1 was upregulated in HNSCC, suggesting HPRT1 contributes to the progression of HNSCC [5]. However, the function and mechanism underlying HPRT1 upregulation in HNSCC remains unknow.

MicroRNAs (miRNAs) play vital roles in various cancers, including HNSCC [11,12]. Aberrant expression of miRNAs regulates carcinogenesis by post-transcriptionally modulating the expression of its target genes, oncogenes, or tumor-suppressive genes [11]. Downregulation of miR-125b-5p suppresses the progression of HNSCC [13,14]. Interestingly, HPRT1 is a putative target of miR-125b-5p and negatively correlates with miR-125b-5p, suggesting that miR-125b-5p and HPRT1 may interact with each other to participate in HNSCC. Although the oncogenic role of HPRT1 has been reported in several cancers [3,7], its roles and underlying mechanism in HNSCC remain elusive. Thus, the present study sought to explore the expression, prognostic value, and function of HPRT1 and validate the regulation and interaction between miR-125b-5p and HPRT1 in HNSCC. The results revealed that HPRT1, a target of miR-125b-5p, promoted cell proliferation and invasion and predicted poor prognosis in patients with HNSCC. MiR-125b-5p restoration significantly downregulated HPRT1 and subsequently constrained HNSCC progression. Overall, these findings confirmed the oncogenic role of HPRT1 in NSCC and highlighted the potential application of HPRT1 as a reliable therapeutic target.

2. Materials and methods

2.1. Tissue samples

From September 2015 to September 2017, 60 pairs of fresh HNSCC and para-cancerous samples with clinical-pathological features were obtained from newly diagnosed patients under surgical resection at the department of stomatology of the Third Xiangya Hospital of Central South University. Every sample was divided into two parts: one part was frozen for RNA isolation and another part was used to prepare paraffin sections for immunohistochemical (IHC) analysis.

2.2. Online analysis

Level 3 of HNSCC RNA-sequence data, level 3 BCGSC miRNA Profiling data, and the paired clinical data from TCGA (The Cancer Genome Atlas, https://portal.gdc.cancer.gov/) were used to analyze the expression, clinical relation, and prognostic value of HPRT1 and miR-125b-5p in HNSCC via R (version 3.6.3) as previous description [15]. Oncomine (http://www.oncomine.org) was used to validate the expression of HPRT1 in HNSCC patients [16]. UALCAN (http://ualcan.path.uab.edu/) was adopted to investigate the promoter methylation status of HPRT1 in HNSCC [17]. The miRNAs showing negative correlation with HPRT1 in HNSCC were analyzed by LinkedOmics (http://linkedomics.org/) and the miRNAs potentially targeting HPRT1 were predicted by Starbase 2.0 (https://starbase.sysu.edu.cn/index.php) [18,19].

2.3. IHC assay

IHC was performed as previously described [20]. Briefly, paraffin-embedded sections were sequentially subjected to dewaxing, hydration, antigen retrieval and endogenous peroxidase activity blocking. Then, slides were incubated overnight at 4 °C with the rabbit anti-HPRT1 antibody (dilution:1:50, BBI, Shanghai, China). After washing with phosphate-buffered saline, slides were subsequently incubated with goat anti-rabbit IgG-biotin, streptavidin-HRP, and DAB (diaminobenzidine) solution. Slides were counterstained with hematoxylin and visualized under a microscope (Leica, Solms, Germany). The IHC outcomes were scored based on the staining intensity and area following the previous description [20].

2.4. Cell lines

HNSCC cell lines, including FaDu, CAL-27, and SCC-4, immortalized human oral keratinocyte HOK, and HEK-293T were maintained in Dulbecco's Modified Eagle Medium (DMEM, BI, Jerusalem, Israel) plus 10% fetal bovine serum (FBS, BI, Jerusalem, Israel) in a humidified cell incubator at 37 °C with 5% CO_2.

2.5. RNA isolation and quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Total RNA of cells and tissues were isolated using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) as previously described [20].

Then, the total RNAs were reversely transcribed into cDNA using miDETECT A TrackTM miRNA qRT-PCR Starter Kit and HiScript III 1st Strand cDNA Synthesis Kit (Vazyme, Nanjing, China), respectively. Subsequently, the expression of miR-125b-5p and HPRT1 in HNSCC cells and tissues were detected using AceQ qRT-PCR SYBR Green Master Mix (Vazyme, Nanjing, China) with CFX Connect Real-Time System (Bio-Rad, CA, USA) following manufacturer's instructions. The specific primer sequences are listed as follow: HPRT1 forward primer: (5′- CCTGGCGTCGTGATTAGTGAT-3′), HPRT1 reverse primer: (5′- AGACGTTCAGTCCTGTCCATAA-3′), HPRT1 hnRNA forward primer: (5′- TCCAGATTCCATGCTGACCG-3′), HPRT1 hnRNA reverse primer: (5′- ACCAGTGAGGGTGGAGCTAA-3′), GAPDH forward primer: (5′- CTGGGCTACACTGAGCACC-3′), GAPDH reverse primer: (5′- AAGTGGTCGTTGAGGGCAATG-3′). Primers for miR-125b-5p and 5S were purchased from RioBio Inc (Guangzhou, China).

2.6. Lentivirus package and stable cell line construction

A lentivirus plasmid GV248-shHPRT1, which expresses short hair RNA (shRNA) targeting HPRT1, was constructed using the ClonExpress Ultra One Step Cloning Kit (Vazyme, Nanjing, China) with GV248-shNC as the vector backbone. The shRNA sequences were 5′-CCAGGTTATGACCTTGATTTACTCGAGTAAATCAAGGTCATAACCTGG-3'. Lentivirus particles of shHPRT1 were packaged by co-transfecting HEK-293T cells with GV248-shHPRT1, pSPAX2, and pMD2.G plasmids as previously described [21]. FaDu and SSC-4 cells were infected with shHPRT1 and control lentivirus particles. Stable cell lines with HPRT1 knockdown and control cell lines were obtained via culturing cells with a selected medium containing puromycin (1 μg/ml) for two weeks.

2.7. MiRNA and plasmid transfection

MiR-125b-5p mimic, pENTER-HPRT1, and corresponding control RNAs and plasmids were transfected into cells using Lipofectamine™ 2000 (Invitrogen, CA, USA) as previously described [20,22]. MiR-125b-5p mimic and controls were synthesized by RioBio (Guangzhou, China). pENTER-HPRT1 and vector plasmids were purchased from Vigene Bioscience Inc (MD, USA).

2.8. Western blot

Western blot analysis was performed as previously described [20,22]. Briefly, 30 μg of total protein was resolved on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and then transmitted onto a polyvinylidene difluoride membrane, followed by blocking for 1 h at room temperature. The membrane was then incubated with corresponding primary antibodies overnight at 4 °C, rinsed thrice with Tris-buffered saline buffer with Tween 20 (TBST), incubated with corresponding horseradish peroxidase (HRP)-conjugated secondary antibodies for 1 h at room temperature, and rinsed thrice with TBST. Protein expression was visualized using a chemiluminescent substrate of HRP (Millipore, MA, USA) via a FluorChem FC3 system (Proteinsimple, CA, USA). The used primary antibodies were rabbit anti-HPRT1 (1:200, BBI Life Sciences Corp., Shanghai, China), mouse anti-Tubulin (1:1000, Proteintech, Wuhan, China), and HRP-conjugated goat anti-mouse/rabbit IgG (1:3000, BBI Life Sciences Corp., Shanghai, China).

2.9. In vivo proliferation assay

The tumor proliferation assay was performed as previously described [20,22]. Briefly, HNSCC cells with HPRT1 knockdown and corresponding control cells, at the density of 1×10^6 cells/mice, were subcutaneously injected into the flanks of 4-week-old female nude mice (n = 5), which were raised in a pathogen-free environment. The volume (mm^3) of xenografts was measured every 4 days according to a modified ellipse formula (volume = length × width 2/2). After 21 days later, mice were killed, and tumors were dissected, photographed, and weighed before formalin fixation and paraffin sectioning. The study was approved by the Scientific Investigation Board of Central South University.

2.10. Cell counting kit-8 (CCK-8) assay

The CCK-8 assay was performed as previously described [22]. Briefly, HNSCC cells were seeded in 96-well plates at a density of 1×10^3 cells/well and cultured for 5 days. Then, 10 μl CCK-8 was added into the well for 1 h. The absorbance at 450 nm using the Epoch microplate spectrophotometer (Bio-Tek, VT, USA). Growth curves were drawn based on the absorbance value. All assays were performed in triplicate.

2.11. Plate clone formation assay

Plate clone assay was applied to as previously described [10]. Simply, HNSCC cells were seeded into 6-well plates at a density of 1000 cells per well. 7 days later, the cell clones were fixed with methanol and stained using 0.1% crystal violet. The clone containing more than 50 cells was calculated. All assays were performed in triplicate.

2.12. Transwell invasion assay

The transwell assay was carried out as previously described [7]. Briefly, 2.5×10^4 cells resuspended in 400 μl pure Dulbecco's Modified Eagle Medium (DMEM) medium were seeded into 8-μm pore size transwell chambers (Costar, ME, USA) pre-coated with

Matrigel (Corning, NY, USA), and placed in 24-well plates containing 750 μl DMEM medium with 10% fetal bovine serum. After 24 h, cells were fixed with methanol and stained by 0.5% crystal violet. Then, non-invasive cells on the upper side of the membrane were swabbed and invasive cells were photographed and counted using an inverted microscope (Leica, Solms, Germany).

2.13. Luciferase reporter assay

The dual-luciferase reporter system assay was performed as previously described [20,22]. Briefly, luciferase reporter plasmids expressing wild-type HPRT1 3′-UTR (untranslated region) or mutant HPRT1 3′-UTR, and Renilla control reporter plasmids were co-transfected with miR-125b-5p mimic or mimic control into HNSCC cells, respectively. After 48 h, cells were harvested and luciferase activity was analyzed using the Dual-Luciferase Reporter detection System (Promega, WI, USA) The relative luciferase activity was indicated by the ratio of firefly luciferase to Renilla luciferase activity.

2.14. Ethics statement

This study was approved by the Ethics Committee of the Third Xiangya Hospital of Central South University, Hunan, China (#22015). Written informed consent was obtained from enrolled participants. All animal experiments were performed following the Guide for the Care and Use of Laboratory Animals of Central South University, with the approval of the Scientific Investigation Board of

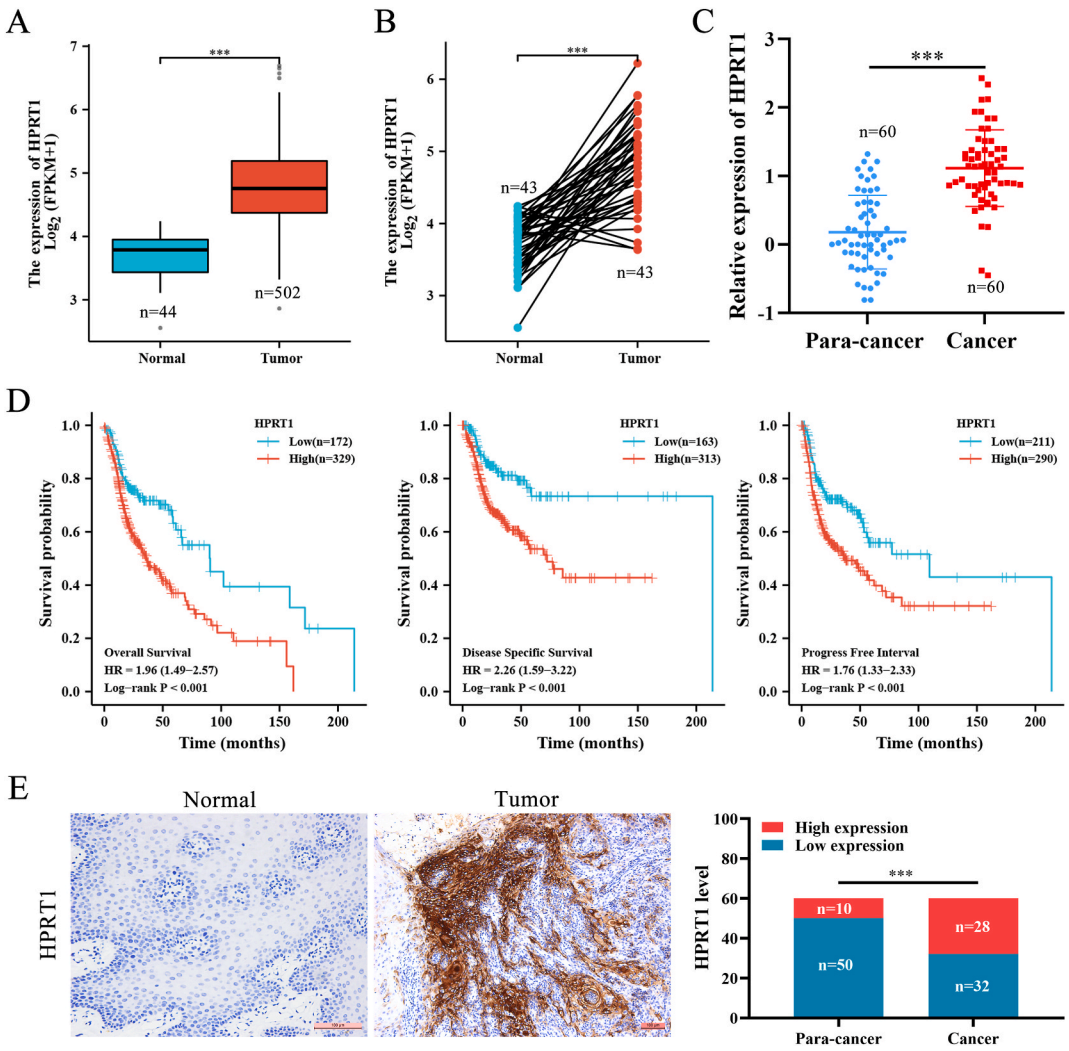

Fig. 1. HPRT1 is upregulated and predicts a poor prognosis in HNSCC. (A, B) Expression of HPRT1 in both unpaired and paired HNSCC and normal tissues were evaluated using TCGA data. (**C**) qRT-PCR analysis of the expression of HPRT1 mRNA in HNSCC and para-cancer tissues. (**D**) TCGA data indicating the prognostic values of HPRT1 in HNSCC, including overall survival (OS), disease-specific survival (DSS), and progression-free interval (PFI). (**E**) Representative images of immunohistochemical (IHC) staining for HPRT1 protein in HNSCC and para-cancer tissues. ***$P < 0.001$.

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2.15. Statistical analysis

Statistical analyses were performed using SPSS Statistics version 22.0 (IBM, IL, USA) and GraphPad version 6.0. The Student's t-test or Fisher's exact test was applied to analyze the statistical difference between two groups. Pearson correlation was adopted to analyze the correlation between two groups. All statistical tests were two-sided, and $P < 0.05$ were considered statistically significant.

3. Results

3.1. HPRT1 is upregulated and predicts poor prognosis in HNSCC

Firstly, the expression of HPRT1 was analyzed using RNA-sequencing data of HNSCC and OSCC in TCGA. Compared with normal tissues, HPRT1 expression was upregulated in both non-paired and paired cancerous tissues of HNSCC (Fig. 1A and B). The upregulation of HPRT1 in HNSCC was also validated using several Gene Expression Omnibus datasets (Supplementary Fig. 1A-D). Importantly, the upregulation of HPRT1 was confirmed in the local cohort (Fig. 1C). Besides, HPRT1 upregulation was positively correlated with poor prognosis of patients with HNSCC, including OS (Overall Survival), DSS (Disease Specific Survival), and PFI (Progress Free Interval), of HNSCC patients (Fig. 1D). Moreover, the HPRT1 level in HNSCC was validated using the IHC assay. Compared with paracancerous tissues, HPRT1 was significantly upregulated in HNSCC tissues, mainly distributed in the cytoplasm and the cell membrane (Fig. 1E). The level of HPRT1 was positively correlated with the primary tumor (T) stage, lymph node (N) metastasis, and clinical TNM stage of HNSCC patients (Table 1). Therefore, in line with the recent studies [5,6], HPRT1 is upregulated in HNSCC and positively correlates with tumor progression and poor prognosis.

3.2. HPRT1 upregulation promotes HNSCC cell proliferation and invasion in vitro

HPRT1 upregulation in HNSCCs, which indicates a poor prognosis, has been revealed in previous studies [5,6]. However, the role of HPRT1 in HNSCCs remains unknown. The expression of HPRT1 in HNSCCs cells was detected in the present study. Consistently, compared with normal HOK cell, HPRT1 was upregulated in HNSCCs cells (Fig. 2A). To explore the function of HPRT1 in HNSCCs, the expression of HPRR1 was successfully depleted in FaDu and SCC-4 cells by infection with lentivirus expressing shHPRT1 (Fig. 2B). Subsequently, the effects of HPRT1 knockdown were detected using CCK-8, plate clone formation, and transwell invasion assays. The results revealed that HPRT1 silencing significantly inhibited cell viability, proliferation, and invasion in HNSCC cells (Fig. 2C–E). Moreover, ectopic expression of HPRT1 could enhance cell growth and invasion in HNSCC cells (Supplementary Fig. 2A-C). Accordingly, HPRT1 knockdown significantly decreased the level of MMP2 (Fig. 2F), a critical enzyme promoting invasion. Together, these findings demonstrated that HPRT1 upregulation could promote proliferation and invasion of HNSCC *in vitro*.

Table 1
The correlation between HPRT1 expression and the clinicopathological parameters of patients with HNSCC (n = 60, Chi-Squared Test).

Variable	Expression level			
	n	Low (0–3)	High (4–6)	*P*
Gender				
Male	49	26	23	0.929
Female	11	6	5	
Age (y)				
<50	25	14	11	0.726
≥50	35	18	17	
Smoking history				
Yes	40	18	22	0.098
No	20	14	6	
Histological grade				
Well	26	16	10	0.265
Moderate/poor	34	16	18	
Primary tumor (T) stage				
T1-2	36	23	13	**0.044**
T3-4	24	9	15	
lymph node (N) metastasis				
pN0	32	24	8	**<0.001**
pN1-3	28	8	20	
Clinical TNM stage				
I-II	23	20	3	**<0.001**
III-IV	37	12	25	

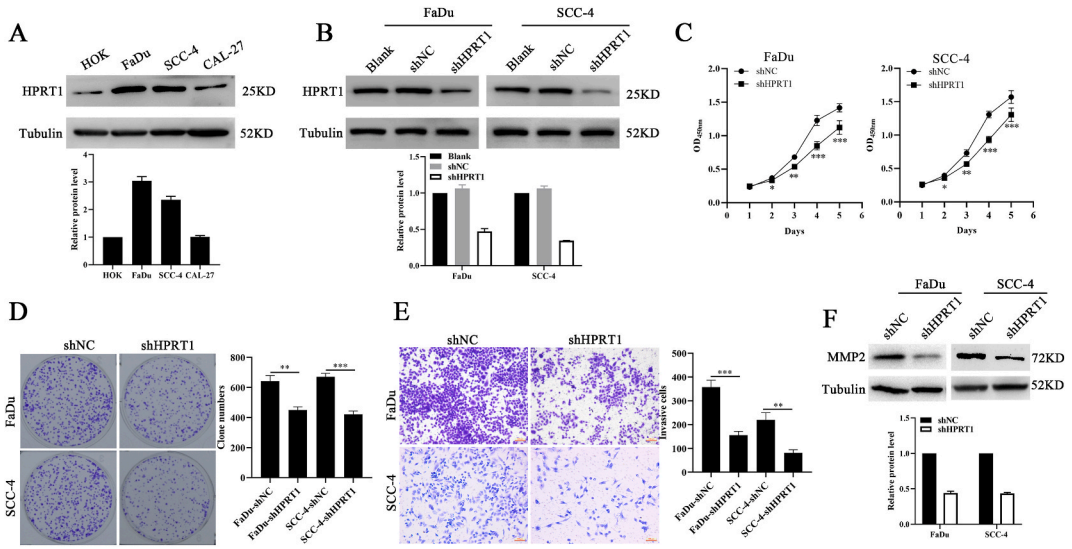

Fig. 2. HPRT1 depletion suppresses proliferation and invasion of HNSCC cells *in vitro*. (**A**) Western blot analysis of HPRT1in HNSCC cells including HOK, FaDu, SCC-4, and CAL-27 cells. (**B**) Western blot analysis of HPRT1 in HNSCC cells infected with shHPRT1 lentivirus particles. (**C**) CCK-8, (**D**) plate clone formation, and (**E**) transwell invasion assays indicating the cell viability, proliferation, and invasion of HNSCC cells with HPRT1 depletion. (**F**) Western blot analysis of MMP2 in HNSCC cells with HPRT1 knockdown. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

3.3. HPRT1 depletion suppresses the growth of HNSCC cells in vivo

Next, the effect of HPRT1 on the growth of HNSCC cells *in vivo* were assessed by using a nude mice xenograft model. Accordingly, HPRT1 depletion significantly constrained the growth of HNSCC cells *in vivo,* as demonstrated by the smaller volume and lower weight of xenograft tumors (Fig. 3A–C). Besides, IHC staining results showed that lower expression of HPRT1 and lower positive rate of Ki67 in xenograft tumor derived from HNSCC cells with HPRT1 depletion (Fig. 3D), which further confirmed the anti-proliferative effects of HPRT1 depletion on HNSCC cells *in vivo*.

3.4. HPRT1 is a target of miR-125b-5p in HNSCC

The mechanisms underlying HPRT1 upregulation were further analyzed. Epigenetic regulators such as DNA methylation and miRNAs play vital roles in the modulation of gene expression. Thus, the promoter methylation status of HPRT1 was detected by analyzing online data. The results revealed that the promoter methylation status of HPRT1 was comparable between normal and cancerous tissues (Supplementary Fig. 3A). Besides, no significant difference was found in the level of HPRT1 hnRNA (heterogeneous nuclear RNA) between normal and cancerous cells (Fig. 4A), suggesting that post-transcriptional mechanisms accounted for HPRT1 upregulation in HNSCC [20]. Therefore, candidate miRNAs possibly targeting HPRT1 were screened. MiRNAs that significantly negatively correlated with HPRT1 were obtained via LinkedOmics (Supplementary Fig. 3B). Four miRNAs that significantly negatively correlated with HPRT1 were selected for further analysis. Most miRNAs were dramatically downregulated in HNSCC tissues (Supplementary Fig. 3C) and were positively associated with the prognosis of patients with HNSCC (Supplementary Fig. 3D). MiR-125b-5p (a mature form of miR-125b-2) and miR-139 with potential binding sites of HPRT1 were selected for experimental validation (Supplementary Fig. 3E). The qRT-PCR and Western blot results indicated that ectopic expression of miR125b-5p, but not for miR-139, significantly downregulated the expression of HPRT1 in HNSCC cells (Fig. 4B and **C**, Supplementary Fig. 4A and **4B**). The expression of miR-125b-5p was negatively correlated with HPRT1 expression in the local HNSCC tissues (Fig. 4D). Furthermore, miR-125b-5p overexpression could significantly decreased the relative luciferase activity of HNSCC cells transfected with wild-type HPRT1, but did not affect HNSCC cells transfected with mutant HPRT1 in the dual-luciferase reporter assay (Fig. 4E). Thus, HPRT1 is a target of miR-125b-5p in HNSCC.

3.5. MiR-125b-5p is downregulated and serves as an independent predictor of poor prognosis in HNSCC

Next, the expression and prognostic value of miR-125b-5p in HNSCC were explored by analyzing TCGA data online. Compared with normal tissues, miR-125b-5p was markedly downregulated in HNSCC tissues (Fig. 5A and B). Besides, the level of miR-125b-5p was negatively associated with T stages (Supplementary Table 1). Survival analysis revealed that miR-125b-5p downregulation was positively correlated with the poor prognosis of HNSCC patients, including OS, DSS, and PFI (Fig. 5C). Moreover, the downregulation of miR-125b-5p was experimentally validated in the local HNSCC cohort and cells (Fig. 5D and **E**). Thus, miR-125b-5p is downregulated and predicts a poor prognosis in HNSCC.

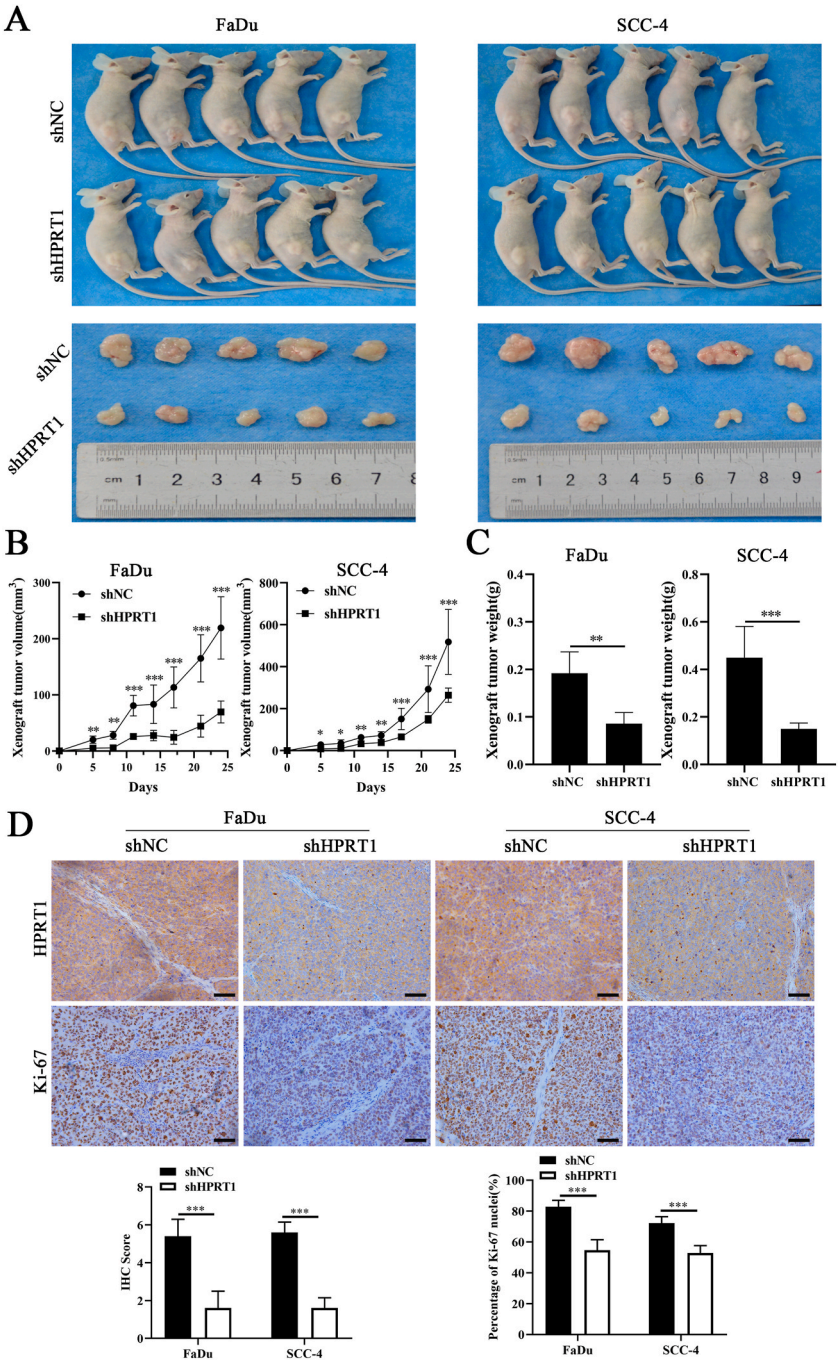

Fig. 3. HPRT1 depletion suppresses the proliferation of HNSCC cells *in vivo*. (A) Images of nude mice and xenograft tumors derived from HNSCC cells with HPRT1 depletion. (**B**) Volume curve of xenograft tumors. (**C**) Weight of xenograft tumors at the endpoint. (D) IHC staining of HPRT1 and Ki-67 in xenograft tumors. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

3.6. MiR-125b-5p suppresses proliferation and invasion of HNSCC cells

Subsequently, the functions of miR-125b-5p in HNSCC were investigated. MiR-125b-5p mimics were introduced into HNSCC cells and the effects on cell proliferation and invasion were explored. The results of CCK-8, plate clone formation, and invasion assays revealed that miR-125b-5p mimic significantly decreased MMP2 (Fig. 4B) and impaired the growth, proliferation, and invasion capacities of HNSCC cells, as demonstrated by a lower optical density (OD_{450nm}), fewer clones and invasive cells (Fig. 5F–H). Therefore, miR-125–5p could inhibit proliferation and invasion of HNSCC *in vitro*.

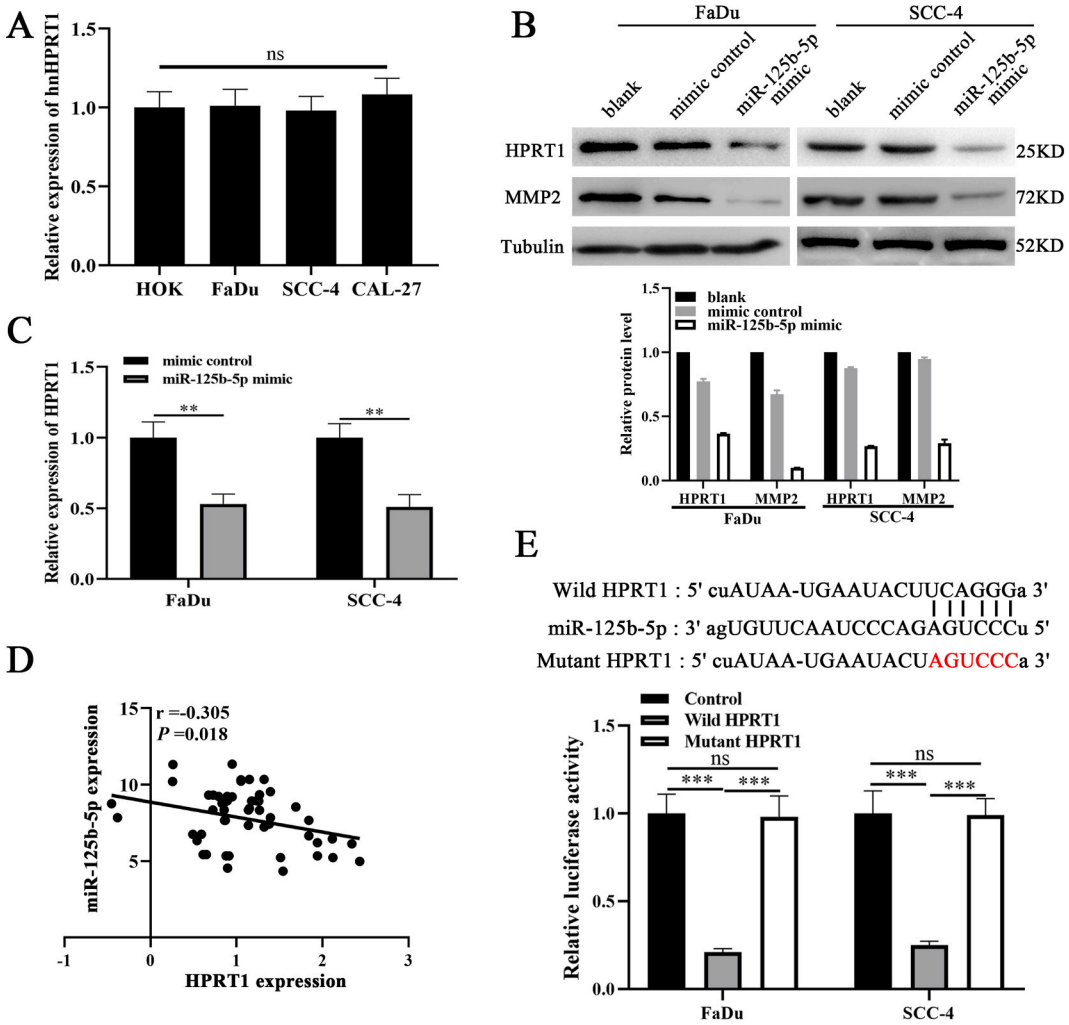

Fig. 4. MiR-125b-5p targets HPRT1 in HNSCC. (**A**) qRT-PCR results analysis of HPRT1 hnRNA in HOK and HNSCC cells. (**B**) qRT-PCR and (**C**) Western blot analyses of HPRT1 in FaDu and SCC-4 cells transfected with miR-125b-5p mimic. (**D**) Pearson correlation analysis between miR-125b-5p and HPRT1 expression in HNSCC tissues. (**E**) Dual-luciferase reporting assay was used to detect the relative luciferase intensity of FaDu and SCC-4 cells co-transfected with miR-125b-5p mimic and wild-type/mutant HPRT1. $^{**}P < 0.01$, $^{***}P < 0.001$.

3.7. MiR-125b-5p partially suppresses proliferation and invasion of HNSCC cells by targeting HPRT1

Since HPRT1 is a target of miR-125b-5p, the role of HPRT1 in the anti-tumor effects of miR-125b-5p was further explored. The Western blot results confirmed that co-transfected HPTR1 expression plasmids with miR-125b-5p mimic successfully restored the level of HPRT1 and MMP2 in HNSCC cells (Fig. 6A). Consistently, ectopic expression of HPRT1 significantly abolished the inhibitory effects of miR-125b-5p on proliferation and invasion of HNSCC cells, as demonstrated by the results of CCK-8, clone formation, and transwell invasion assays (Fig. 6B–D). Therefore, miR-125b-5p could partially suppress proliferation and invasion of HNSCC cells by targeting HPRT1.

4. Discussion

The increased nucleotide synthesis is necessary to fuel the rapid proliferation of cancer cells [22–24]. As one of the vital enzymes in the salvage nucleotide synthesis pathway, HPRT1 has been upregulated in several cancers [3,7]. In the present study, both HPRT1 mRNA and protein were upregulated in HNSCC cells, which is consistent with previous studies [5]. Besides, it was further proved that HPRT1 upregulation boosted cell proliferation and invasion both *in vitro* and *in vivo*. Mechanistically, our study revealed that miR-125b-5p, which was downregulated in HNSCC tissues and predicts favorable prognosis, directly downregulated HPRT1 and subsequently suppressed the malignant progression of HNSCC.

Historically, HPRT1 is a well-known reporter gene of carcinogenesis, whose mutational frequency serves as an established

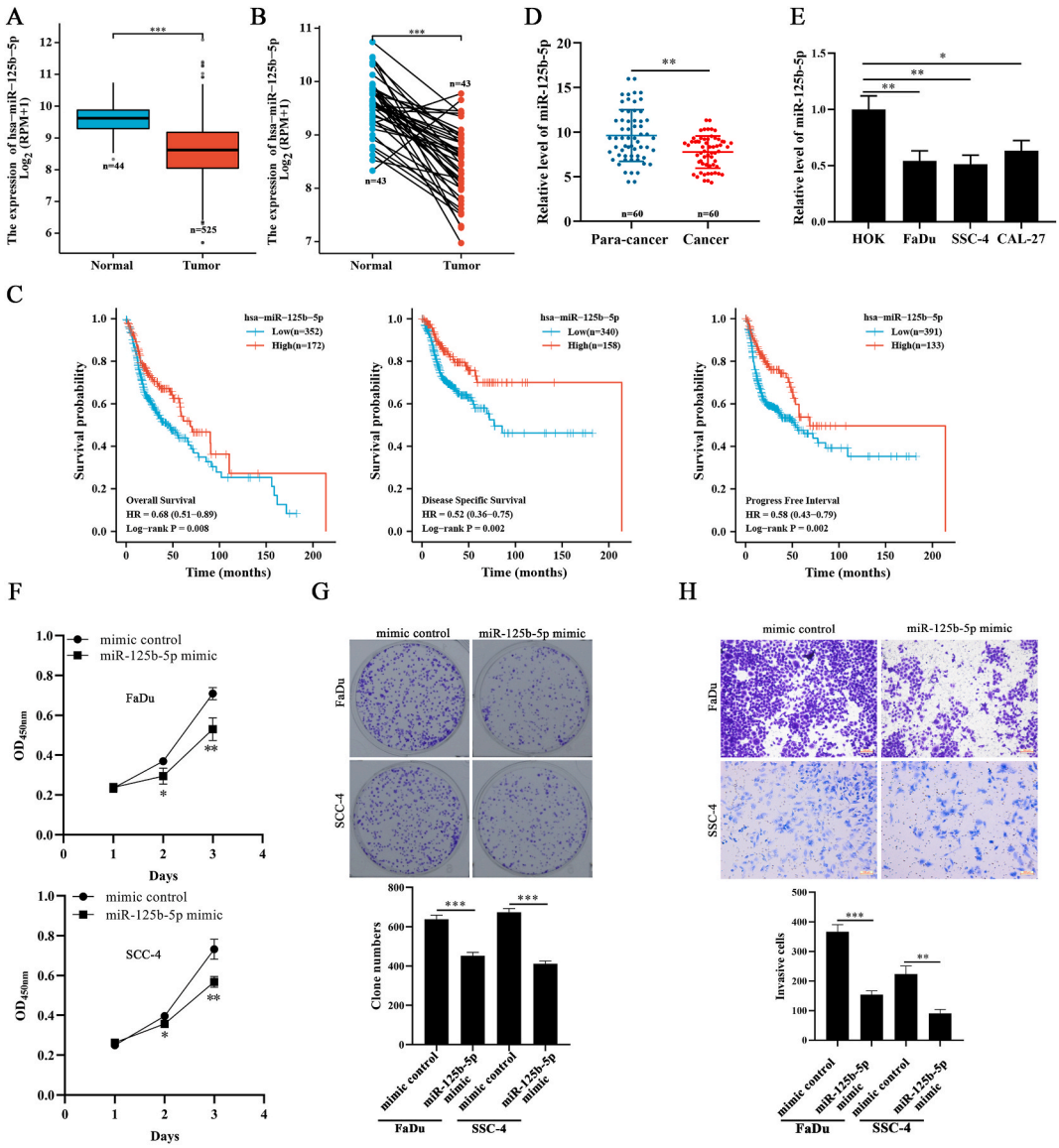

Fig. 5. MiR-125b-5p is downregulated, predicts favorable prognosis, and inhibits cell proliferation and invasion in HNSCC. (**A**) and (**B**) Expression of miR-125b-5p in both unpaired and paired HNSCC and normal tissues were evaluated using TCGA data. (**C**) Prognostic values of miR-125b-5p in HNSCC including OS, DSS, and PFI were evaluated using TCGA data. (**D**) qRT-PCR analysis of the expression of miR-125b-5p in HNSCC and para-cancer tissues. (**E**) qRT-PCR analysis of the expression of miR-125b-5p in HOK and HNSCC cells. (**F**) CCK-8, (**G**) plate clone formation, and (**H**) transwell invasion assays were used to evaluate cell viability, proliferation, and invasion of HNSCC cells transfected with miR-125b-5p mimic. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

biomarker to evaluate cancer risk in patients and determine potential carcinogens [3,10,25]. Recently, accumulating evidence revealed that aberrant upregulation and activation of HPRT1 directly promote malignant progression in cancers, suggesting that HPRT could be a potential promising biomarker for the characterization and treatment of cancer [3,7]. For instance, TCGA data indicated that HPRT1 is generally upregulated and predicts poor prognosis in most cancers, including oral squamous cell carcinoma (OSCC), breast cancer, and HNSCC [5–7]. Functionally and mechanistically, HPRT1 knockdown significantly inhibited proliferation and invasion and impaired chemoresistance of OSCC cells via inactivation of MMP1/PI3K/AKT pathways [6]. Consistently, the current study found that HPRT1 was upregulated, predicted poor outcomes, and promoted cell proliferation and invasion in HNSCC cells, highlighting the notion that HPRT1 is a potential target for HNSCC therapy.

Although the upregulation of HPRT1 and its oncogenic functions have been revealed, the underlying mechanism for HPRT1 upregulation remains elusive. Both transcriptional and post-transcriptional regulators are involved in the modulation of gene expression, which could be distinguished by detecting the level of hnRNA, the primary transcript directly reflecting the transcriptional activity [20,26–28]. Our data demonstrated that HPRT1 hnRNA was comparable between HNSCC cells and normal HOK cells and

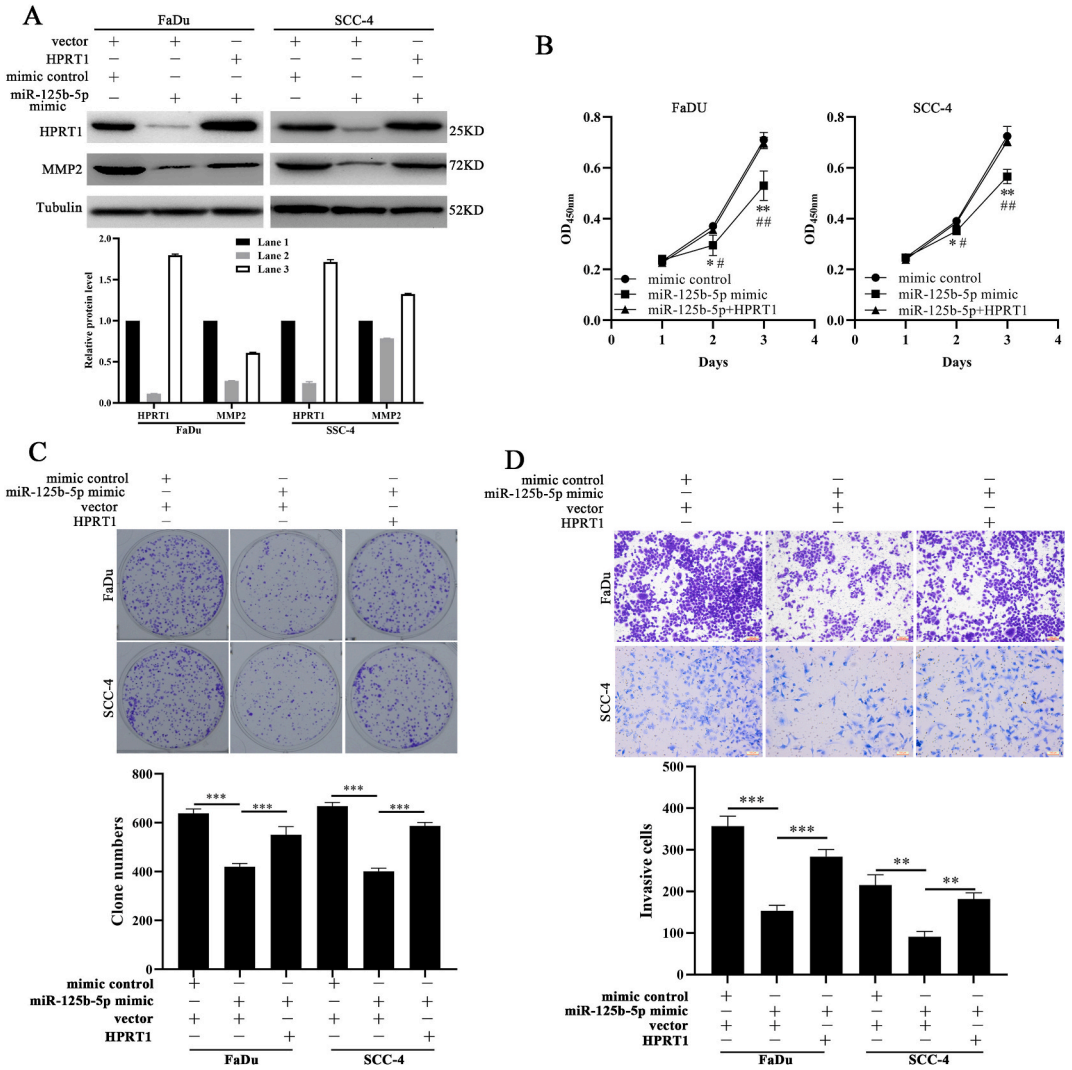

Fig. 6. miR-125b-5p inhibits HNSCC cell proliferation and invasion by targeting HPRT1. (**A**) Western blot analysis of HPRT1 and MMP2 in HNSCC cells co-transfected with HPRT1 and miR-125b-5p. (**B**) CCK-8, (**C**) plate clone formation, and (**D**) transwell invasion assays were used to evaluate cell viability, proliferation, and invasion of HNSCC cells co-transfected with HPRT1 and miR-125b-5p. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. $\#P < 0.05$, $\#\#P < 0.01$.

subsequently validated miR-125b-5p, with downregulation and negative correlation with HPRT1 in HNSCC, could directly target and downregulate HPRT1 expression, originally uncovering that loss of miR-125b-5p, at least partially, causes the upregulation of HPRT1 in HNSCC.

Downregulation of miR-125b-5p is widely observed in multiple cancers, such as bladder, breast, liver, lung, colon, esophageal, and laryngeal cancers, and predicts poor prognosis [29–34]. Accordingly, miR-125b-5p inactivation inhibits apoptosis and ferroptosis, boosts cell proliferation and invasion, and promotes glycolysis and chemoresistance, due to liberation of the inhibitory effects on its downstream targets and activation of oncogenic signaling [31,34–36]. For example, miR-125b-5p downregulation negatively correlated with favorable prognosis and promoted cell proliferation, invasion, and cisplatin-resistance via upregulating HK2, subsequently activating PI3K/AKT signaling and anaerobic glycolysis in the bladder, pancreatic, laryngeal, and colon cancers [29,34,35,37]. Besides, more targets of miR-125b-5p, such as SphK1, BMPR1B, GLUT5, TXNRD1, Bcl-2, *etc*., have mediated the oncogenic roles of miR-125b-5p inactivation in cancers [30–32,38,39]. The downregulation of miR-125b (miR-125b-1) and its anti-tumor functions have also been reported in HNSCC [40]. miR-125b-1 significantly inhibited invasion, anchorage-independent growth, and colony formation of HNSCC cells by targeting and downmodulating TACSTD2 [40]. Correspondingly, the present study confirmed the suppressive effects of miR-125b-5p on the proliferation and invasion of HNSCC cells. Importantly, we revealed that HPRT1 is a novel target of miR-125b-5p, whose anti-tumor functions largely depend on HPRT1 downregulation in HNSCC.

Of course, our current research has certain limitations that need to be addressed and investigated in subsequent studies. For instance, it is imperative to determine the downstream molecular mechanisms by which HPRT1 promotes the progression of HNSCC.

Additionally, it is crucial to ascertain whether the tumorigenic functionality of HPRT1 is dependent on its enzymatic activity. These are all critical aspects that require attention and resolution in our future research endeavors.

5. Conclusion

In summary, the present study validated the upregulation of HPRT1, revealed the pro-tumor roles of HPRT1, and originally elucidated the underlying mechanism of HPRT1 upregulation in HNSCC, which provides the rationale for the assessment of HPRT1 as a clinical target in HNSCC.

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Author contribution statement

Ruohuang Lu: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Zhiqiang Xiao: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Li Yuan: Performed the experiments; Analyzed and interpreted the data.

Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e20174.

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