

Research Paper

PKM2 is the Metabolic Checkpoint for Stemness Features in Head and Neck Cancer

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Abstract

Owing to their self-renewal, differentiation capabilities, and increased chemoresistance, cancer stem cells (CSCs) are associated with poor prognosis and an elevated chance of relapse and metastasis. CSCs survive under therapy-imposed pressure and aggravate tumor malignancy through metabolic reprogramming, leading to treatment failure. The RNA level of pyruvate kinase isoenzyme M2 (PKM2) is increased in head and neck cancer (HNC) tissues and is linked to poor prognosis, whereas PKM2 deletion was found to accelerate tumorigenesis and tumor growth *in vivo*, leading to a paradox between PKM2 expression and tumorigenesis. To date, the role of PKM2 inhibition in HNC progression remains unclear. In the present study, we found that low PKM2 protein expression was significantly associated with later stages and higher grades of cancer in HNC tissues. Following PKM2 silencing, HNC cells exhibited significantly increased ATP levels, decreased lactate production, and upregulated mitochondrial respiratory activity. In addition, PKM2 knockdown promoted clonogenicity, motility, nuclear-activated β -catenin, CSC formation, and *in vivo* tumorigenicity in HNC cells. PKM2 inhibition further augmented stemness and resistance to chemotherapeutic drugs in HNC CSCs. Whole-genome transcriptome analysis of PKM2-knockdown CSCs revealed upregulation of differentially expressed genes (DEGs) in the Wnt signaling pathway. The present study concluded that decreased PKM2 is associated with aggressive clinicopathological features. PKM2 inhibition-promoted stemness may be mediated by increased nuclear β -catenin localization, which potentially contributes to tumorigenesis and disease progression in HNC. Our findings suggest that PKM2 modulates HNC stemness by linking metabolic rewiring with oncogenic signaling, highlighting its potential as a molecular target for adjuvant therapy in patients with HNC.

Keywords: PKM2, cancer metabolism, cancer stemness, Wnt/ β -catenin, head and neck cancer.

Introduction

Head and neck cancer (HNC) is one of the top ten cancer types among cancer-associated diseases with poor prognosis, even after therapeutic intervention [1]. The lack of clinically evident antecedent premalignant lesions often leads to delayed diagnosis. Consequently, most patients suffer from late-stage HNC, which has poor susceptibility to conventional chemoradiotherapy (CRT) and a high risk of subsequent recurrence. Although the application of

epidermal growth factor receptor monoclonal antibody (cetuximab) and immune enhancement therapy (pembrolizumab and nivolumab) has contributed to improved performance [2], the elucidation of the molecular genetic landscape and oncogenic hallmarks is indispensable for mining novel, effective, and least-toxic therapeutic interventions for HNC.

Cancer stem cells (CSCs) self-renew and

differentiate into multiple lineages, thereby aggravating tumor formation, metastasis, CRT resistance, and relapse. CSCs were previously regarded as a group of quiescent cells. However, studies have found that this population is distinct and metabolically hyperactive, and that homeostasis and tropism are intricately reprogrammed [3]. Pyruvate kinase (PK) converts phosphoenolpyruvate to pyruvate and effluxes it for lactate fermentation or the tricarboxylic acid (TCA) cycle. Pyruvate kinase has four isozymes with distinct tissue distributions: PKL, PKR, PKM1, and PKM2. *PKM1* and *PKM2* are alternatively spliced from *PKM*, and include exons 9 and 10, respectively. *PKM2* has two structural isoforms, homotetramers and homodimers. The *PKM2* tetramer harbors higher catalytic activity to fulfill metabolic demands, whereas the *PKM2* dimer serves as a transcriptional co-regulator by nuclear translocation [4].

PKM2 has been reported as a tumor promoter and is involved in many malignant processes, including cancer growth, apoptosis, metastasis, drug resistance, and tumor initiation, by regulating metabolism and signal transduction [4]. Clinically, high *PKM2* levels are correlated with poor prognosis in lung cancer, ovarian cancer, and multiple myeloma [5-7]. In HNC, increased RNA level of *PKM2* is associated with worse outcomes [8]. *PKM2* protein level is positively associated with advanced clinical stage and poorer survival in oral squamous cell carcinoma (OSCC) and head and neck squamous cell carcinoma (HNSCC) [9,10]. From a subcellular perspective, nuclear *PKM2* mediates the EGF-promoted cell proliferation and motility in nasopharyngeal carcinoma [11] and drives epithelial-mesenchymal transition (EMT) through proteasome-mediated TGIF2 repression in OSCC [12]. Under glucose restriction, *PKM2* nuclear translocation sustains pancreatic CSCs and expression of stemness genes [13]. Clinically, elevated nuclear *PKM2* expression strongly predicts poor prognosis in patients with esophageal squamous cell carcinoma [14]. However, the germline loss of *PKM2* promotes hepatocellular carcinoma [15]. Deletion of *PKM2* in a mouse model of breast cancer caused by the absence of *Brca1* hastens tumor growth [16]. This evidence has led to a paradox between *PKM2* expression and tumorigenesis. However, the relationship between *PKM2* inhibition and HNC progression also remains unclear.

Here, we demonstrate that *PKM2* down-regulation was associated with aggressive clinicopathological features and disease progression in HNC. *PKM2* silencing induced nuclear *PKM2*/β-catenin co-localization and metabolic rewiring toward

mitochondrial respiration, suggesting that *PKM2* integrates metabolic and oncogenic signaling to enhance CSC formation, chemoresistance, and *in vivo* tumorigenicity.

Materials and Methods

Immunohistochemistry (IHC)

Tissue microarrays were purchased from US Biomax, Inc. (HN802). The disease features and clinical information of the patients with HNC are presented in Table S1. Briefly, the slides were deparaffinized, followed by antigen retrieval and blocking using a Dual Endogenous Enzyme Block (Dako). Anti-*PKM2* antibody (1:800, #4053, Cell Signaling Technology) was hybridized at 4 °C overnight, and processed using the EnVision Dual Link System-HRP (DAB) Kit (Dako). Following hematoxylin counterstaining, digital scoring was performed using an Aperio ImageScope. The final score was calculated by adding the percentage of stained areas (0, 1, 2, 3, and 4) and intensity (0, 1, 2, and 3) of the stained cells.

Cell culture, cell growth, and clonogenic assay

The SAS cell line was derived from a poorly differentiated human squamous cell carcinoma of the tongue and grown in DMEM supplemented with 10% fetal bovine serum (FBS) (Gibco). All experiments were performed using mycoplasma-free cells. Cells (2500/100 μL) were seeded into a 96-well plate, and the MTS assay (Promega) was used to assess cell growth after 24, 48, and 72 h. Cells (50/3 mL) were seeded in a 6-well plate and cultured for 9 days. Colonies were fixed in 100% methanol, stained with 3% crystal violet, and counted to assess clonogenicity.

PKM2 knockdown

Short-hairpin RNA (shRNA) oligonucleotides against *PKM2* were synthesized (Mission Biotech) and cloned into the pLKO.1-puro plasmid (Academia Sinica, Taiwan). The target sequences [13] were as follows: sense oligonucleotide: 5'-CCGGCCATAATCGTCCTCACCAACTCGAGTTGGTGAGGACGATTATGGTTT-3'; antisense oligonucleotide: 5'-AATTAAAAACCATAATCGTCCTCACCAACTCGAGTTGGTGAGGACGATTATGG-3'. The pCMVΔR8.91, pMD.G, and pLKO.1-puro plasmids were co-transfected into HEK293T cells by TransIT-LT1 reagent (Mirus Bio) to package lentivirus particles as per the instructions from Academia Sinica (Taiwan). HNC cells were transduced with either shLuc or sh*PKM2* lentivirus (multiplicity of infection (MOI) = 1) in the presence of 8 μg/mL of protamine sulfate (Merck Millipore). Clones resistant to puromycin (1 μg/mL, Sigma-Aldrich) were grown for 14 days to

generate a stable population.

Real-time quantitative polymerase chain reaction (RT-qPCR)

Cells ($1 \times 10^6/3$ mL) were placed in a 6 cm dish and incubated overnight. RNA was harvested using TRIzol Reagent (Invitrogen), converted to cDNA using the Maximal First Strand cDNA Synthesis Kit (Thermo Scientific), and analyzed using Fast SYBR Green Master Mix with a StepOnePlus real-time PCR system (Applied Biosystems). The primer sequences are listed in Table S2.

Nuclear/cytoplasm fractionation and western blot

Cells ($4 \times 10^5/3$ mL) were seeded in a 6 cm dish for 48 h. Cell lysates were prepared using RIPA lysis buffer containing a protease inhibitor cocktail (Thermo Scientific). Cell lysates were fractionated into cytoplasmic (Cyto) and nuclear fractions (Nuc) using column-based extraction kits (Invent Biotechnologies), separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and transferred onto polyvinylidene fluoride membranes. Protein expression levels were assayed using primary antibodies against PKM1, PKM2, activated β -catenin, Sox2, Nanog, histone H3 (Cell Signaling Technology), and β -actin (Sigma-Aldrich), and then visualized by horseradish peroxidase-conjugated secondary antibodies (Jackson ImmunoResearch) coupled with a Pierce ECL substrate (Thermo Scientific).

Lactate and ATP production

A total of 1×10^4 cells/200 μ L were placed in a 96-well plate for 24 h and refreshed with serum-free medium (100 μ L) for another 24-h incubation. The lactate levels in the supernatant were determined using an L-Lactate Assay kit (Abcam).

Parental cells ($4 \times 10^5/3$ mL) were seeded in a 6 cm dish for 48 h. Parental or spheroid cells (4×10^4) were collected and resuspended in 200 μ L of distilled water. After three freeze-thaw cycles, ATP production was determined using an ATP Determination Kit (Invitrogen).

Measurement of oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) by Seahorse XF24 Analyzer

Cells ($3 \times 10^4/250$ μ L) were seeded in a XF24 cell culture microplate for 24 h and refreshed with XF Base Medium Minimal DMEM (Agilent) with supplements (for OCR: 10 mM glucose, 2 mM glutamine, 1 mM sodium pyruvate; for ECAR: 2 mM glutamine). Cells were treated by sequential injection of the following compounds: for OCR: 15 μ M oligomycin, 2.5 μ M

carbonyl cyanide-4-(trifluoromethoxy)phenyl hydrazone, and 5 μ M rotenone/antimycin; for ECAR: 100 μ M glucose, 10 μ M oligomycin, and 500 mM 2-deoxy-D-glucose. OCR and ECAR measurements were performed using a Seahorse XF24 Analyzer (Agilent).

Transwell migration and invasion assay

For migration, SAS cells ($3 \times 10^4/100$ μ L serum-free DMEM) were placed in a Transwell insert (8- μ m pore, Corning), and 10% FBS-containing DMEM (800 μ L) was added to the bottom chamber and incubated for 30 h.

For invasion, SAS cells (3×10^4 cells/500 μ L serum-free DMEM) were seeded in a Matrigel Invasion insert (Corning), and the lower chamber was filled with 10% FBS-containing DMEM (750 μ L) for 48 h of incubation. Liu's stain was used to visualize the migrated and invaded cells.

Sphere formation

SAS shLuc and shPKM2 cells (2.5×10^2 cells/100 μ L) were seeded in 96-well ultra-low attachment plates with serum-free DMEM/F12 medium supplemented with 20 ng/mL human epidermal growth factor (EGF), 20 ng/mL human basic fibroblast growth factor (bFGF), and 1% N2 supplement (defined CSC medium; Gibco). The number of spheres was counted after 12 days of incubation. For iCRT-3 (HY-103705; MedChem Express) treatment, the cells were cultured with the indicated concentrations of iCRT-3; 0.1% dimethyl sulfoxide (DMSO) served as the vehicle control. The half-maximal inhibitory concentration (IC_{50}) value was determined using nonlinear regression analysis.

Chemodrug sensitivity assay of PKM2 knockdown in primary CSCs

SAS cells ($1 \times 10^4/2$ mL) were cultured in defined CSC medium for 12 days to form primary spheres (1S) and then separated into single cells using 1X Accumax™. Primary spheroid single cells were knocked down by lentiviral shPKM2 infection (MOI: 20) for 3 h. Luciferase shRNA lentivirus (shLuc) was used as a control. shLuc and shPKM2 1S spheroids were cultured in defined CSC medium for 12 days to form secondary spheres (2S).

The secondary spheroid single cells ($2 \times 10^3/100$ μ L) were seeded with varying concentrations of chemodrugs. Cell viability was determined using the CellTiter-Glo Luminescent Cell Viability Assay (Promega) after 48 h of incubation. Cisplatin (P4394), gemcitabine (G6423), and etoposide (E1383) were purchased from Sigma-Aldrich.

Whole transcriptome analysis

RNA sequencing was performed by Welgene Biotech, Taiwan. In brief, total RNA from pooled biological replicates of shLuc 2S and shPKM2 2S cells was extracted using TRIzol reagent (Thermo Fisher Scientific). RNA was qualified by using a Bioanalyzer 2100 (Agilent Technologies, USA) with an RNA 6000 LabChip kit (Agilent Technologies, USA). A SureSelect XT HS2 mRNA Library Preparation Kit (Agilent Technologies) was used for library construction, and AMPure XP beads (Beckman Coulter) were used for size selection. Sequencing was performed using Illumina sequencing-by-synthesis (SBS) technology, generating 150-bp paired-end reads. Sequencing data (FASTQ reads) were processed using Welgene Biotech's in-house pipeline based on Illumina's base-calling program bcl2fastq v2.20. Transcript assembly and abundance estimation were performed using the StringTie software (v2.1.4). Differential expression analysis was performed using DESeq2 (v1.39.0) with genome bias detection/correction using Welgene Biotech's in-house pipeline. *P*-values were calculated by DESeq with non-grouped samples used in blind mode. Differentially expressed genes (DEGs) were defined as transcripts per million (TPM) > 0.5 and an absolute fold change ($|FC|$) > 2. For pathway analysis, gene set enrichment analysis (GSEA, software version 4.4.2) was performed in the pre-ranked mode. The gene list was ranked using the following formula: $\text{sign}(\log_2(FC)) \times \log(p\text{-value})$. The molecular signatures databases (MSigDB) H (H gene sets), C5 (Gene Ontology: BP gene sets), and C2 (curated gene sets, Kyoto Encyclopedia of Genes and Genomes (KEGG medicus)) collections were used. Gene sets with a false discovery rate (FDR) < 0.25 were considered significantly enriched.

Dual-luciferase reporter assay

SAS tumorspheres were dissociated into single cells after 12 days of culture. Cells (4×10^5) were seeded in 6-well plates in sphere medium and co-transfected with the TCF/LEF-driven firefly luciferase construct (Fluc) and a constitutively expressing *Renilla* luciferase construct (Rluc; CCS-018L, QIAGEN). After 48 h of incubation, firefly and *Renilla* luciferase activities were measured using the Twinlite™ Glow Reporter Gene Assay System (Revvity). The Wnt/ β -catenin transcriptional activity is expressed as the Fluc/Rluc ratio and normalized to the shLuc control group.

Subcutaneous xenograft tumor model

NOD/SCID mice (NOD.Cg-*Prkdc*^{scid}/JNarl; 6-week-old, male) were obtained from BioLASCO and raised at the National Yang Ming Chiao Tung University Animal Center according to Institutional

Animal Care and Use Committee regulations. The SAS parental cells were resuspended in serum-free DMEM, and 1×10^4 or 1×10^5 cells in a total volume of 100 μ L were subcutaneously injected into the dorsal flanks of three mice. Tumors were measured using calipers at the indicated time points and photographed after excision.

Statistical analysis

All statistical analyses were conducted using the SPSS software (IBM Corp., Armonk, NY, USA). All experiments were performed with at least three independent biological replicates, and representative data or images are shown. The quantitative data are presented as the mean $\pm$ standard deviation (SD). The statistical significance of the data from the cell-based assays was examined using a two-tailed Student's *t*-test. The two-tailed Pearson's chi-square test was conducted to identify the association between PKM2 protein expression and clinicopathological characteristics. The Mann-Whitney U test was performed to examine the variance in PKM2 protein expression between different stages and grades of cancer.

Results

Low PKM2 expression is associated with advanced stages and higher grades of cancer in HNC tissues

The HN802 tissue microarray was selected for its comprehensive representation of HNC cases across a diverse spectrum of clinical stages (I-IV) and pathological grades (1-3), which is essential for robust statistical evaluation of the association between PKM2 expression and disease progression. We performed IHC staining of HNC tissue microarrays to explore the clinical relevance of PKM2 protein expression (**Fig. 1A**). The tissues were scored from 2 to 7 as follows: 2 ($n = 1$), 3 ($n = 11$), 4 ($n = 32$), 5 ($n = 11$), 6 ($n = 8$), and 7 ($n = 7$). The median expression level is the most frequently used criterion for expression-based patient stratification. Therefore, we separated the cohort into a low expression group (scores ≤ 4 , $n = 44$) and a high expression group (scores ≥ 5 , $n = 26$) (**Table 1**). Although PKM2 expression levels were not associated with sex, age, T stage, node involvement (N), or metastasis (M), a higher percentage of PKM2 low levels was significantly associated with advanced stages (31/41, 75.6%) and high grades (10/10, 100%) in patients with HNC (**Table 1**). PKM2 levels were significantly higher in grade 1 than in grade 3 in all HNC and in the squamous cell carcinoma cohort (**Fig. 1B, 1C**). These results revealed that PKM2 expression negatively correlated with HNC progression.

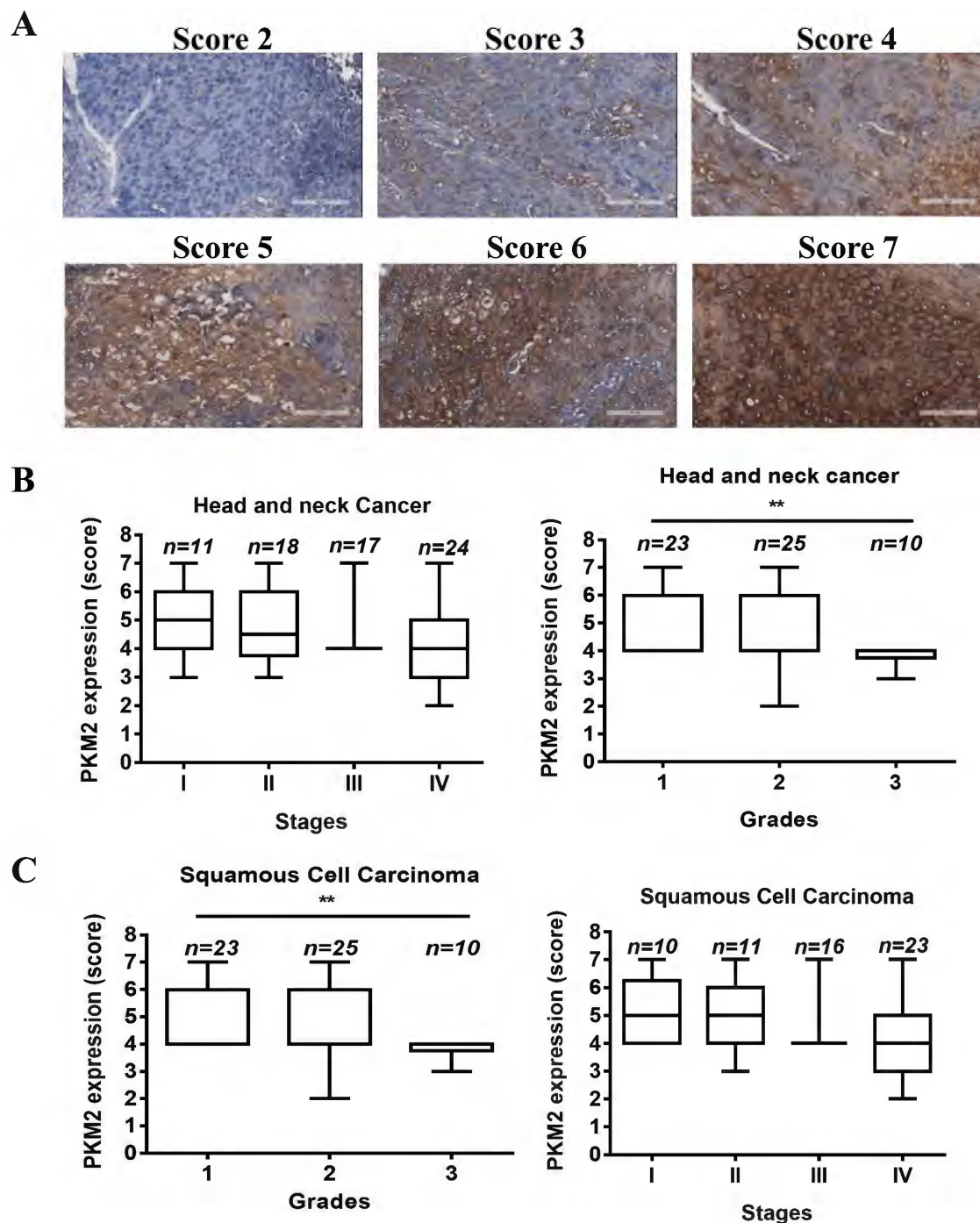


Figure 1. Lower PKM2 expression is associated with advanced HNC tumor grades. (A) Representative immunohistochemical images of HNC tissues with different PKM2 expression scores. Scale bar, 100 μ m. **(B, C)** Association of PKM2 expression levels with clinical stages and pathological grades in the overall HNC cohort **(B)** and the squamous cell carcinoma sub-cohort **(C)**. The Mann–Whitney U test was performed to examine the variance in PKM2 protein expression between different stages and grades of cancer. ** $p < 0.01$

Increased cytoplasmic PKM1, nuclear PKM2, and nuclear activated β -catenin in PKM2-silenced HNC cells

The SAS cell line, a well-characterized human papilloma virus-negative cell line harboring a *TP53* nonsense mutation (p.E336Ter), is derived from a poorly differentiated human OSCC of the tongue. The oral cavity is the most prevalent and lethal subtype of

HNC [17]. This specific virologic and genomic profile makes it the most clinically challenging and therapy-resistant subtype of HNC [18], thereby providing a highly relevant model to investigate the factors driving disease progression. To investigate the role of PKM2 in HNC progression, an shRNA lentivirus system was used to knockdown *PKM2* (shPKM2) in SAS cells. PKM2 expression was decreased by approximately 50% at both the RNA **(Fig. 2A)** and

protein levels (**Fig. 2B**) in shPKM2 SAS cells. Because PKM2 can translocate into the cell nucleus, we fractionated the cell lysates into cytoplasmic and nuclear fractions and performed western blotting to determine the cellular localization of the proteins. PKM2 was found in both the cytoplasm and the nucleus, whereas PKM1 was found only in the cytoplasm (**Fig. 2C**). Furthermore, PKM2 silencing upregulated cytoplasmic PKM1, nuclear PKM2, and nuclear activated β -catenin in SAS cells.

Decreased lactate, increased ATP production, and upregulated mitochondrial respiratory activity in PKM2-silenced HNC cells

We analyzed the effects of PKM2 knockdown on cellular metabolism. Increased ATP levels and

decreased lactate production were observed in the PKM2-silenced SAS cells (**Fig. 3A**). Through real-time live-cell assessment using a Seahorse XF24 analyzer, we measured the OCR, which represents mitochondrial oxidative phosphorylation, and the ECAR, which measures glycolysis. PKM2 silencing induced a significant increase in OCR profiles, such as basal respiration, ATP production, maximal respiration, and respiratory capacity in SAS cells (**Fig. 3B**). However, basal glycolysis in the ECAR profiles remained unaffected, and glycolytic capacity and reserves were slightly increased in shPKM2 SAS cells (**Fig. 3C**). Moreover, PKM2 knockdown increased the mRNA expression of LDHA, PGK1, PDH, HK2, PDK, GAPDH, and PKM1 in SAS cells (**Fig. 3D**). Therefore, PKM2 inhibition promotes mitochondrial oxidative phosphorylation in SAS cells.

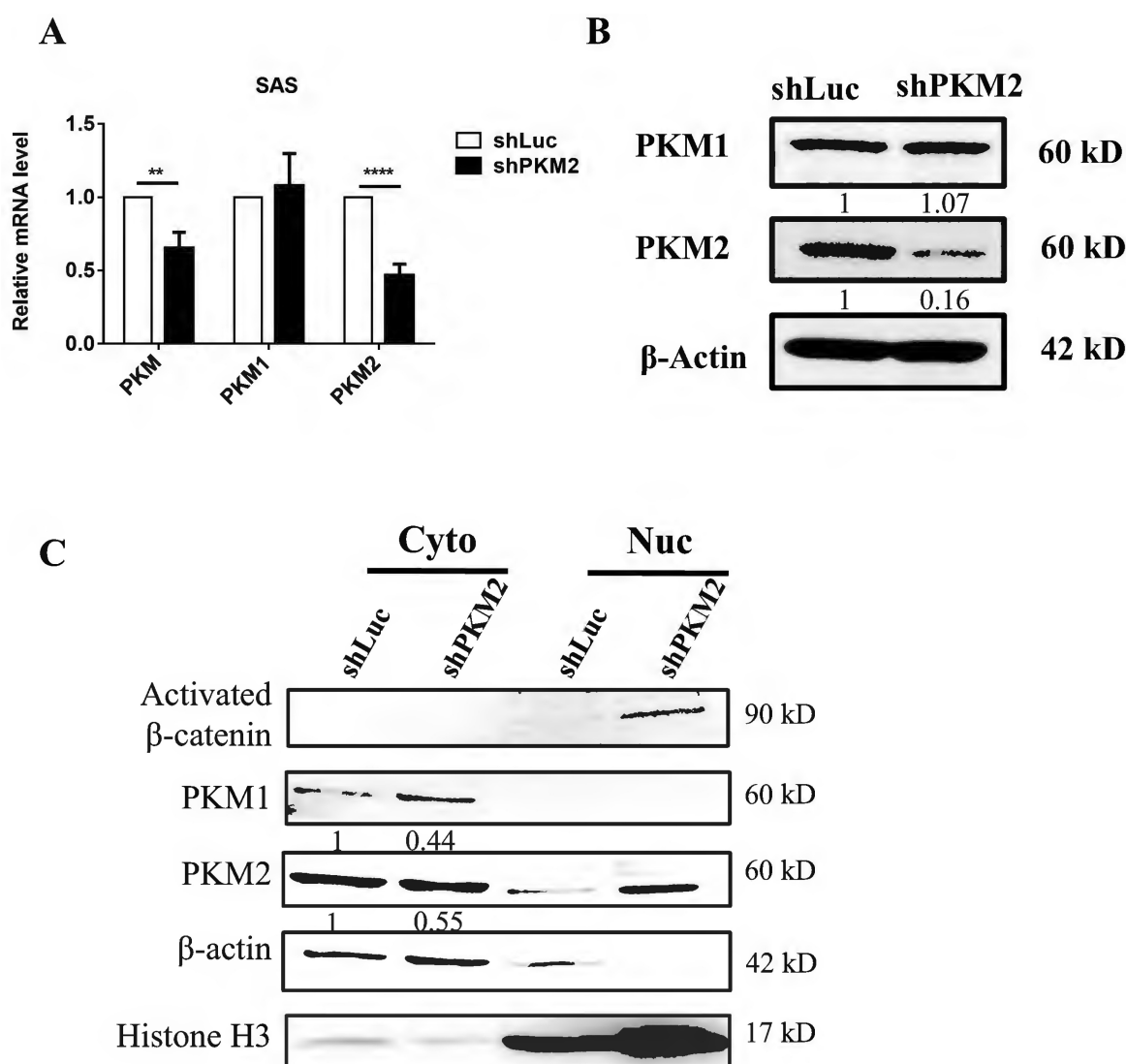


Figure 2. Nuclear PKM2 and activated β -catenin levels are increased in PKM2 knockdown cells. (A) Relative mRNA expression levels of PKM, PKM1, and PKM2 in SAS shLuc and shPKM2 parental cells determined by RT-qPCR. (B, C) Western blot analysis of the indicated proteins in whole-cell lysates (B) and subcellular fractions (Cyto: cytoplasmic; Nuc: nuclear) (C) of SAS shLuc and shPKM2 parental cells. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$.

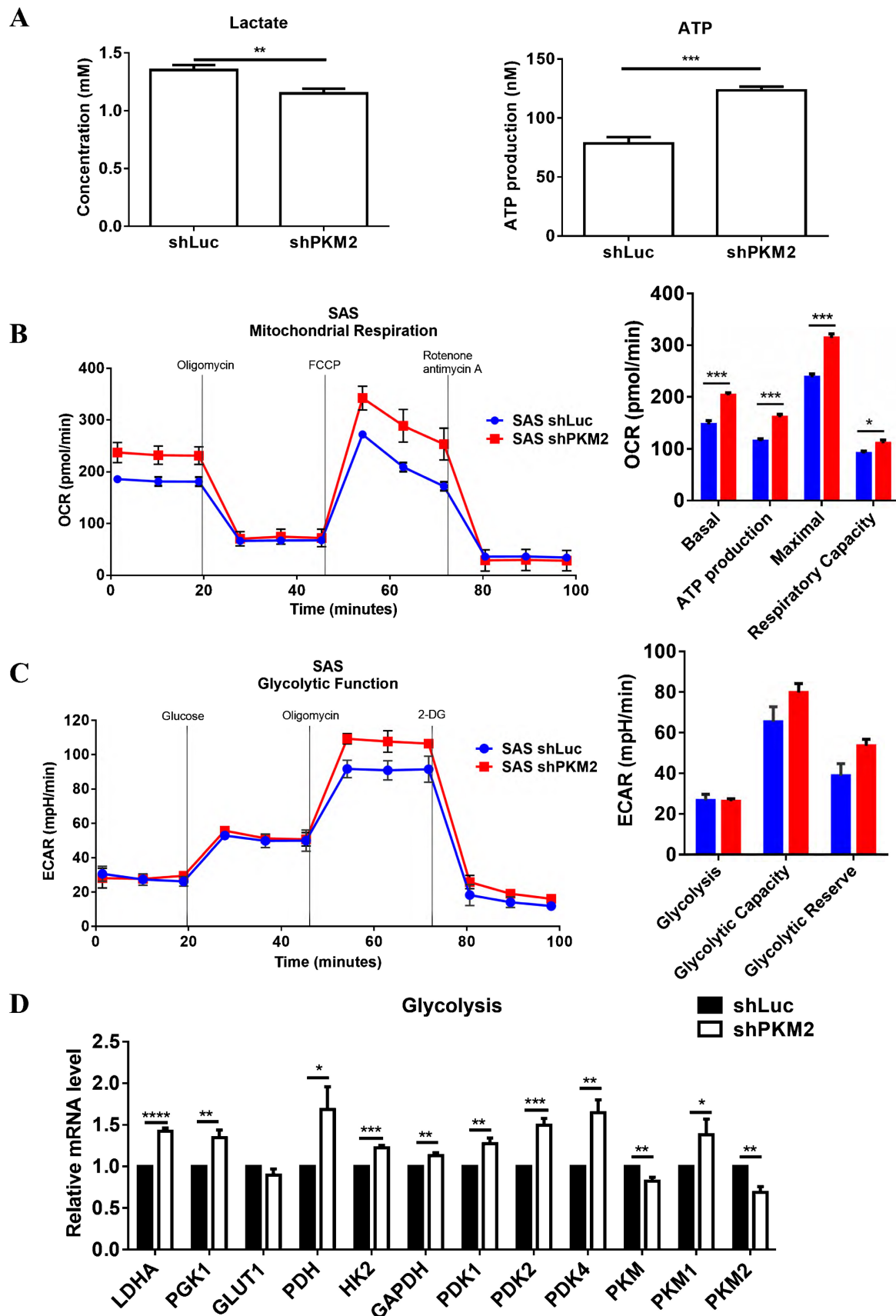


Figure 3. PKM2 knockdown increases mitochondrial respiratory capacity. (A) Relative extracellular lactate production and intracellular ATP levels in SAS shLuc and shPKM2 parental cells. (B, C) Real-time oxygen consumption rate (OCR) (B) and extracellular acidification rate (ECAR) (C) measured by Seahorse XF analysis. (D) Relative mRNA expression of metabolism-related genes in SAS shLuc and shPKM2 parental cells determined by RT-qPCR. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

PKM2 knockdown promoted clonogenicity, migration, and invasion ability in SAS cells

We investigated the effect of *PKM2* knockdown on cell growth and motility. *PKM2* silencing did not alter the 3-day cell proliferation (**Fig. 4A**). However, *PKM2* knockdown significantly promoted the ability of a single cell to grow into a colony (clonogenicity) after a 9-day culture (**Fig. 4B**). In addition, *PKM2* silencing significantly increased the Transwell migration (**Fig. 4C**) and invasion activity (**Fig. 4D**) of SAS cells. We constructed a subcutaneous xenograft model to investigate the effects of *PKM2* inhibition on tumorigenesis *in vivo*. We found that sh*PKM2* cells showed increased tumor incidence after 21 days and increased tumor volume at the end of the experiment compared to shLuc cells (**Fig. 4E, 4F**).

PKM2 inhibition augments the sphere-forming capability, stemness, and chemoresistance in CSCs

CSCs have self-renewal and differentiation capabilities and increased chemotherapy resistance. Therefore, we investigated the effects of *PKM2* inhibition on the properties of CSCs. *PKM2* silencing significantly augmented sphere formation (**Fig. 5A**). The stem cell transcription factors Sox2 and Nanog increased in sh*PKM2* sphere cells after 12-day CSC culture (**Fig. 5B**). The mRNA levels of stem cell markers (*OCT4*, *CD44*, and *CD133*) and glycolytic genes (*LDHA*, *PGK1*, *PDH*, *PDK*, *GAPDH*, and *PKM1*) were upregulated in *PKM2*-silenced spheres (**Fig. 5C**). Furthermore, decreased lactate production and increased ATP production were observed in sh*PKM2* spheroid cells (**Fig. 5D**). Thus, *PKM2* inhibition increases the sphere-forming capability and stemness of HNC cells.

PKM2 was knocked down in CSC-enriched SAS 1S to investigate its role in maintaining the stemness of HNC CSCs. SAS 1S cells were infected with shLuc or sh*PKM2* lentiviruses and cultured to form secondary spheres (2S). After verifying the inhibition of *PKM2* at the gene and protein levels, we observed increased Sox2 and Nanog protein levels (**Fig. 6A**, left panel) in sh*PKM2* 2S cells. Additionally, the mRNA levels of *PKM1*, stemness genes (*OCT4*, *SOX2*, *NANOG*, *CD44*, *CXCR4*, and *CXCR7*), and multiple drug resistance genes (*ABCC* and *ABCB*) were upregulated (**Fig. 6A**, right panel). Moreover, the suppression of *PKM2* in SAS 1S cells promoted sphere formation, with larger size and greater numbers (**Fig. 6B**) and chemotherapeutic resistance (cisplatin, gemcitabine, etoposide; **Fig. 6C**) in CSC-enriched SAS cells. Thus,

PKM2 downregulation enhances the self-renewal capacity of SAS CSCs.

DEGs of *PKM2* knockdown in HNC CSCs showed upregulated Wnt signaling

To investigate the molecular alterations underlying *PKM2* silencing in CSCs, a whole-genome transcriptome analysis was performed using sh*PKM2* and shLuc 2S cells. Analysis of DEGs using a cutoff change of over 2-fold identified 819 genes, of which 475 were upregulated and 344 were downregulated (**Fig. 7A**). DEGs included protein-coding RNA, microRNAs, and long non-coding RNA. GSEA was performed for GO terms in metabolic pathways (**Fig. 7B**). *GSEA recommends an FDR threshold of 0.25* in the setting of exploratory discovery. These results revealed a set of enriched processes related to glycolysis, oxidative phosphorylation, and fatty acid metabolism. In addition, stemness-related pathways examined by GO and KEGG enrichment analysis indicated that the TGF- β , Wnt, JNK, and ERK signaling pathways were upregulated (normalized enrichment score, NES >1) in sh*PKM2* CSC 2S cells (**Fig. 7C**). The upregulated Wnt signaling pathway was found in both the GO and KEGG enrichment analyses (**Fig. 7C**); and the core enrichment genes in the Wnt signaling pathway are listed (**Fig. 7D**). The Wnt family (*WNT16*, *WNT3A*, *WNT10A*, *WNT9A*), frizzled receptors (*FZD4*, *FZD10*), and *RSPO3* (activator of the Wnt/ β -catenin signaling) were increased prominently. The upregulation of *WNT9A* and *RSPO2* in sh*PKM2* CSCs was confirmed using RT-qPCR (**Fig. 7E**). A firefly/*Renilla* dual-luciferase reporter assay with TCF/LEF responsive elements confirmed the enhanced transcriptional activity of Wnt/ β -catenin signaling in *PKM2*-silenced CSCs (**Fig. 7F**). To examine the dependency on β -catenin activity in *PKM2* silencing-promoted cancer stemness, we performed sphere formation assay with iCRT-3, an inhibitor of Wnt/ β -catenin-responsive transcription. Five μ M iCRT-3 attenuated the sphere number that was increased by *PKM2* silencing. Ten μ M iCRT-3 exhibited a higher inhibitory effect on the sphere-forming capacity of *PKM2*-silenced cells compared with shLuc control cells. The decreased IC₅₀ value to iCRT-3 treatment indicated that sh*PKM2* tumorspheres were more susceptible to Wnt/ β -catenin signaling inhibition (**Fig. 7G**). Increased nuclear β -catenin was observed in sh*PKM2* parental cells (**Fig. 2C**). Taken together, the findings indicate that increased Wnt signaling activators and nuclear β -catenin may potentially mediate the sh*PKM2*-promoted HNC cell stemness.

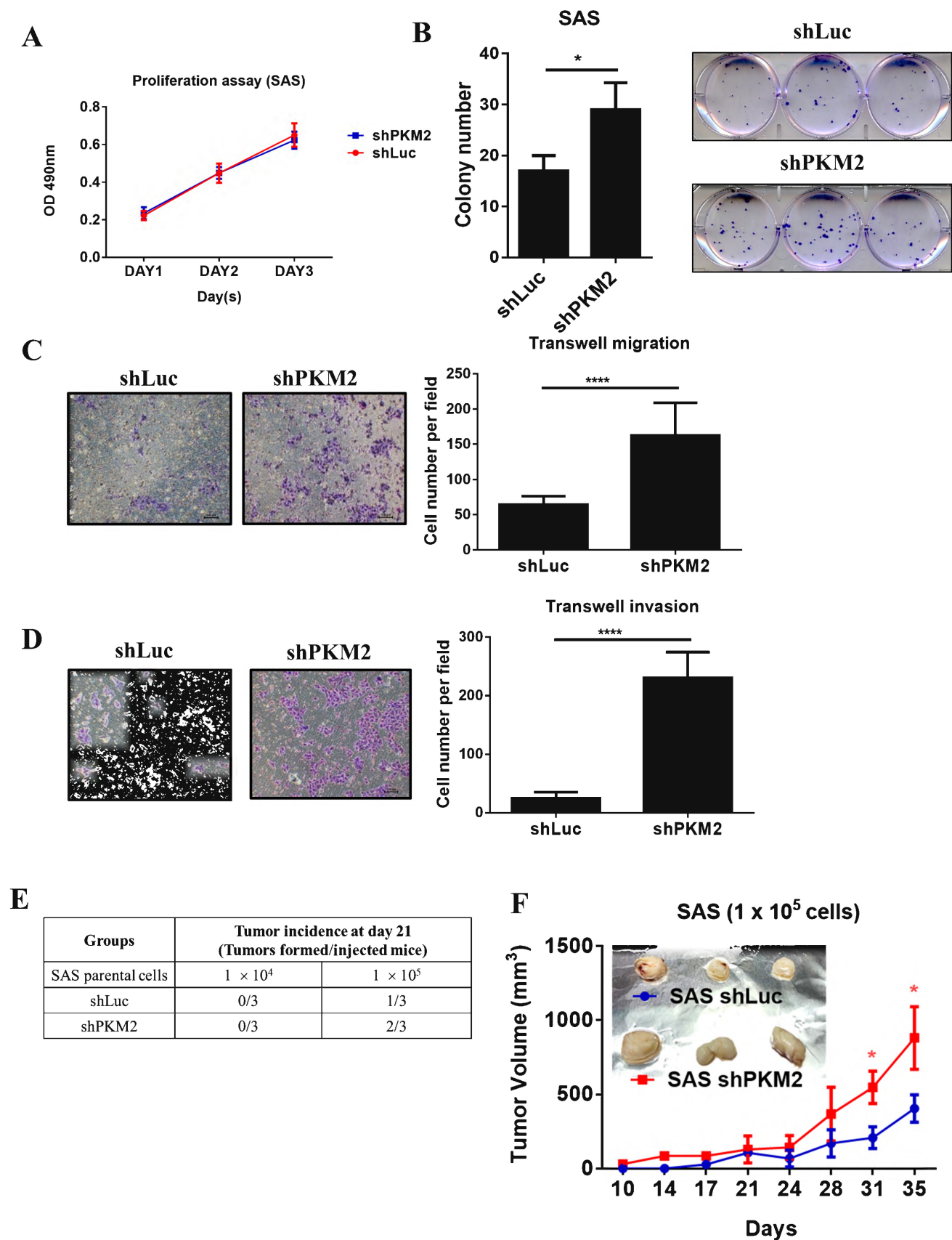


Figure 4. PKM2 knockdown increases long-term growth and motility of cells. (A) Proliferation of SAS parental cells assessed by MTS assay. (B) Clonogenicity of SAS shLuc and shPKM2 cells evaluated by colony formation assay. (C, D) SAS parental cell migration (C) and invasion (D) capabilities determined by Transwell assays. Scale bars, 100 μm . * $p < 0.05$, **** $p < 0.0001$.

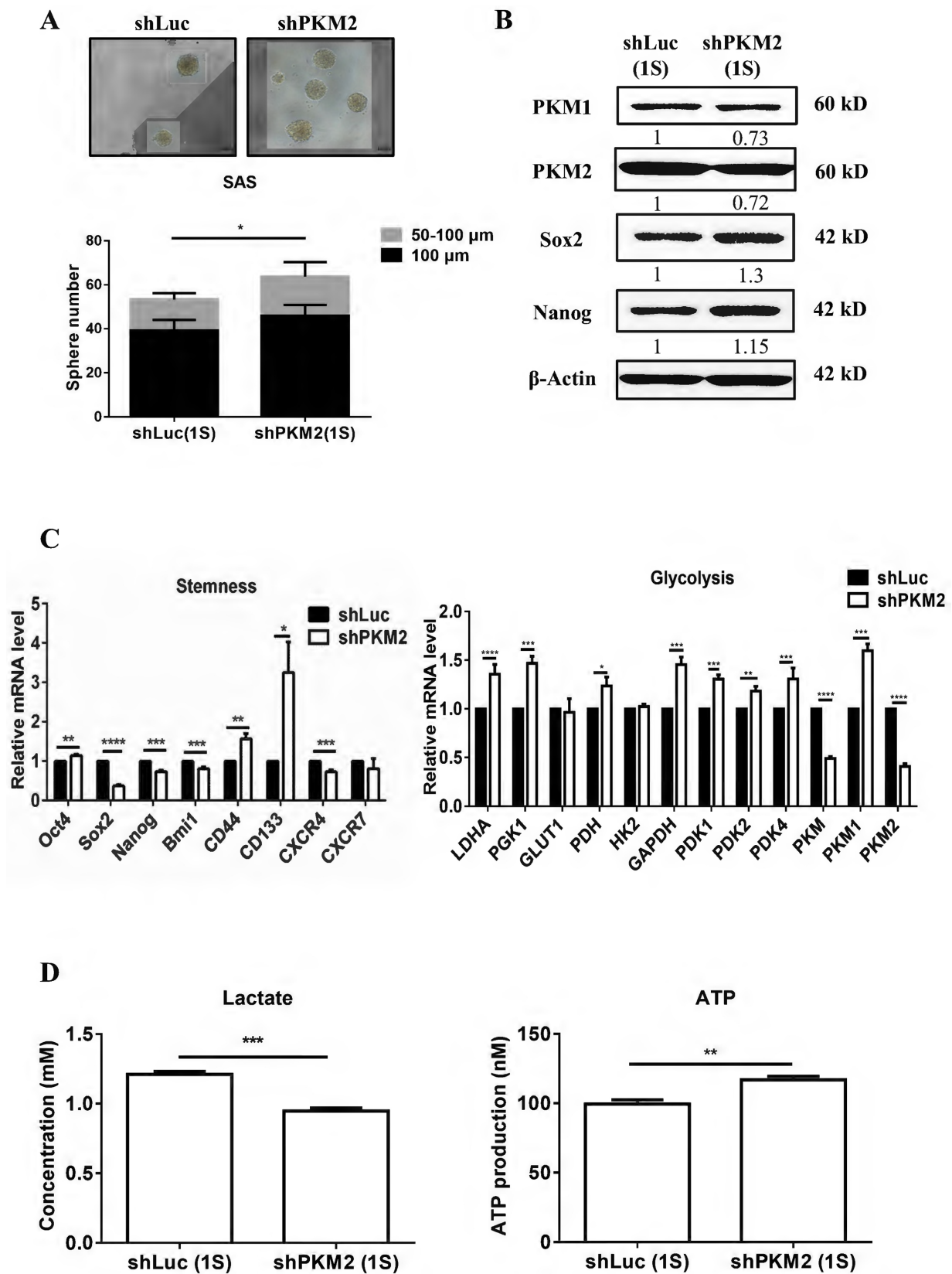


Figure 5. PKM2 knockdown promotes tumorsphere formation and cancer stemness properties. (A) Representative images and quantification of primary tumorspheres (IS) formed by SAS shLuc and shPKM2 cells. Scale bar, 100 μm . (B) Western blot analysis and corresponding quantification of stemness-related markers, PKM1, and PKM2 in IS cells. (C) Relative mRNA expression of stemness and metabolism genes in SAS IS cells. (D) Relative extracellular lactate production and intracellular ATP levels in IS cells. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Discussion

CSCs have different metabolic features and plasticity based on their oncogenic background, tissue origin, and tumor microenvironment. The metabolic plasticity of CSCs promotes self-renewal, CRT resistance, immune evasion, metastasis, and recurrence [3]. Co-targeting of CSCs with metabolic inhibitors and systemic therapeutic drugs is more effective in overcoming CRT resistance and prolonging survival in patients with cancer [3]. Thus, exploring the molecular mechanisms underlying the metabolic plasticity of CSCs may reveal novel metabolic targets for combination therapies in CSC-driven tumor advancement.

The allosteric switch between the cytoplasmic tetramer and nuclear dimer of PKM2 is crucial for cancer cells to protect against environmental stress by coordinating metabolic and signaling activities. The low glycolytic activity of dimeric PKM2 shifts metabolic tropism from the TCA cycle to lactate production and upregulates nucleotide biosynthesis via the pentose phosphate pathway to promote tumor growth. In addition, it functions as a transcriptional coactivator in the nucleus to regulate cancer cell growth, metastasis, stemness, and drug resistance [4]. After *PKM2* silencing, we observed metabolic rewiring with decreased lactate and increased ATP production in *PKM2*-silenced SAS parental cells (**Fig. 3A**) and CSCs (**Fig. 5D**) led by decreased cytoplasmic distribution (**Fig. 2C**). Moreover, *PKM2* knockdown upregulated fatty acid metabolism in CSCs (**Fig. 7B**), compensating for the glycolytic deficit and promoting CSC proliferation.

PKM2 silencing and inhibition of *PKM2* nuclear translocation attenuates EGF-enhanced nasopharyngeal carcinoma cell motility [11]. *PKM2* inhibition significantly reduces lung cancer growth in mice [19]. In addition, si*PKM2* suppresses the proliferation and migration of OSCC and HNSCC cells [20,21]. However, Tanaka *et al.* demonstrated that si*PKM2* only inhibited OSCC cell motility under EMT induction by TGF- β 1 [12]. In the present study, we found that *PKM2* knockdown did not affect 3-day cell proliferation, but promoted the clonogenicity and motility of SAS cells (**Fig. 4**). Therefore, the experimental approach and conditions may have affected the effects of *PKM2* inhibition *in vitro*. Oncogenic background, tissue origin, and context-specific metabolic tropism may determine the function of *PKM2*.

PKM2-promoted cancer stemness through the Wnt/ β -catenin pathway was first found in breast tumors [22]. Yang *et al.* demonstrated that nuclear *PKM2* is critical for β -catenin-driven transcriptional

activity and cancer progression by forming a physical interaction complex [23]. Glucose restriction-induced nuclear co-translocation of *PKM2*/AMPK promotes the binding of *PKM2* to Oct4, transactivates stemness-related genes, and induces *in vivo* metastasis [13]. Endogenous p-Tyr105 *PKM2* levels correlate with CD44, a stemness marker, in HNC cells [24]. ErbB2-mediated p-Tyr105 induces *PKM2* dimerization and nuclear translocation, and promotes cancer stem-like cells by increasing YAP nuclear translocation [25]. Shikonin, a *PKM2* phosphorylation inhibitor, exhibits antitumor efficacy by impeding the stemness population during combination treatment [20,21,26,27]. Suzuki *et al.* found that *PKM2* targeted by shRNA or an inhibitor (2825-0900) downregulated cytoplasmic *PKM2* but upregulated nuclear *PKM2* in lung cancer cells [19]. Our study demonstrated the increased nuclear localization of *PKM2* and β -catenin after *PKM2* silencing (**Fig. 2C**), which may contribute to the Wnt/ β -catenin signaling activation and be associated with the enhanced cancer stemness (**Fig. 7E-G**).

The *PKM2* inhibitor (TT-232) showed promise in a phase II clinical trial for metastatic kidney cancer [28]. The nuclear translocation of *PKM2* induced by TT-232 was sufficient to induce cell death [29]. Paradoxically, *PKM2*-knockout mice develop spontaneous hepatocellular carcinoma and show accelerated onset of spontaneous breast cancer tumorigenesis [15,16]. Germline loss of the *PKM2* can induce spontaneous hepatocellular carcinoma and lead to a unique metabolite profile, accompanied by multiple carcinogenic driving events, abnormal fatty acid metabolism, liver inflammation, and increased hepatocyte proliferation [15]. *PKM2* deletion results in *PKM1* compensation and sustained glucose metabolism in *PKM2*-null spontaneous breast tumors [16]. Systemic *PKM2* knockout increases plasma cholesterol levels and promotes allograft breast tumor growth [30]. We confirmed that *PKM2* silencing promoted HNC stemness in *PKM2*-knockout SAS cells, which recapitulated the decreased lactate secretion, increased ATP production, enhanced tumorsphere formation, and upregulated the expression of stemness markers in SAS CSCs (data not shown). Our findings suggested that the loss of *PKM2*'s canonical glycolytic function may serve as a metabolic trigger driving stemness features, mirroring *in vivo* studies where *PKM2* deficiency accelerated spontaneous tumorigenesis with altered glucose and lipid homeostasis in cancers [15,16,30]. Our shRNA model further suggested that an additional layer of the *PKM2* inhibition-exacerbated phenotype was associated with increased nuclear translocation of the residual *PKM2* pool (**Fig. 2C**). Therefore, broad *in vitro*

and *in vivo* models should be further investigated to consider the generalizability of PKM2 inhibition-promoted HNC stemness properties and the long-

term clinical use of PKM2 inhibitors, which may induce CSC formation, CRT resistance, and cancer relapse.

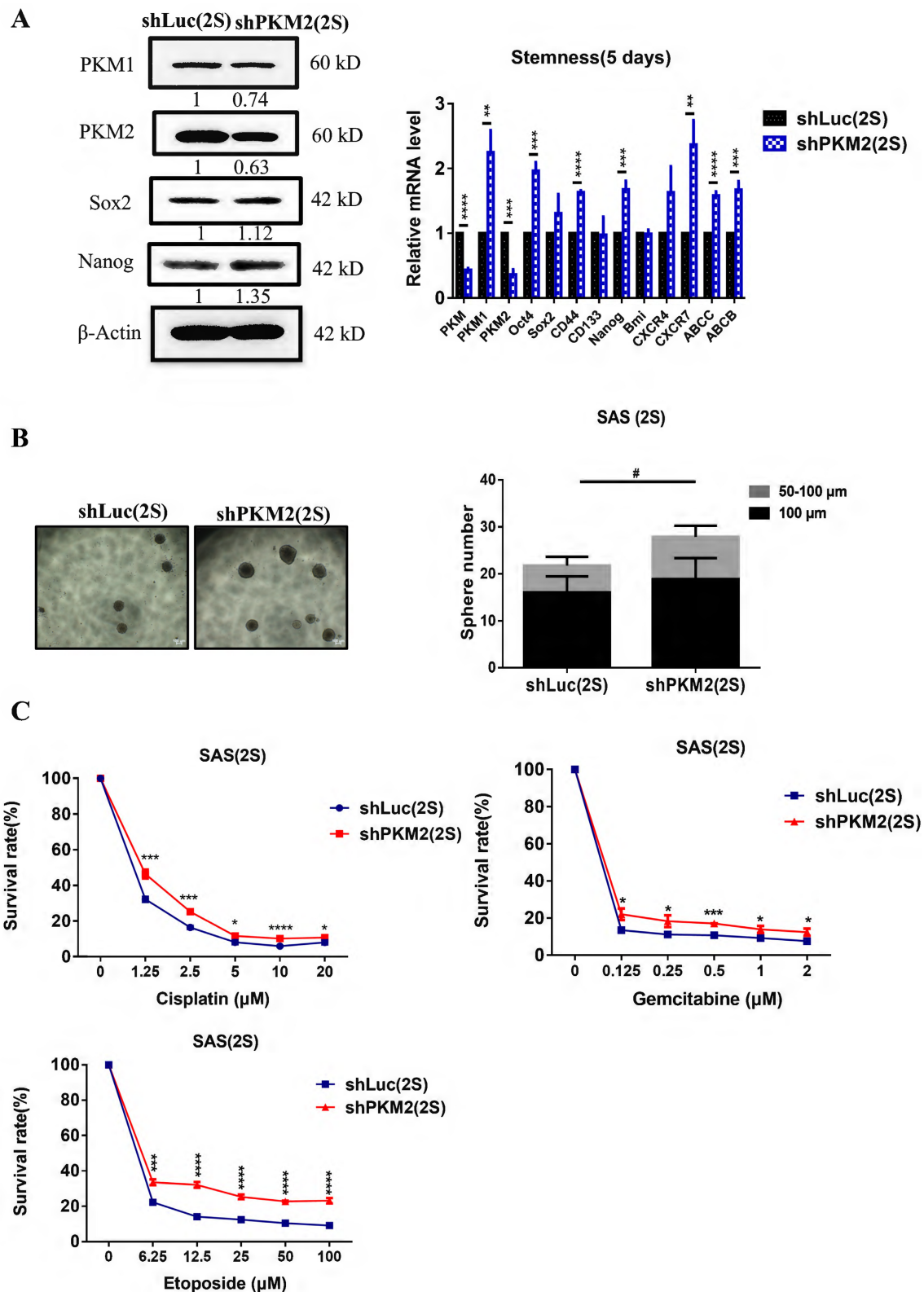


Figure 6. PKM2 knockdown in cancer stem cells increases motility and chemodrug resistance. (A) Protein (left panel) and mRNA (right panel) expression levels of stemness markers in SAS secondary spheres (2S). **(B)** Representative images and quantification of 2S formation. Scale bar, 100 μ m. **(C)** Cell viability of 2S cells following 48-h treatment with the indicated concentrations of chemodrugs. * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001.

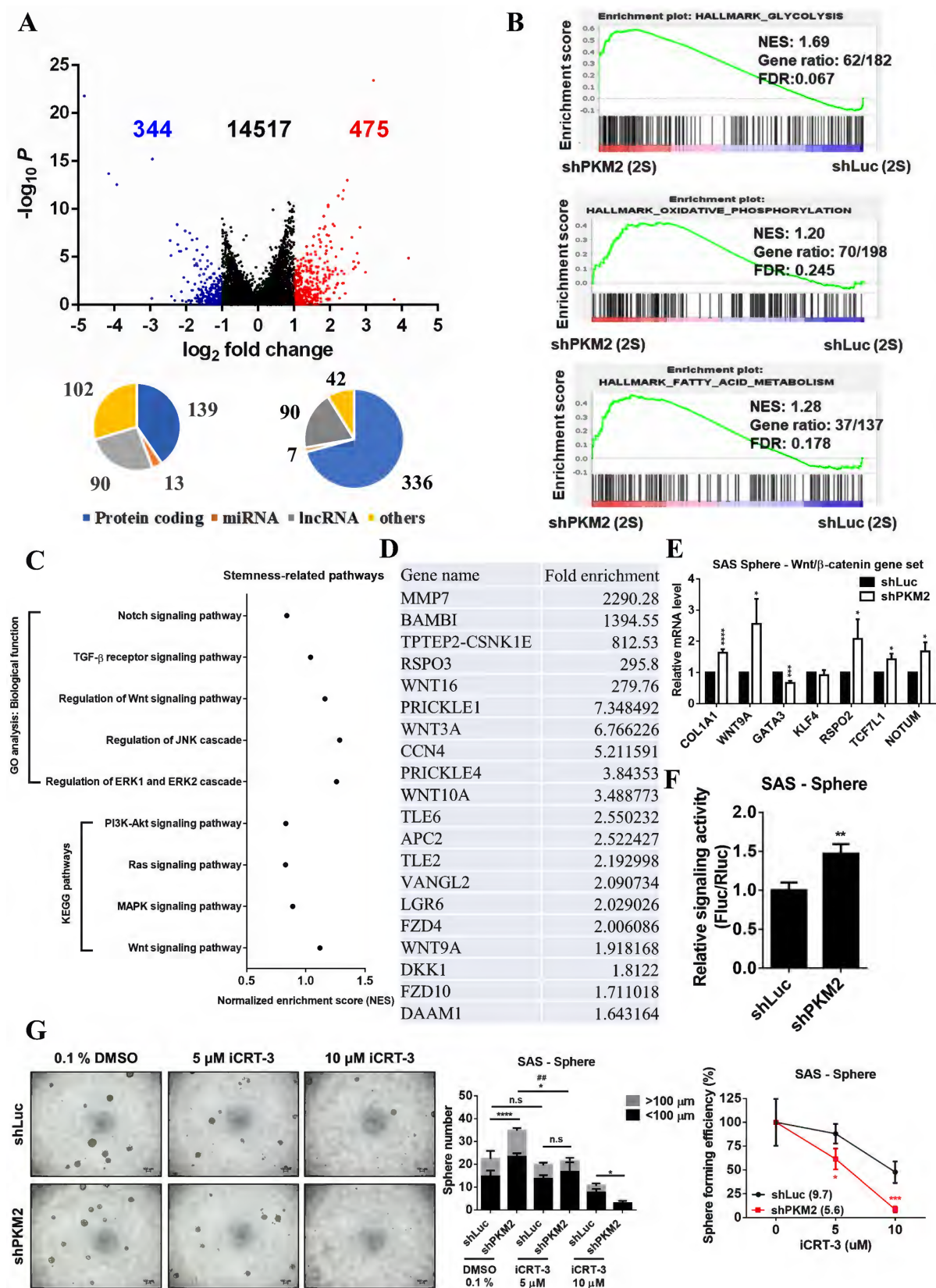


Figure 7. Differentially expressed genes (DEGs) determined by RNA sequencing of PKM2 knockdown in cancer stem cells. (A) Volcano plot of DEGs in shPKM2 versus shLuc 2S cells. Significant DEGs (TPM > 0.5 and [fold change] > 2) are highlighted in red (upregulated) and blue (downregulated). **(B, C)** Normalized enrichment scores (NES) of metabolism-related **(B)** and stemness-related **(C)** pathways identified by Gene Set Enrichment Analysis (GSEA). **(D)** List of core enrichment genes within the Wnt signaling pathway (hsa04310). **(E)** mRNA expression levels of Wnt/β-catenin in SAS IS. **(F)** Wnt/β-catenin signaling activity in SAS IS was determined by TCF/LEF reporter assay. **(G)** Representative images and quantification of primary tumorspheres (IS) formed by SAS shLuc and shPKM2 cells with the indicated concentrations of iCRT-3 or 0.1% DMSO (vehicle control). Scale bar, 100 μm. *: comparison of smaller tumorspheres; #: comparison of larger tumorspheres. Sphere-forming efficiency is expressed as a percentage relative to the corresponding vehicle group.

Table 1. Association of PKM2 expression with clinical features of patients with HNC.

	No. of patients with HNC	PKM2 ^a		p-value
		Low	High	
Sex				
F	14	7	7	0.266
M	56	37	19	
Stage				
I+II	29	13	16	0.009
III+IV	41	31	10	
Age (years)				
< 55	40	26	14	0.668
≥ 55	30	18	12	
T				
T1+T2	37	20	17	0.107
T3+T4	33	24	9	
N				
N0	51	19	32	0.253
N1+N2	19	7	12	
M				
M0	67	41	26	0.264
M1	2	2	0	
Grade				
1+2	48	26	22	0.007
3	10	10	0	

PKM2^a: Low score =2–4, High score =5–7. T, primary tumor size; N, lymph node metastasis; M, distant metastasis. HNC, head and neck cancer. Two-tailed Pearson's chi-square test was conducted to identify the association between PKM2 protein expression and clinicopathological characteristics.

Studies have linked high *PKM2* mRNA expression to poor prognosis in various malignancies [5,6,8]. Increased *PKM2* protein expression is associated with aggressive clinicopathological features and unfavorable prognosis in OSCC and HNSCC [9,10]. In the present study, quantification of total *PKM2* protein by IHC staining showed that low *PKM2* expression was significantly associated with advanced stages and higher grades of HNC (**Fig. 1 and Table 1**). Biologically, high *PKM2* expression translates into an abundant protein pool that simultaneously supports the formation of tetramers (cytosolic: glycolysis) and dimers (nuclear: oncogenic transcription), cooperatively driving tumor progression. Post-transcriptional regulation may contribute to the discrepancy between high mRNA and low protein levels (e.g., miR-139-5p) [31]. Furthermore, the function of *PKM2* depends on its subcellular localization [4], which cannot be distinguished using transcriptomic analysis.

Notably, our *in vitro* data suggest that the discrepancy in reduced total *PKM2* protein expression drives a poorly differentiated status by forcing the remaining *PKM2* pool to undergo nuclear translocation (**Fig. 2B, 2C**). Accumulated evidence consistently indicates that elevated nuclear *PKM2* is driven by specific post-translational modifications, including Y105 phosphorylation [25], S37 phosphorylation [32], K433 acetylation [33], and K62 deacetylation [34]. These post-translational

modifications serve as promoters of EMT, advanced tumor stage, and poor prognosis across various malignancies [10,12,14]. Furthermore, *PKM2*-inhibitor non-responding tumors demonstrated low cytoplasmic staining and a more than 38% increase in nuclear *PKM2* staining [19]. Notably, Apostolidi *et al.* found that the expression of S37-phosphorylated nucleus-localized *PKM2*, but not total *PKM2*, was correlated with decreased overall survival in patients with triple-negative breast cancer [32]. This growing evidence indicates that quantification of the nuclear/cytosol ratio, which remains limited in the present study, and the post-translational modification status of *PKM2* may be more decisive factors in evaluating clinical outcomes and patient stratification than bulk mRNA or total protein expression. In addition, context-dependent roles and tumor heterogeneity should be carefully considered when interpreting the clinical relevance of *PKM2*.

In this study, we demonstrated that *PKM2* silencing mediates metabolic reprogramming, promotes tumorigenesis *in vivo*, and contributes to CSC properties in HNC cells, which may be attributed to the increased *PKM2* nuclear localization and Wnt/ β -catenin activation. We suggest that nuclear *PKM2* is a potential theranostic target for combined treatment of HNC. Inhibitors that block *PKM2* post-translational modifications and nuclear translocation may improve therapeutic efficacy. Notably, the potential risk of long-term *PKM2* inhibition leading to CSC expansion, therapeutic resistance, and tumor recurrence should be carefully considered (**Fig. 8**).

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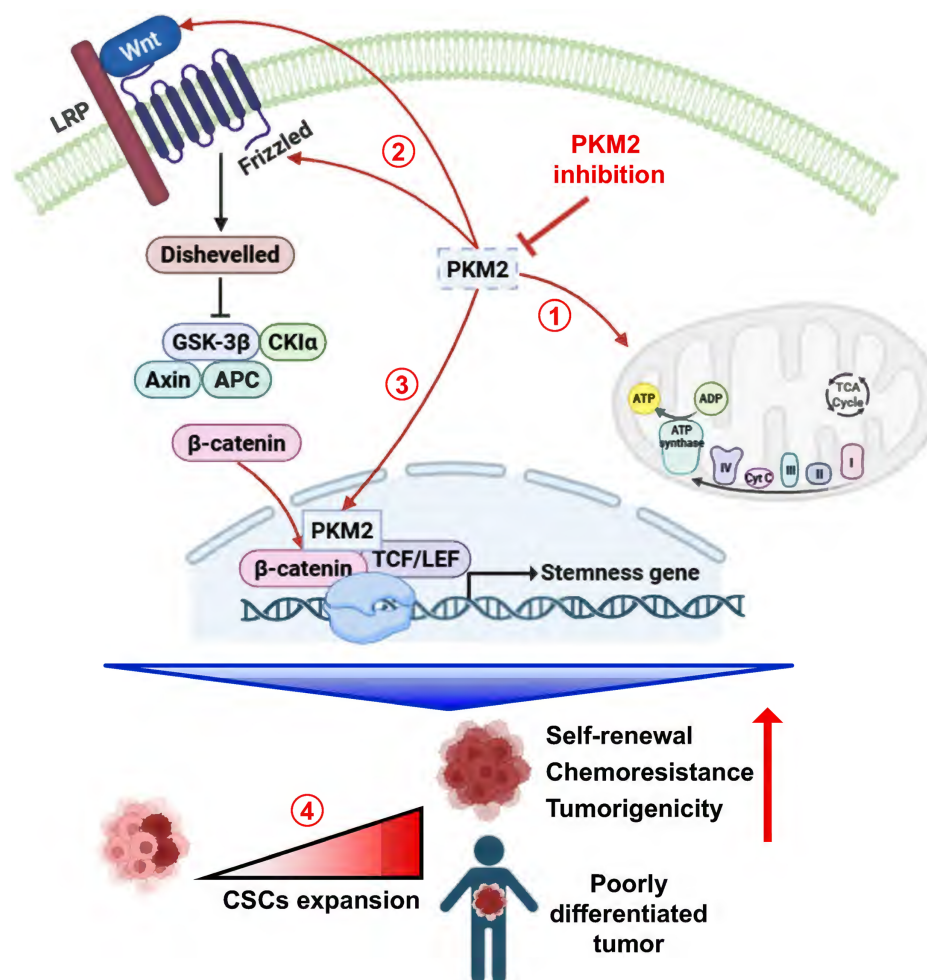


Figure 8. Graphical abstract. PKM2 inhibition enhances mitochondrial respiratory activity (1) and upregulates Wnt-FZD expression, thereby driving β -catenin nuclear translocation (2). The increased nuclear PKM2 translocation may form a complex with β -catenin and promote signaling transactivation (3), which consequently augments CSC formation, self-renewal capacity, chemoresistance, tumorigenicity, and initiates poorly differentiated tumors in patients with head and neck cancer (4).

Data availability statement

Data is available on request from the authors.

Authors' contributions

W-H Sun: writing - original draft, conceptualization, funding acquisition, formal analysis, and revision of the manuscript; K-H Sun: conceptualization, funding acquisition, supervision, formal analysis, and writing - review & editing; Ta-Jung Peng, Hsueh-Jou Fang, Chia-Ling Chang, and Yu-Wen Tang: data acquisition, validation, formal analysis, and visualization. The authors declare that all data were generated in-house and that no paper mill was used.

Ethics approval and consent to participate

A waiver for the use of human tissues was granted by the IRB of Taipei City Hospital. The animal study was approved by the Institutional Animal Care and Use Committee (IACUC; 1120310) of the National Yang Ming Chiao Tung University.

Patient consent for publication

US Biomax provided certified documents in which all human tissues were collected with informed consent from the donors.

Supplementary Material

Supplementary tables.

<https://www.jcancer.org/v17p1504s1.pdf>

Competing Interests

The authors have declared that no competing interest exists.

References

- [1] Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. *CA Cancer J Clin.* 2023; 73(1): 17-48.
- [2] Johnson DE, Burtneis B, Leemans CR, Lui VWY, Bauman JE, Grandis JR. Head and neck squamous cell carcinoma. *Nat Rev Dis Primers.* 2020; 6(1): 92.
- [3] Kaur J, Bhattacharyya S. Cancer Stem Cells: Metabolic Characterization for Targeted Cancer Therapy. *Front Oncol.* 2021; 11: 756888.
- [4] He S, Liang Y, Tan Y, Liu Q, Liu T, Lu X, Zheng S. Positioning determines function: Wandering PKM2 performs different roles in tumor cells. *Cell Biol Int.* 2024; 48(1): 20-30.

- [5] Papadaki C, Sfakianaki M, Lagoudaki E, et al. PKM2 as a biomarker for chemosensitivity to front-line platinum-based chemotherapy in patients with metastatic non-small-cell lung cancer. *Br J Cancer*. 2014; 111(9): 1757-1764.
- [6] Panchabhai S, Schlam I, Sebastian S, Fonseca R. PKM2 and other key regulators of Warburg effect positively correlate with CD147 (EMMPRIN) gene expression and predict survival in multiple myeloma. *Leukemia*. 2017; 31(4): 991-994.
- [7] Chao TK, Huang TS, Liao YP, et al. Pyruvate kinase M2 is a poor prognostic marker of and a therapeutic target in ovarian cancer. *PLoS One*. 2017; 12(7): e0182166.
- [8] Jing C, Qu X, Li Z, et al. EGFRwt/vIII-PKM2- β -catenin cascade affects proliferation and chemo-sensitivity in head and neck squamous cell carcinoma. *Am J Cancer Res*. 2017; 7(12): 2491-2502.
- [9] Wang Y, Zhang X, Zhang Y, Zhu Y, Yuan C, Qi B, Zhang W, Wang D, Ding X, Wu H, Cheng J. Overexpression of pyruvate kinase M2 associates with aggressive clinicopathological features and unfavorable prognosis in oral squamous cell carcinoma. *Cancer Biol Ther*. 2015; 16(6): 839-845.
- [10] Chang H, Xu Q, Li J, Li M, Zhang Z, Ma H, Yang X. Lactate secreted by PKM2 upregulation promotes Galectin-9-mediated immunosuppression via inhibiting NF- κ B pathway in HNSCC. *Cell Death Dis*. 2021; 12(8): 725.
- [11] Chen S, Youhong T, Tan Y, et al. EGFR-PKM2 signaling promotes the metastatic potential of nasopharyngeal carcinoma through induction of FOSL1 and ANTXR2. *Carcinogenesis*. 2020; 41(6): 723-733.
- [12] Tanaka F, Yoshimoto S, Okamura K, Ikebe T, Hashimoto S. Nuclear PKM2 promotes the progression of oral squamous cell carcinoma by inducing EMT and post-translationally repressing TGF β 2. *Oncotarget*. 2018; 9(73): 33745-33761.
- [13] Yang YC, Chien MH, Liu HY, et al. Nuclear translocation of PKM2/AMPK complex sustains cancer stem cell populations under glucose restriction stress. *Cancer Lett*. 2018; 421: 28-40.
- [14] Zhang X, He C, He C, et al. Nuclear PKM2 expression predicts poor prognosis in patients with esophageal squamous cell carcinoma. *Pathol Res Pract*. 2013; 209: 510-515.
- [15] Dayton TL, Gocheva V, Miller KM, et al. Germline loss of PKM2 promotes metabolic distress and hepatocellular carcinoma. *Genes Dev*. 2016; 30(9): 1020-1033.
- [16] Israelsen WJ, Dayton TL, Davidson SM, et al. PKM2 isoform-specific deletion reveals a differential requirement for pyruvate kinase in tumor cells. *Cell*. 2013; 155(2): 397-409.
- [17] Sun H, Yu M, An Z, et al. Global burden of head and neck cancer: epidemiological transitions, inequities, and projections to 2050. *Front Oncol*. 2025; 15: 1665019.
- [18] de Bakker T, Journe F, Descamps G, et al. Restoring p53 function in head and neck squamous cell carcinoma to improve treatments. *Front Oncol*. 2022; 11: 799993.
- [19] Suzuki A, Puri S, Leland P, Puri A, Moudgil T, Fox BA, Puri RK, Joshi BH. Subcellular compartmentalization of PKM2 identifies anti-PKM2 therapy response in vitro and in vivo mouse model of human non-small-cell lung cancer. *PLoS One*. 2019; 14(5): e0217131.
- [20] Werner M, Lyu C, Stadlbauer B, et al. The role of Shikonin in improving 5-aminolevulinic acid-based photodynamic therapy and chemotherapy on glioblastoma stem cells. *Photodiagnosis Photodyn Ther*. 2022; 39: 102987.
- [21] Lyu C, Stadlbauer B, Wang L, Buchner A, Pohla H. Identification of a novel combination treatment strategy in clear cell renal cell carcinoma stem cells with shikonin and ipilimumab. *Front Immunol*. 2023; 14: 1186388.
- [22] Zhao Z, Song Z, Liao Z, et al. PKM2 promotes stemness of breast cancer cell by through Wnt/ β -catenin pathway. *Tumour Biol*. 2016; 37(3): 4223-4234.
- [23] Yang W, Xia Y, Ji H, et al. Nuclear PKM2 regulates β -catenin transactivation upon EGFR activation. *Nature*. 2011; 480(7375): 118-122.
- [24] Boschert V, Teusch J, Müller-Richter UDA, Brands RC, Hartmann S. PKM2 Modulation in Head and Neck Squamous Cell Carcinoma. *Int J Mol Sci*. 2022; 23(2): 775.
- [25] Zhou Z, Li M, Zhang L, et al. Oncogenic kinase-induced PKM2 tyrosine 105 phosphorylation converts nononcogenic PKM2 to a tumor promoter and induces cancer stem-like cells. *Cancer Res*. 2018; 78(9): 2248-2261.
- [26] Zhao X, Zhu Y, Hu J, et al. Shikonin Inhibits Tumor Growth in Mice by Suppressing Pyruvate Kinase M2-mediated Aerobic Glycolysis. *Sci Rep*. 2018; 8: 14517.
- [27] Wang L, Stadlbauer B, Lyu C, Buchner A, Pohla H. Shikonin enhances the antitumor effect of cabazitaxel in prostate cancer stem cells and reverses cabazitaxel resistance by inhibiting ABCG2 and ALDH3A1. *Am J Cancer Res*. 2020; 10(11): 3784-3800.
- [28] Wang Q, Liu J, Chen Z, et al. Targeting metabolic reprogramming in hepatocellular carcinoma to overcome therapeutic resistance: A comprehensive review. *Biomed Pharmacother*. 2024; 170: 116021.
- [29] Steták A, Veress R, Ovádi J, Csérmely P, Kéri G, Ullrich A. Nuclear translocation of the tumor marker pyruvate kinase M2 induces programmed cell death. *Cancer Res*. 2007; 67(4): 1602-1608.
- [30] Liu F, Ma M, Gao A, et al. PKM2-TMEM33 axis regulates lipid homeostasis in cancer cells by controlling SCAP stability. *EMBO J*. 2021; 40(22): e108065.
- [31] Chen J, Yu Y, Chen X, et al. MiR-139-5p is associated with poor prognosis and regulates glycolysis by repressing PKM2 in gallbladder carcinoma. *Cell Prolif*. 2018; 51(6): e12510.
- [32] Apostolidi M, Vathiotis IA, Muthusamy V, et al. Targeting pyruvate kinase M2 phosphorylation reverses aggressive cancer phenotypes. *Cancer Res*. 2021; 81(16): 4346-4359.
- [33] Lv L, Xu YP, Zhao D, et al. Mitogenic and oncogenic stimulation of K433 acetylation promotes PKM2 protein kinase activity and nuclear localization. *Mol Cell*. 2013; 52(3): 340-352.
- [34] Zhang R, Shen M, Wu C, et al. HDAC8-dependent deacetylation of PKM2 directs nuclear localization and glycolysis to promote proliferation in hepatocellular carcinoma. *Cell Death Dis*. 2020; 11(12): 1036.