

hnRNPC promotes oral squamous cell carcinoma progression via N6-methyladenosine-dependent stabilization of BCAT1 to enhance branched-chain amino acid metabolism

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Abstract. Oral squamous cell carcinoma (OSCC) remains a major cause of cancer-associated mortality worldwide, with current treatments limited by chemoresistance and long-term toxicities. N6-methyladenosine (m⁶A) modification regulates mRNA fate to drive cancer progression. However, how m⁶A modification regulates OSCC progression remains to be elucidated. The present study integrated bioinformatics analysis of The Cancer Genome Atlas and Genotype-Tissue Expression datasets and experimental validation to explore the role of heterogeneous nuclear ribonucleoprotein C (hnRNPC), an m⁶A-associated RNA-binding protein, in OSCC. Bioinformatics analysis of 515 patients with head and neck squamous cell carcinoma (HNSCC) suggested that hnRNPC may have prognostic relevance in HNSCC. High hnRNPC expression associated with advanced pathological stages, poor overall survival and served as a potential risk factor for HNSCC. Functional experiments demonstrated that hnRNPC knockdown in OSCC cell lines inhibited cell proliferation, colony formation, tumor sphere formation and *in vivo* tumor growth, while downregulating stemness-associated genes (Oct4, Sox2 and Nanog). Mechanistically, hnRNPC promoted branched-chain amino acid (BCAA) metabolism in OSCC. High hnRNPC expression was associated with activated BCAA metabolic pathways and hnRNPC knockdown reduced BCAA uptake, glutamate levels, oxygen consumption rate and glutathione levels. Furthermore, hnRNPC stabilized BCAA transaminase 1 (BCAT1) mRNA in an m⁶A-dependent manner. BCAT1 inhibition via ERG240 enhanced the tumor-suppressive effects of hnRNPC knockdown. Exogenous

supplementation of α -ketoglutarate rescued energy deficiency and functional defects in hnRNPC-knockdown OSCC cells. Collectively, the present study findings revealed a novel mechanism driving OSCC progression. In this mechanism, hnRNPC regulates BCAA metabolism by stabilizing BCAT1 mRNA in an m⁶A-dependent manner. The present study supports the prognostic relevance of hnRNPC and may offer a promising therapeutic direction in improving OSCC treatment outcomes by disrupting tumor-specific metabolic and epigenetic adaptations.

Introduction

Oral squamous cell carcinoma (OSCC) is a heterogeneous malignant neoplasm originating from the epithelial tissue of the oral mucosa, which remains one of the leading causes of cancer-associated mortality worldwide (1). The 5-year cumulative overall survival rate for surgically treated patients with OSCC ranges from 47 to 71% across different published studies (2,3). OSCC accounts for 90% of all malignant tumors in the oral cavity and serves as the most common subtype of head and neck squamous cell carcinoma (HNSCC) globally (2,3). Clinically, drug resistance, markedly reduces the efficacy of traditional chemotherapeutics such as cisplatin, fluorouracil and paclitaxel (4,5). Furthermore, while high-dose radiotherapy is often combined with drug therapy, it causes acute toxicities (such as soft-tissue necrosis and xerostomia) that develop into long-term sequelae, further compromising treatment tolerance and the quality of life of patients (6). Thus, current OSCC drug-based treatments are far from optimal, warranting further effective and tailored solutions in the future.

Branched-chain amino acids (BCAAs; including leucine, isoleucine and valine) are essential amino acids that require catabolism. Branched-chain amino acid transaminase (BCAT) is the rate-limiting enzyme driving BCAA transamination, a reaction that transfers the amino group of BCAAs (leucine, isoleucine and valine) to α -ketoglutarate (α -KG), generating branched-chain α -ketoacids (BCKAs) and glutamate (7). BCKAs enter the tricarboxylic acid (TCA) cycle to produce ATP for tumor energy needs, while glutamate acts as a key precursor for glutathione (GSH) synthesis: BCKAs

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promote GSH production (supporting the first rate-limiting step via glutamate-cysteine ligase), scavenge reactive oxygen species (ROS) to protect tumors from oxidative stress and contribute to chemoresistance (8-10). This BCAT-mediated metabolism (energy supply via BCKAs and antioxidant defense via glutamate-GSH) is a tumor-specific adaptation, making BCAT1 a potential therapeutic target for tumor therapy (11). A recent study further validated the clinical and functional importance of BCAT1 in OSCC, demonstrating its involvement in malignant progression and metabolic reprogramming (12).

N⁶-methyladenosine (m⁶A) modification is the most abundant epigenetic modification in eukaryotic mRNA, which is co-regulated by methyltransferase complexes ('writers') that catalyze m⁶A deposition, demethylases ('erasers') that remove the methylation mark, and m⁶A-binding proteins ('readers') that recognize m⁶A and mediate downstream effects such as mRNA splicing, translation or stability (13,14). By influencing mRNA splicing, transport, translation and degradation, m⁶A serves a key role in physiological processes such as cell proliferation and differentiation (15). Increasing evidence has also implicated multiple m⁶A regulatory factors in OSCC tumorigenesis, prognosis and therapeutic response (16,17). Heterogeneous nuclear ribonucleoprotein C (hnRNP) is a classic RNA-binding protein that mainly recognizes uracil-rich sequences in precursor mRNA to participate in RNA splicing, stability maintenance and nucleocytoplasmic transport, serving as a key regulatory factor in the RNA metabolic network (18,19). Previous studies have demonstrated that hnRNP consistently exerts oncogenic functions. hnRNP promotes tumor progression through multiple mechanisms, such as regulating the stability of mRNA or microRNA and mediating alternative splicing, with well-documented roles in glioma, acute myeloid leukemia and breast cancer (20-23).

Although both m⁶A regulators and BCAT1 have been individually implicated in OSCC pathogenesis (12,16,17,24), no previous studies have established a mechanistic or functional link associating these two pathways, to the best of our knowledge. Notably, it remains to be elucidated whether hnRNP governs BCAT1 expression and BCAA metabolism through m⁶A modification in OSCC. Therefore, the novelty of the present study does not lie in identifying m⁶A regulators or BCAT1 as individual OSCC-associated molecules, but in establishing a novel functional axis associating hnRNP, m⁶A modification and BCAT1-dependent BCAA metabolism.

The present study utilized The Cancer Genome Atlas (TCGA) datasets to conduct an integrated bioinformatics analysis, systematically exploring the relationship between m⁶A modification patterns and OSCC malignancy.

Materials and methods

Collection of m⁶A transcriptome data and expression profiling. Transcriptome datasets, comprising 515 HNSCC and 44 normal tissue samples, were retrieved from the TCGA database via the UCSC Xena database (<https://xenabrowser.net>) and the GDC portal (<https://portal.gdc.cancer.gov>). Specifically, the Genomic Data Commons (GDC) TCGA Head and Neck Cancer cohort was accessed through the UCSC Xena database, and the Spliced Transcripts Alignment to a Reference-Fragments Per

Kilobase of transcript per Million mapped reads data under the gene expression RNA sequencing module were downloaded. Clinical information, including pathological stage, T stage, N stage and M stage, was extracted from the TCGA-HNSC clinical data files. Tumor staging was based on the American Joint Committee on Cancer staging system (25). A total of 23 m⁶A-associated genes were selected from the dataset based on findings from previous studies (15,16). All bioinformatics analyses were performed using R software (version 4.2.1; <https://www.r-project.org/>) and RStudio (version 2023.06.1; <https://posit.co/>; Posit Software, PBC). The following R packages (all obtained from CRAN, <https://cran.r-project.org/>) were used: i) 'ggplot2' (version 3.5.2) and 'boxplot' (version 1.3.1) for constructing heatmaps and boxplots to visualize gene expression patterns; ii) 'survival' (version 3.7-0) for Cox regression analysis; 'forestplot' (version 3.13) and 'pheatmap' (version 1.0.12) for hazard ratio visualization; iii) 'survminer' (version 0.5.1) for Kaplan-Meier curves; iv) 'rms' (version 6.8-2) for nomogram construction; v) 'gghalves' (version 0.1.4) and 'Hmisc' (version 5.1-3) for clinical correlation analysis; and vi) 'ggExtra' (version 0.10.1), 'cowplot' (version 1.1.3), 'ggpubr' (version 0.6.0), 'ggstatsplot' (version 0.14.0) and 'ggside' (version 0.3.1) for Pearson's correlation analysis between hnRNP and BCAT1. To identify genes associated with hnRNP expression, patients with HNSCC from TCGA database were stratified into hnRNP-high and hnRNP-low groups based on the median expression level of hnRNP. Differentially expressed genes between the two groups were identified using the 'limma' package (version 3.50.0) in R software. Genes with $\log_2$ fold-change > 1 and adjusted P-value (false discovery rate) < 0.05 were considered significantly differentially expressed.

Development of Cox risk regression model. Study samples were randomly allocated into a training cohort and a validation cohort at a 3:7 ratio. Univariate and multivariate Cox regression analyses were applied to identify independent prognostic factors for patients with HNSCC. The robustness of these factors was further assessed using the validation cohort. Therefore, three genes, insulin-like growth factor 2 mRNA-binding protein 2 (IGF2BP2), hnRNP and YTHDC2, were incorporated into the final Cox risk regression model.

Cell culture and lentivirus preparation. The human HNSCC cell lines CAL-27 and SCC-15 were acquired from The Cell Bank of Type Culture Collection of The Chinese Academy of Sciences. These cell lines were maintained in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum (FBS; Corning, Inc.). For stem cell (27-CSC) induction, OSCC cells were cultured in DMEM/F12 medium supplemented with basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF). These induced stem cells were grown as tumor spheres in DMEM/F12 medium with 20 ng/ml each of bFGF (cat. no. HY-P7331; MedChemExpress) and EGF (cat. no. AF-100-15-100; Peprotech, Inc.; Thermo Fisher Scientific, Inc.).

For lentivirus-mediated knockdown, two independent short hairpin RNA (shRNA) sequences targeting distinct regions of hnRNP and methyltransferase-like 3 (METTL3) transcripts were designed and cloned into the pLKO.1-puro lentiviral vector

[Hanheng Biotechnology (Shanghai) Co., Ltd.]. Lentiviral particles were produced by co-transfection of 293T cells with the pLKO.1-shRNA transfer plasmid, the psPAX2 packaging plasmid and the pMD2.G envelope plasmid. Transfection was performed when 293T cells reached 70-80% confluence using Lipofectamine 3000 (Thermo Fisher Scientific, Inc.) with 3 μ g of total plasmid DNA (pLKO.1-shRNA:psPAX2:pMD2.G=4:3:1) at 37°C for 6 h, after which the culture medium was replaced. Viral supernatants were collected at 48 and 72 h post-transfection, filtered through 0.45 μ m filters and used immediately or stored at -80°C. For transduction, CAL-27 and SCC-15 cells were incubated with viral supernatants supplemented with 8 μ g/ml polybrene for 24-48 h at 37°C. The negative control (NC) group was transduced with lentiviral particles carrying a non-targeting scrambled shRNA sequence cloned into the same pLKO.1-puro backbone. Stably transduced cells were selected and maintained in medium containing puromycin. The corresponding shRNA sequences are provided in Table SI.

Subcutaneous tumor xenograft model. Male BALB/c nude mice (6 weeks old; 18-22 g) were purchased from Cyagen Biosciences, Inc. A total of 15 mice were randomly assigned to three groups (n=5 per group: sh-NC control, sh-hnRNP#1 and sh-hnRNP#2). Mice were housed in a specific pathogen-free animal facility under controlled environmental conditions: Temperature, 20-26°C; relative humidity, 40-70%; a 12/12 h light/dark cycle; and free access to food and water. The present study was approved by the Ethics Committee of Zhenjiang Stomatological Hospital (approval no. PJ2023-08-10; Zhenjiang, China). SCC-15 cells stably expressing sh-NC or sh-hnRNP were collected, resuspended at a density of 1×10^7 cells per 100 μ l in a 1:1 mixture of PBS and Matrigel (as recommended by ATCC for subcutaneous tumor formation in nude mice; <https://www.atcc.org/products/crl-1623>), and injected subcutaneously into the right flank of each mouse. Tumor growth was monitored every 3 days starting from 1 week post-injection; tumor volume was calculated using calipers with the formula: Volume=(length x width²)/2. Humane endpoints were predefined as: i) Tumor diameter >20 mm; ii) tumor ulceration; iii) body weight loss >20%; or iv) signs of severe distress. No mice reached these endpoints before day 21 and tumor growth remained within humane limits throughout the experiment. At 21 days post-injection, mice were euthanized by gradual CO₂ inhalation (30-50% in an airtight chamber) followed by cervical dislocation after loss of consciousness. Death was confirmed by cessation of heartbeat and respiration, absence of corneal/pedal reflexes and graying of mucous membranes. Tumors were then dissected, weighed and processed for subsequent analyses. All procedures complied with institutional animal welfare guidelines.

Reagents. For reagent treatment, the indicated cells were treated with various concentrations (5, 10, 20 and 30 μ M) of ERG240 (cat. no. HY-W193545A; MedChemExpress). Dimethyl 2-oxoglutarate were purchased from MedChemExpress (cat. no. HY-44134). The m⁶A RNA methylation assay kit were purchased from Abcam (cat. no. ab185912) and the assays were conducted according to the manufacturer's instructions.

RNA extraction and reverse transcription-quantitative PCR (RT-qPCR). Total RNA was isolated from cultured cells with the Axygen® RNA Extraction Kit (cat. no. AP-MN-MS-RNA-250; Corning, Inc.). Subsequent to extraction, the RNA samples were converted into complementary DNA via reverse transcription, a process carried out using the Takara PrimeScript™ RT Master Mix Kit (cat. no. RR036A; Takara Bio, Inc.) according to the manufacturer's protocol (37°C for 15 min, followed by 85°C for 5 sec). For the analysis of gene expression, reactions were run in triplicate using the Takara SYBR® Premix Ex Taq™ II kit (cat. no. RR036A; Takara Bio, Inc.) on an Applied Biosystems™ 7500 instrument (Thermo Fisher Scientific, Inc.). The thermocycling conditions were as follows: 95°C for 30 sec, followed by 40 cycles of 95°C for 5 sec and 60°C for 34 sec, with a final dissociation stage to generate melting curves. The 2^{- $\Delta\Delta C_q$} algorithm was employed to determine the relative expression levels of each target gene (26). β -actin served as the endogenous reference gene to normalize the data. Detailed information regarding the primers utilized in the qPCR assays is provided in Table SII.

Western blotting. SDS-PAGE and western blotting procedures were conducted according to a previous study (27). For cell lysis, RIPA buffer (cat. no. P0013D; Beyotime Biotechnology) was used, supplemented with both protease inhibitors (cat. no. 43002700; Roche Diagnostics) and phosphatase inhibitors (cat. no. 43002700; Roche Diagnostics). Protein concentrations was determined using the BCA Protein Assay kit (cat. no. K813-2500; BioVision, Inc.; Abcam) according to the manufacturer's protocol. Equal protein aliquots were separated by SDS-PAGE and transferred to 0.45 μ m PVDF membranes (cat. no. IPVH00010; MilliporeSigma; Merck KGaA). Following membranes were blocked with skim milk before incubation with primary antibodies, followed by a 1-h incubation with horseradish peroxidase-conjugated secondary antibodies (Beijing Zhongshan Jinqiao Biotechnology Co., Ltd.). Immunoreactive bands were visualized using an ECL detection kit (MilliporeSigma; Merck KGaA), with β -actin serving as the loading control. Primary antibodies included: Rabbit anti-hnRNP (cat. no. ab133607; 1:1,000; Abcam), rabbit anti-BCAT1 (cat. no. ab197941; 1:1,000; Abcam) and mouse anti- β -actin (cat. no. sc58673; 1:10,000; Santa Cruz Biotechnology, Inc.).

Colony formation assay. Cells were seeded in 6-well plates at a density of 1,000 cells per well and maintained in a 5% CO₂ atmosphere at 37°C for 14 days in adherent culture (without soft agar). Following PBS washes, cells were fixed with 4% para-formaldehyde at room temperature for 15 min and stained with 0.5% crystal violet at room temperature for 15 min. Colony images were captured using a Nikon bright-field microscope. Colony number and area were quantified using ImageJ software (version 1.54f; National Institutes of Health). A distinct cluster was scored as a positive colony only if it contained ≥ 50 cells. To ensure the accuracy of the analysis in distinguishing adjacent or overlapping colonies, all software-based quantification was subjected to manual verification. Results are expressed as mean $\pm$ standard deviation (SD) from three independent biological replicates.

Cell proliferation assay. Relative cell proliferation was evaluated through viability measurements using the Cell Counting Kit-8 (CCK-8; cat. no. CK04; Dojindo Laboratories, Inc.) and 5-ethynyl-20-deoxyuridine (EdU) incorporation assays with the EdU Cell Proliferation Assay Kit (Guangzhou RiboBio, Co., Ltd.), following previously described methods (28).

Limiting dilution assay. Isolated stem cells were plated in 96-well plates at varying densities: 5, 10, 20, 50, 100 or 200 cells per well. After an 8-day incubation period, tumor sphere formation in each well was examined. Stem cell frequency was calculated using the extreme limiting dilution analysis web tool (<http://bioinf.wehi.edu.au/software/elda>).

Seahorse-based bioenergetic analysis. Oxygen consumption rate (OCR) was assessed using the Seahorse XFe24 extracellular flux analyzer (Agilent Technologies, Inc.) following the manufacturer's protocol. Cells were seeded onto collagen-coated 24-well Seahorse plates at a density of 1×10^5 cells per well and allowed to adhere overnight at 37°C. OCR measurements were performed in basal assay medium containing 1 mM pyruvate, 10 mM glucose and 2 mM glutamine. This was followed by sequential injections of 1 μ M oligomycin, 2 μ M FCCP and 0.5 μ M rotenone/antimycin A into each well.

BCAA and glutamate production quantification. BCAA and glutamate production were measured using the BCAA Assay Kit (cat. no. ab83374; Abcam) and Glutamate Assay Kit (cat. no. MAK004-1KT; MilliporeSigma; Merck KGaA), respectively, following the manufacturer's instructions. BCAA was determined by subtracting the measured BCAA concentration in the medium from the initial concentration. All results were normalized to cell number.

Metabolite level detection. ATP levels were quantified using the ATP Assay Kit (colorimetric/fluorometric; cat. no. ab83355). The ATP Assay kit employs a standard protocol for fluorometric and colorimetric ATP determination involving three key reactions: i) Glycerol phosphorylation by glycerol kinase (ATP-dependent) to form glycerol 3-phosphate; ii) conversion of glycerol 3-phosphate to glycero phosphate and hydrogen peroxide by glycerol phosphate oxidase; and iii) peroxidase-mediated reaction of hydrogen peroxide with a probe to generate a red color ($\lambda=570$ nm) and fluorescence (excitation/emission=535/587 nm). Succinate levels were measured using the Succinate Assay Kit (colorimetric; cat. no. ab204718, Abcam) following the manufacturer's instructions. This assay detects succinate through its conversion (along with ATP and CoA) to succinyl-CoA, ADP and inorganic phosphate by succinyl-CoA synthase, with colorimetric detection at 450 nm. Fumarate concentrations were determined using the Fumarate Assay Kit (cat. no. MAK492; Merck KGaA), which utilizes a colorimetric method ($\lambda=450$ nm) for quantification. GSH levels were measured with the GSH Assay Kit (colorimetric; cat. no. ab239709; Abcam), where GSH concentration is determined by absorbance readings at 412 nm.

RNA stability assay. CAL-27 and SCC-15 cells (4×10^5) were seeded in 6-well plates at 37°C overnight and treated with 5 μ g/ml actinomycin D (cat. no. HY-17559; MedChemExpress)

at 37°C for various durations (0, 2, 4 and 6 h). At each designated time point, cells were harvested for RNA isolation and subsequent RT-qPCR analysis as aforementioned.

RNA immunoprecipitation (RIP). RIP assays were performed using the PureBinding® RNA Immunoprecipitation Kit (cat. no. P0101/P0102; Guangzhou Genesee Biotech. Co., Ltd.) according to the manufacturer's protocol. Briefly, 5 μ g of hnRNP antibody (cat. no. ab133607; 1:1,000; Abcam) was bound to 200 μ l protein A/G magnetic beads (provided in the kit) at room temperature for 30 min, followed by three washes with wash buffer (prepared from the kit components). The antibody-conjugated beads were then incubated with pre-cleared nuclear extracts in RIP buffer at 4°C overnight with rotation (10 rpm). For each IP reaction, 400 μ l of pre-cleared nuclear lysate was used. After immunoprecipitation, the beads were washed three times with wash buffer (prepared from the kit components) with vortexing for 2 min each wash, followed by magnetic separation using a magnetic stand. Protein complexes were eluted by boiling in 1X loading buffer for 5 min and isolated via magnetic separation. RNA was purified using the DR Columns and RC Columns provided in the kit, with centrifugation steps performed according to the manufacturer's protocol (including 10,000 x g for 1 min for DNA removal, 10,000 x g for 30-60 sec for column washing, and 12,000 x g for 3 min for RNA elution). Total RNA served as the input control and extracted RNA was analyzed by RT-qPCR, which included U1 small nuclear RNA as a negative control. All RIP-qPCR data were normalized to U1 levels to account for non-specific background binding. All RIP experiments were conducted in triplicate using three biological replicates. Primer sequences are provided in Table SIII.

Methylated RNA immunoprecipitation (MeRIP)-qPCR. The m⁶A modification status of a specific gene was analyzed using the MeRIP Kit (MilliporeSigma; Merck KGaA) following previously described procedures (2). Briefly, 5 μ g of anti-m⁶A antibody (cat. no. CS220007; MilliporeSigma; Merck KGaA) or normal mouse IgG (cat. no. CS200621; MilliporeSigma; Merck KGaA) was prewashed and then incubated with Magna chromatin IP protein A/G magnetic beads (cat. no. CS203152; MilliporeSigma; Merck KGaA) for 30 min at room temperature. This antibody-bead complex was then incubated with purified poly-(A) RNA. The degree of enrichment of m⁶A-containing mRNA was subsequently quantified using RT-qPCR as aforementioned, with the relevant primer sequences listed in Table SIII.

Gene expression visualization via gene expression profiling interactive analysis (GEPIA). To evaluate the correlation between hnRNP and BCAT1 expression across different cancer types and normal tissues, expression data were analyzed using the GEPIA web server (<http://gepia.cancer-pku.cn/>), which integrates RNA-sequencing expression data from the TCGA and GTEx projects. Pearson's correlation analysis between hnRNP and BCAT1 expression was performed using the correlation analysis module of GEPIA. Specifically, the pan-cancer correlation was assessed using TCGA tumor samples across all available cancer types, and the pan-tissue correlation was assessed using GTEx normal tissue samples.

Scatter plots were generated with $\log_2(\text{TPM}+1)$ -transformed expression values, and the Pearson's correlation coefficient (R) and corresponding P-value were calculated by GEPIA to quantify the strength and significance of the association.

Statistical analysis. All experiments were performed with at least three independent biological replicates ($n \geq 3$), unless otherwise indicated in the figure legends. Data are presented as mean $\pm$ SD. Normality of data distribution was assessed using the Shapiro-Wilk test. Homogeneity of variances was assessed using Levene's test. For comparisons between two groups, a two-tailed Student's t-test was applied when both normality and variance homogeneity assumptions were met; otherwise, the Mann-Whitney U test was used. For comparisons among three or more groups, one-way ANOVA followed by Tukey's post hoc test was used for normally distributed data with equal variances; the Kruskal-Wallis test followed by Dunn's post hoc test was used for non-normal distributions or unequal variances. All statistical analyses were performed using GraphPad Prism (version 9.0; Dotmatics) or R software (version 4.1.0; R Development Core Team). $P < 0.05$ was considered to indicate a statistically significant difference.

Results

hnRNPC represents a novel independent prognostic biomarker in HNSCC. To investigate the impact of m⁶A regulatory genes on HNSCC, the present study analyzed transcriptome data from 515 patients with HNSCC and 44 normal tissues from TCGA database. Expression profiles of 23 m⁶A-associated genes were extracted, among which 18 demonstrated aberrant expression in HNSCC (Fig. 1A), suggesting a close association between m⁶A modification and HNSCC development. Feature selection of m⁶A regulators was performed using univariate Cox regression analysis (Fig. 1B), identifying five genes (hnRNPC, ALKBH5, IGF2BP1, IGF2BP2 and YTHDC2) significantly associated with HNSCC survival. Subsequent multivariate Cox regression analysis of these candidates revealed that three genes (hnRNPC, IGF2BP2 and YTHDC2) were significantly associated with survival (Fig. 1C). Univariate analysis (Fig. 1D) was also conducted on clinical variables from HNSCC samples and significant clinical factors (including sex, age and tumor stage) along with the three candidate genes were incorporated into a multifactorial Cox model (Fig. 1E). The results indicated that hnRNPC, IGF2BP2 and YTHDC2 could serve as independent prognostic factors for HNSCC. Specifically, YTHDC2 was identified as a protective factor, while IGF2BP2 and hnRNPC were risk factors. Patients were stratified into high- and low-risk groups based on a risk score derived from the model, with the optimal cut-off value (4.64) determined by maximally selected rank statistics based on the log-rank test (Fig. S1). Survival analysis demonstrated that patients in the high-risk group had significantly shorter overall survival (OS; Fig. 1F), a finding consistent in the test set (Fig. 1G and H). A nomogram and calibration curves based on the final multivariate Cox model further supported these results (Fig. 1I and J). Although the roles of YTHDC2 and IGF2BP2 in HNSCC have been previously reported (29,30), the present study focused on hnRNPC. Survival analysis based on hnRNPC expression alone indicated that high expression

was associated with worse prognosis (Fig. 1K). Furthermore, hnRNPC expression was positively associated with stage III and IV and T stage (Fig. 1L and M).

hnRNPC exerts oncogenic functions in OSCC. Prior to functional studies, the present study examined the endogenous mRNA expression level of hnRNPC in a panel of six commonly used OSCC cell lines (CAL-27, SCC-15, SCC-9, SCC-25, HSC-3 and FaDu) by qPCR. Among these, CAL-27 and SCC-15 exhibited the markedly highest basal levels of hnRNPC mRNA (Fig. S2A), the present study thus knocked down hnRNPC expression in both cell lines using two distinct shRNA sequences to verify hnRNPC function (Fig. 2A and B). Results from CCK-8 and colony formation assays demonstrated that hnRNPC knockdown significantly inhibited the proliferative capacity of OSCC cells compared with the NC group, confirming the oncogenic function of hnRNPC (Fig. 2C-F). Furthermore, EdU incorporation assay indicated that hnRNPC knockdown significantly suppressed the proliferation rate of OSCC cells (Fig. 2G and H). Consistently, nude mouse subcutaneous xenograft experiments demonstrated that hnRNPC silencing led to a significant reduction in both volume and weight of subcutaneously formed OSCC tumors (Fig. 2I-K). Furthermore, hnRNPC knockdown significantly decreased the frequency of tumor sphere formation by CAL-27-derived stem cells, as determined by an *in vitro* limiting dilution assay (Fig. 2L). The present study analyzed the expression levels of key stemness genes, including Oct4, Sox2 and Nanog, which are well-known in promoting tumor cells with self-renewal capabilities, using qPCR. hnRNPC knockdown resulted in a significant downregulation of these genes (Fig. 2M). Therefore, hnRNPC emerges as a key regulator of OSCC cell proliferation and the maintenance of stem-like properties.

hnRNPC promotes energy production and antioxidant capacity in OSCC via BCAA metabolism. To investigate the mechanism underlying hnRNPC-mediated regulation of OSCC progression, the present study analyzed transcriptomic data of HNSC from TCGA database. The results revealed that the BCAA metabolic pathway was active in HNSC tissues with high hnRNPC expression (Fig. 3A). Since the BCAA metabolic pathway is involved in energy production and redox homeostasis, the present study also observed changes in pathways associated with oxidative phosphorylation and stress (Fig. 3B and C). Consistently, BCAA uptake capacity was significantly impaired in OSCC cell lines with hnRNPC knockdown (Fig. 3D). Since BCAAs generate large amounts of glutamate and BCKAs through catabolic pathways, where glutamate contributes to the synthesis of reduced GSH for antioxidant defense, and BCKAs promote the TCA cycle and oxidative phosphorylation via acetyl-CoA production, the present study examined these indicators in OSCC cells (31,32). The present study findings demonstrated that hnRNPC knockdown led to significantly decreased glutamate levels, reduced OCR and lower GSH levels in OSCC cells compared with the negative control group (Fig. 3E-J). In accordance, gene set enrichment analysis (GSEA) based on TCGA data also revealed a significant enrichment of GSH metabolism pathway in hnRNPC highly expressed OSCC tissues (Fig. 3K and L). These results indicated that hnRNPC can enhance tumor

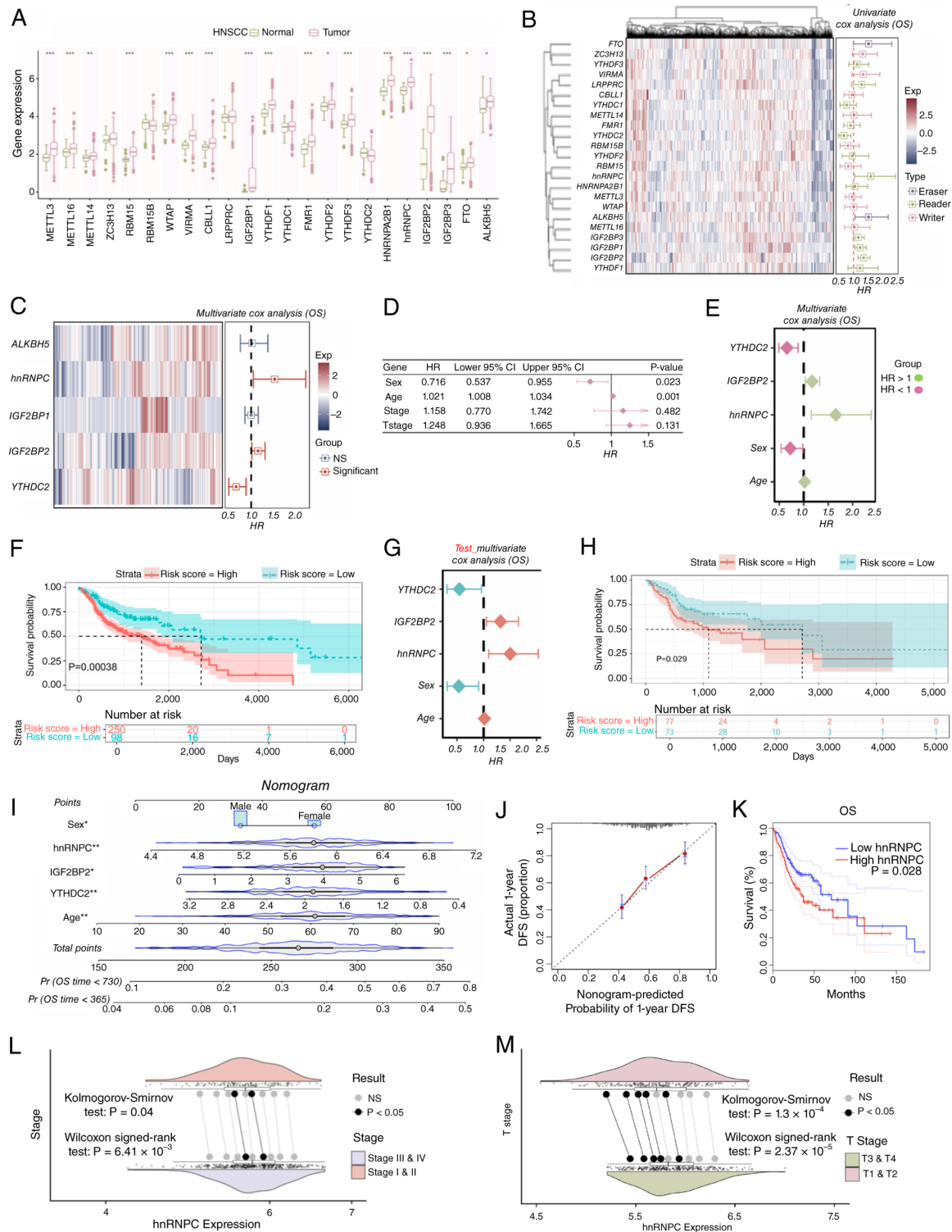


Figure 1. hnRNPC represents a novel independent prognostic biomarker in HNSCC. (A) Boxplot visualizing the expression levels of 23 m⁶A-associated genes in 515 HNSCC and 44 normal tissues. (B) Heatmap (left) of the 23 m⁶A-associated genes and univariate Cox regression analysis (right) based on m⁶A-associated genes associated with the OS of HNSCC. (C) (left) Heatmap of the significant genes (hnRNPC, ALKBH5, IGF2BP1, IGF2BP2 and YTHDC2) in the univariate Cox regression analysis and (right) multivariate Cox regression analysis based on candidate genes with the OS of HNSCC. (D) Forest plot presents the univariate Cox regression analysis of the clinical information of the training set. (E) Forest plot presents the multivariate regression analysis of the candidate genes in the training set and clinical information (pink, HR <1; green, HR >1). (F) Kaplan-Meier OS curves for patients in the training set assigned to high- and low-risk groups. (G) Forest plot presents the results of a multivariate regression analysis of candidate genes in the test set and clinical information (red, HR >1; blue, HR <1). (H) Kaplan-Meier OS curves for patients in the test set assigned to high- and low-risk groups. (I) Nomogram integrating the HNSCC risk score with age, sex, and expression levels of hnRNPC, IGF2BP2 and YTHDC2 to predict 1- and 2-year OS. (J) Calibration curves confirming good agreement between nomogram-predicted and -observed survival probabilities. (K) Kaplan-Meier curves depicting OS according to the hnRNPC expression based on The Cancer Genome Atlas data. (L) Box and whisker plots comparing hnRNPC expression across pathological stages (stage I and II vs. III and IV) and (M) T classifications (T3 and T4 vs. T1 and T2). Boxes represent median and IQR; whiskers indicate 1.5X IQR. Unless otherwise stated, data are expressed as mean $\pm$ SD. *P<0.05, **P<0.01 and ***P<0.001. hnRNPC, heterogeneous nuclear ribonucleoprotein C; HNSCC, head and neck squamous cell carcinoma; NS, not significant; OS, overall survival; DFS, disease-free survival; HR, hazard ratio; IQR, interquartile range; IGF2BP2, insulin-like growth factor 2 mRNA-binding protein 2.

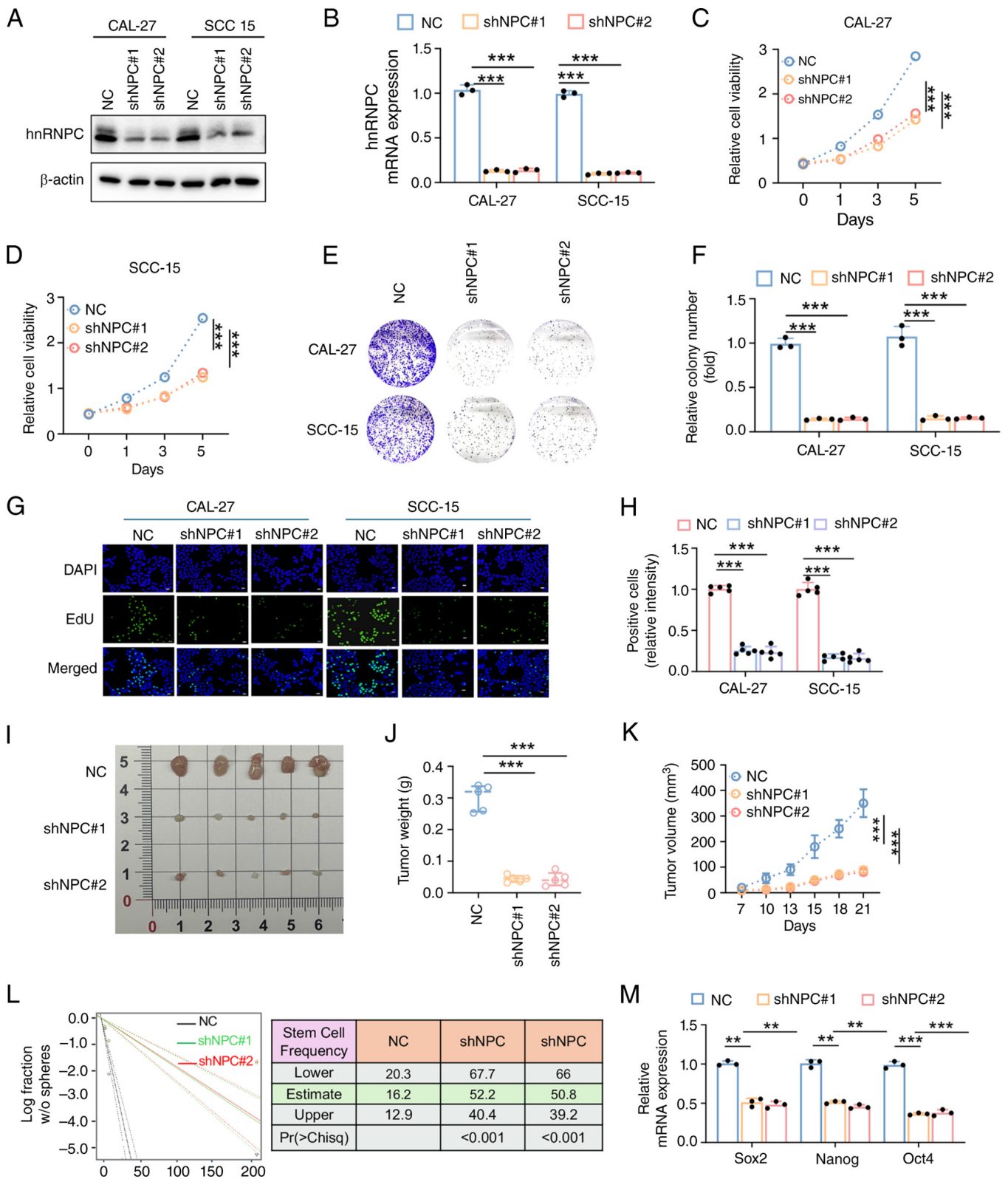


Figure 2. hnRNPC exerts oncogenic functions in oral SCC. (A) Western blotting and (B) qPCR were used to detect protein expression after stable knockdown expression level of hnRNPC in CAL-27 and SCC-15 cell lines. Cell Counting Kit-8 assay was used to detect the effect of hnRNPC knockdown on the viability of (C) CAL-27 and (D) SCC-15 cells. (E) Representative images and (F) relative quantifications of colony formation assays of CAL-27 and SCC-15 cells. (G) 5-ethynyl-20-deoxyuridine solution was incorporated for 2 h to evaluate the proliferation in CAL-27 and SCC-15 cells. (H) Percentage of positive cells was quantified. (I-K) Knockdown of hnRNPC can inhibit the growth of subcutaneous tumors. SCC-15 cells with or without hnRNPC knockdown were subcutaneously injected into nude mice. (I) Tumors were dissected from the nude mice of each group and captured at 21 days after (J) the weight of tumors was measured. (K) Tumor size was measured every 3 days and growth curves were plotted. (L) Sphere formation frequency of germline stem cells (27-CSC) with or without hnRNPC knockdown was calculated with (left) extreme limiting dilution assay analysis; (right) stem cell frequencies from 27-CSC cells with or without hnRNPC knockdown were estimated as the ratio 1/x with the upper and lower 95% confidence intervals, where 1=stem cell and x=all cells. (M) qPCR was used to detect the Sox2, Nanog and Oct4 mRNA expression levels. For (A-H) and (L-M), n=3 biologically independent experiments. For (I-K), n=5 mice per group. Unless stated otherwise, data are expressed as mean $\pm$ SD. **P<0.01 and ***P<0.001. qPCR, quantitative PCR; hnRNPC, heterogeneous nuclear ribonucleoprotein C; SCC, squamous cell carcinoma; CSC, cancer stem cell; NC, negative control; sh, short hairpin RNA.

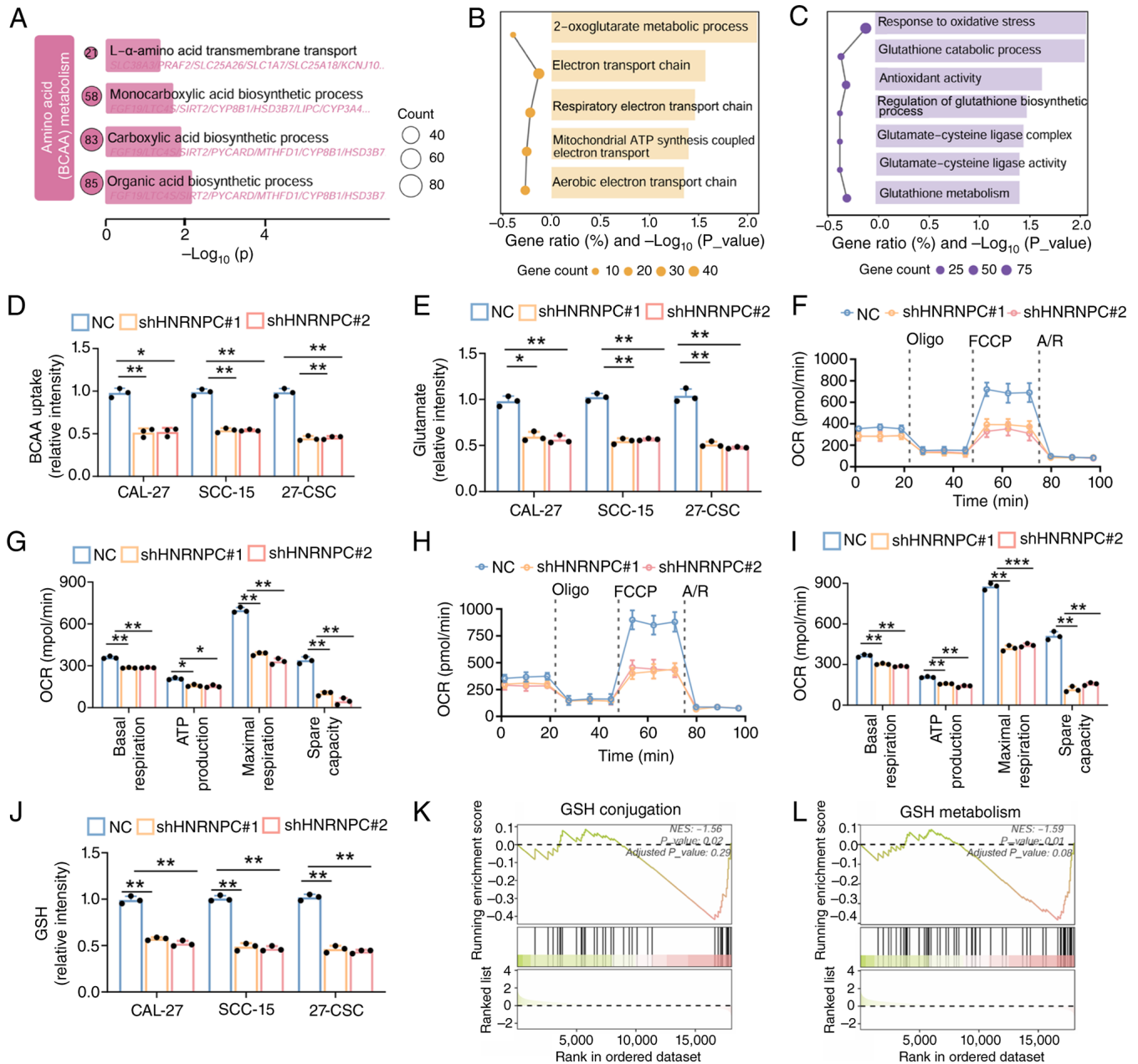


Figure 3. hnRNP C promotes energy production and antioxidant capacity in OSCC via BCAA metabolism. Gene Ontology enrichment analysis of 1.5 fold-change differentially expressed genes. (A) Metabolism of amino acids (BCAA), (B) energy metabolism and (C) oxidative stress pathways. (D) BCAA uptake rate and (E) internal glutamate levels were determined in CAL-27, SCC-15 and 27-CSC cells with or without hnRNP C knockdown. (F and H) Analysis of OCR measurement of one representative experiment in indicated cells after 4 h culture in basal condition and after sequential addition of oligomycin, FCCP and A/R. (G and I) Quantification of basal respiration, ATP-production-coupled respiration, maximal respiration and spare capacity in CAL-27 and SCC-15 cells with or without HNRNP C knockdown. Box and whisker plots display median and minimum to maximum; data in dot and bar plots are mean $\pm$ SD. (J) GSH level was determined in CAL-27, SCC-15 and 27-CSC cells with or without hnRNP C knockdown. (K and L) Gene set enrichment analysis GSH metabolism pathway. Unless stated otherwise, data are expressed as mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. hnRNP C, heterogeneous nuclear ribonucleoprotein C; A/R, antimycin/rotenone; FCCP, carbonyl cyanide-p-trifluoromethoxyphenylhydrazone; GSH, glutathione.

energy production and antioxidant capacity by promoting the BCAA metabolic pathway, thereby facilitating oxidative phosphorylation and increasing GSH levels, which likely represents a key mechanism underlying its tumor-promoting effects.

hnRNP C regulates BCAT1 mRNA stability in an m^6A modification-dependent manner. To explore the specific mechanism by which hnRNP C promotes tumor progression via regulation of the BCAA metabolism, the present study analyzed differentially expressed genes between hnRNP C-high and -low

expressing samples from the HNSC transcriptome database. The present study reported that BCAT1 was significantly upregulated in OSCC tissues with high hnRNP C expression, suggesting that hnRNP C may regulate BCAA metabolism by promoting BCAT1 expression (Fig. 4A and B). Consistent with this observation, western blotting and qPCR results demonstrated that BCAT1 expression was significantly suppressed in OSCC cells following hnRNP C knockdown (Fig. 4C and D). Notably, treatment with the BCAT1 inhibitor ERG240 further enhanced the tumor-suppressive effects of hnRNP C

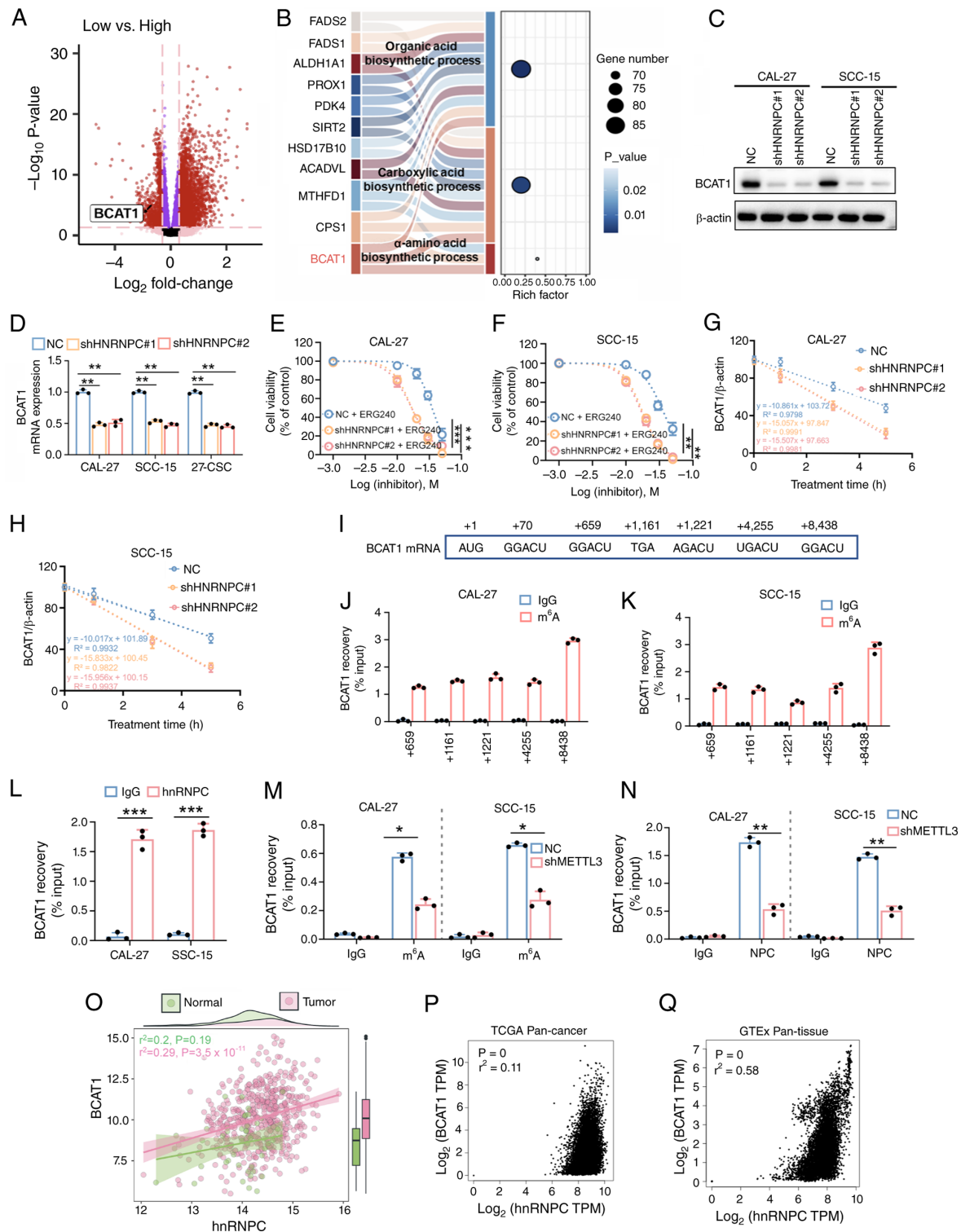


Figure 4. hnRNPC regulates BCAT1 mRNA stability in an m⁶A modification-dependent manner. (A) Volcano plot displays the expression level of BCAT1 in hnRNPC with low vs. high expression based on TCGA-sequencing data. (B) Sankey diagram chart displays the expression levels of genes in different amino acid metabolic pathways. BCAT1 denoted in pink. (C) Western blotting and (D) qPCR analysis of BCAT1 protein and mRNA expressions in in CAL-27, SCC-15 with or without hnRNPC expression. Growth conditions of (E) CAL-27 and (F) SCC-15 cells with or without hnRNPC knockdown after adding the BCAT1 inhibitor ERG240. Detection of the stability of BCAT1 mRNA in (G) CAL-27 and (H) SCC-15 cells after knockdown of hnRNPC. (I) Position map displays the prediction of the top five m⁶A modification sites in the BCAT1 mRNA using the 'SRAMP' algorithm. Analysis of MeRIP assays detecting BCAT1 retrieved by a m⁶A antibody around the high-confidence m⁶A sites in (J) CAL-27 and (K) SCC-15 cells. (L) Assessment of RIP assays detecting BCAT1 mRNA +8,438 site retrieved by a hnRNPC antibody or by IgG in CAL-27 and SCC-15 cells. (M) Analysis of MeRIP assays detecting BCAT1 mRNA +8,438 site recovered with m⁶A antibody in METTL3 knockdown in CAL-27 and SCC-15 cells. (N) Assessment of RIP assays by a hnRNPC antibody or by IgG in CAL-27 and SCC-15 cells after METTL3 knockdown. (O and P) Transcriptomic analysis of TCGA database displays a significant positive correlation between hnRNPC and BCAT1 expression in head and neck SCC tissues. (Q) GTEx database also revealed the significant positive correlation between hnRNPC and BCAT1 expression. Unless stated otherwise, data are expressed as mean $\pm$ SD, *P<0.05, **P<0.01 and ***P<0.001. m⁶A, N6-methyladenosine; hnRNPC, heterogeneous nuclear ribonucleoprotein C; TCGA, The Cancer Genome Atlas; RIP, RNA immunoprecipitation; MeRIP, methylated RIP; BCAT1, branched-chain amino acid transaminase 1; qPCR, quantitative PCR; SCC, squamous cell carcinoma; CSC, cancer stem cell; METTL3, methyltransferase-like 3; sh, short hairpin RNA; TPM, transcripts per million; GTEx, Genotype-Tissue Expression.

knockdown (Fig. 4E and F), indicating that combined targeting of hnRNPc and BCAT1 to interfere with BCAA metabolism could potentially represent a promising therapeutic strategy for OSCC in the future.

Since hnRNPc functions as an m⁶A modification reader protein to regulate mRNA stability of downstream genes, the present study further investigated whether hnRNPc-mediated regulation of BCAT1 is dependent on m⁶A modification. mRNA stability assays demonstrated that BCAT1 mRNA stability was significantly reduced after hnRNPc knockdown (Fig. 4G and H). Using the 'SRAMP' algorithm, the present study predicted the top five m⁶A modification sites in BCAT1 mRNA (Fig. 4I). Further MeRIP-qPCR experiments identified the most notable m⁶A peak at position +8,438 within the BCAT1 3'-UTR (Fig. 4J and K). RIP assays confirmed the direct binding of hnRNPc to BCAT1 mRNA (Fig. 4L), while MeRIP analysis revealed that METTL3 knockdown reduced m⁶A methylation on BCAT1 transcripts (Figs. 4M and S2B). Of note, METTL3 silencing also diminished the association between hnRNPc and BCAT1 mRNA, demonstrating that m⁶A marks are required for hnRNPc recognition (Fig. 4N). Furthermore, transcriptomic analysis of TCGA database revealed a significant positive correlation between hnRNPc and BCAT1 expression in HNSC tissues (Fig. 4O). Consistent with this finding, pan-cancer transcriptomic data from TCGA database and pan-tissue transcriptomic data from the GTEx database also revealed the significant positive correlation between hnRNPc and BCAT1 expression (Fig. 4P and Q). Therefore, hnRNPc may promote BCAT1 expression in an m⁶A-dependent manner during OSCC progression, thereby driving the malignant evolution of OSCC dependent on BCAA metabolism.

α -KG rescues hnRNPc-mediated BCAA-dependent energy deficiency. Since α -KG serves as a key donor for BCAT1-catalyzed glutamate generation in the BCAA metabolic pathway and functions as a key intermediate in mitochondrial TCA cycle, the present study investigated whether exogenous supplementation of α -KG could reverse the hnRNPc-induced suppression of BCAA metabolism and subsequent energy deficiency. Results from the CCK-8 and colony formation assays in the present study demonstrated that α -KG supplementation significantly restored cell viability in hnRNPc-knockdown OSCC cells (Fig. 5A-D). Stem cell viability experiments revealed that α -KG supplementation notably increased the frequency of tumor sphere formation by CAL-27-derived stem cells with hnRNPc knockdown (Fig. 5E), accompanied by significant recovery of Oct4, Sox2 and Nanog expression levels which were downregulated by hnRNPc silencing (Fig. 5F). Furthermore, Seahorse assay results indicated that α -KG supplementation led to significant restoration of OCR and increased ATP levels in OSCC cells (Fig. 5G-K). Therefore, hnRNPc binds to m⁶A-modified BCAT1 mRNA in a METTL3-dependent manner, stabilizing BCAT1 transcripts and enhancing BCAT1 expression. This upregulates BCAA catabolism, producing BCKAs for TCA cycle-driven energy supply and glutamate for GSH-mediated antioxidant defense, ultimately promoting OSCC cell proliferation, stemness and tumor growth (Fig. 6). These findings confirmed

that BCAT1-mediated BCAA metabolic pathway for energy production represents one of the key mechanisms underlying hnRNPc-promoted tumor progression.

Discussion

The present study systematically explored the role and mechanism of hnRNPc in OSCC by integrating bioinformatics analysis and experimental validation. Using data mining for datasets of HNSCC in TCGA database, with OSCC as the predominant subtype, the present study first identified hnRNPc as an associative independent prognostic biomarker for HNSCC. The high expression level of hnRNPc was associated with advanced pathological stages, poor OS and served as a risk factor for clinical outcomes. Functional experiments further confirmed the oncogenic role of hnRNPc in OSCC. Knockdown of hnRNPc inhibited cell proliferation, colony formation and tumor sphere formation *in vitro*, and reduced tumor growth in nude mouse xenografts, while also down-regulating stemness-associated genes (Oct4, Sox2 and Nanog). Mechanistically, the present study revealed that hnRNPc promotes OSCC progression by regulating BCAA metabolism. hnRNPc enhances BCAA uptake, supports BCAT1-dependent energy production (via BCKAs entering the TCA cycle) and redox homeostasis (via glutamate-mediated GSH synthesis). Of note, this regulation of BCAT1 by hnRNPc is dependent on m⁶A modification; hnRNPc binds to m⁶A-modified BCAT1 mRNA (predominantly at the +8,438 site in the 3'-UTR) to maintain its stability and METTL3 is required for this recognition. Lastly, exogenous supplementation of α -KG, a key metabolite in TCA cycle, rescued the energy deficiency and functional impairment of OSCC cells caused by hnRNPc knockdown, further validating the BCAA metabolic pathway as a downstream effector of hnRNPc.

A key innovation of the present study lies in establishing a novel regulatory axis, hnRNPc/m⁶A/BCAT1/BCAA metabolism, that drives OSCC progression, filling key gaps in current knowledge. Previous studies have separately highlighted the roles of m⁶A modification and BCAA metabolism (for example, BCAT1) in OSCC, but their functional association remained uncharacterized (12,30,33). The present study findings demonstrated that hnRNPc, a classic RNA-binding protein with established oncogenic roles in numerous types of cancer, such as cervical and pancreatic cancer (34,35), acts as an m⁶A reader in OSCC to stabilize BCAT1 mRNA. This finding not only expands the functional spectrum of hnRNPc, beyond mRNA splicing and stability regulation to m⁶A-dependent metabolic control but also reveals a unique epigenetic-metabolic crosstalk in OSCC. Specifically, m⁶A modification, by marking BCAT1 mRNA, enables hnRNPc to fine-tune BCAA metabolism, an adaptation that supports tumor energy demands (via BCKA-driven oxidative phosphorylation) and antioxidant defense (via glutamate-GSH axis). Although the present study identified hnRNPc as a key m⁶A reader that stabilizes BCAT1 mRNA in OSCC, emerging evidence indicates that multiple m⁶A readers may coordinately regulate target gene expression (16,17). Previous studies have confirmed that YTHDF1 and IGF2BP2 act as key m⁶A readers involved in OSCC progression (16,17). Therefore, the possibility that YTHDF1, IGF2BP2 or other m⁶A readers may

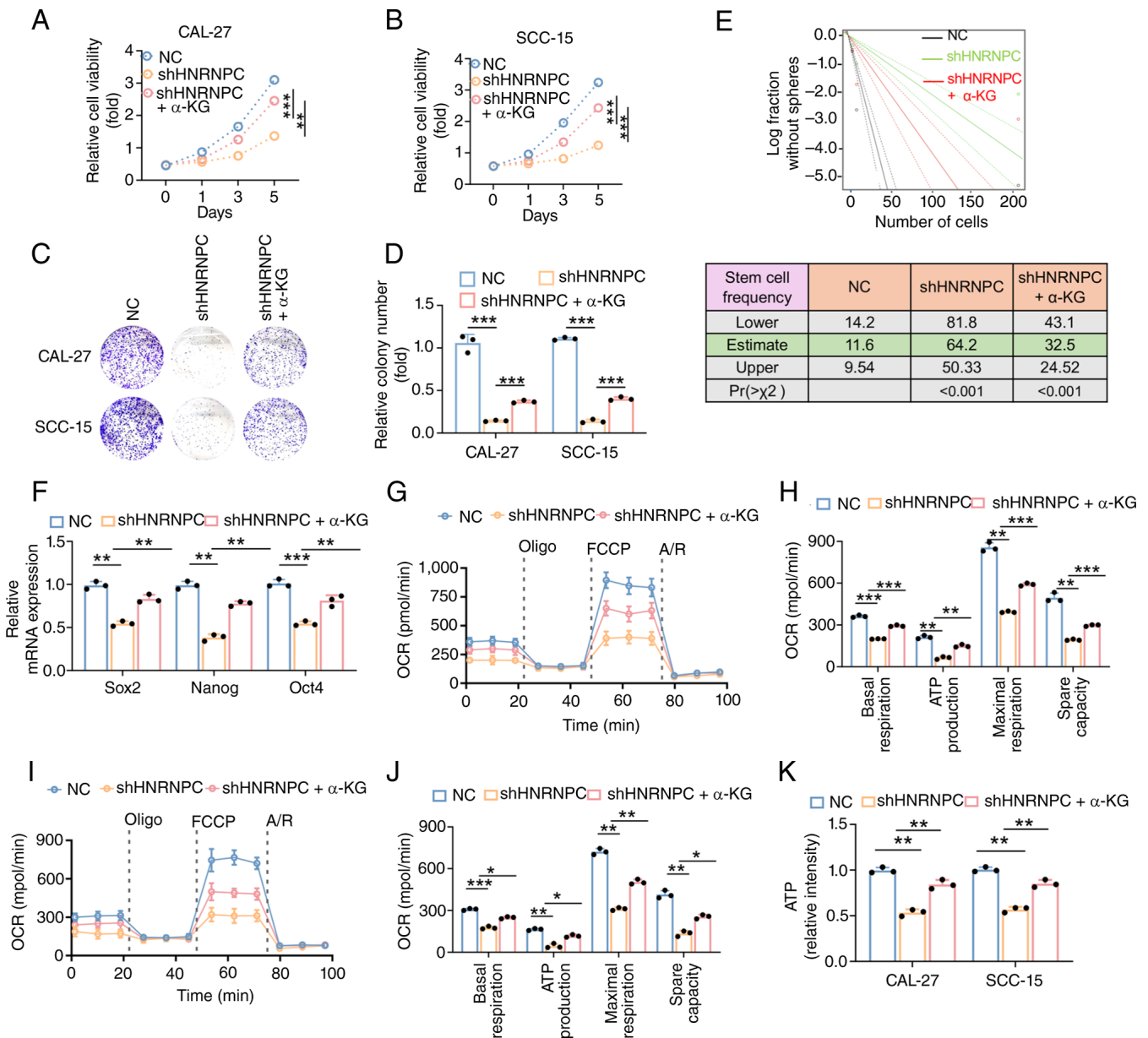


Figure 5. α -KG rescues hnRNP-mediated BCAA-dependent energy deficiency. Cell Counting Kit-8 assay was used to investigate the growth conditions of α -KG in CAL-27 (A) and SCC-15 (B) cells with knocked-down hnRNP. Representative images (C) and relative quantifications (D) of colony formation assays of indicated cells. (E) Sphere formation frequency in 27-CSC of knocked-down hnRNP with conditions of α -KG was calculated with extreme limiting dilution assay analysis (top). Stem cell frequencies from 27-CSC cells were estimated as the ratio $1/x$ with the upper and lower 95% confidence intervals, where 1 =stem cell and x =all cells (bottom). (F) Quantitative PCR was used to detect the Sox2, Nanog and Oct4 mRNA expression levels. (G and I) Analysis of OCR measurement of one representative experiment in indicated cells after 4 h culture in basal condition and after sequential addition of oligomycin, FCCP and A/R. (H and J) Quantification of basal respiration, ATP-production-coupled respiration, maximal respiration and spare capacity in CAL-27 and SCC-15 of knocked-down hnRNP with conditions of α -KG. Box-and-whisker plots show median and minimum to maximum; data in dot and bar plots are mean $\pm$ SD. (K) ATP level was determined in CAL-27 and SCC-15 of knocked-down hnRNP with conditions of α -KG. Unless stated otherwise, data are expressed as mean $\pm$ SD. * P <0.05, ** P <0.01 and *** P <0.001. m⁶A, N⁶-methyladenosine; hnRNP, heterogeneous nuclear ribonucleoprotein C; α -KG, α -ketoglutarate; NC, negative control; sh, short hairpin RNA; FCCP, carbonyl cyanide-p-trifluoromethoxyphenylhydrazone; A/R, antimycin/rotenone; BCAA, branched-chain amino acid; SCC, squamous cell carcinoma; CSC, cancer stem cell.

also contribute to the regulation of BCAT1 mRNA stability or translation in OSCC should not be excluded. The potential crosstalk or redundant functions among these m⁶A readers warrant further exploration in future studies.

Notably, this axis also explains the clinical challenge of chemoresistance in OSCC. Previous studies have reported that BCAT1-mediated GSH synthesis reduces drug-induced ROS and DNA damage (9,36). The present study extended this finding by demonstrating that hnRNP upregulation

enhances this pathway, providing a molecular basis on why high hnRNP expression associates with poor prognosis. This mechanistic association offers a novel perspective in overcoming chemoresistance, suggesting that targeting hnRNP or BCAT1 may hold potential to disrupt the BCAA-GSH antioxidant system and sensitize OSCC cells to cisplatin and other ROS-inducing therapies.

The hnRNP/m⁶A/BCAT1 axis identified in the present study may hold preliminary clinical relevance

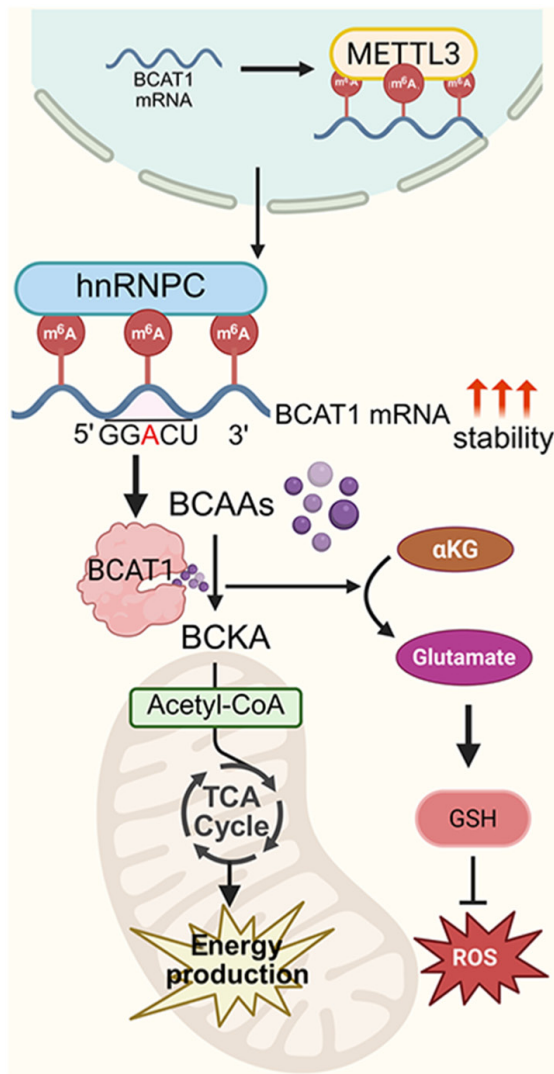


Figure 6. Proposed mechanism of hnRNP C action in regulation of BCAT1 stabilization in m⁶A-dependent manner during oral squamous cell carcinoma progression. Black arrows represent promotion; black T-shaped lines indicate inhibition; red arrows indicate increased mRNA stability; and dashed arrows indicate the TCA cycle. m⁶A, N⁶-methyladenosine; hnRNP C, heterogeneous nuclear ribonucleoprotein C; BCAT1, branched-chain amino acid transaminase 1; METTL3, methyltransferase-like 3; BCKA, branched-chain α-keto acids; α-KG, α-ketoglutarate; GSH, glutathione; ROS, reactive oxygen species; TCA, tricarboxylic acid.

for the management of OSCC in the future. From a prognostic perspective, hnRNP C may serve as a prognostic biomarker. When combined with pathological stage, hnRNP C expression may help improve risk stratification for patients with OSCC and assist clinicians in identifying individuals at higher risk of recurrence or progression. In terms of treatment, the present study preclinical findings suggested that targeting this axis may represent a promising investigative strategy. Combined inhibition of hnRNP C (such as, using small interfering RNAs or small-molecule inhibitors) and BCAT1 (for example, using ERG240) may theoretically synergistically disrupt BCAA metabolism. Such disruption could reduce tumor energy supply, impair antioxidant capacity and increase chemosensitivity in preclinical models. Furthermore, METTL3 shRNA can block the m⁶A modification of BCAT1 mRNA, thereby

eliminating the stabilization of BCAT1 mRNA mediated by hnRNP C. This approach may represent a potential alternative to avoid potential off-target effects associated with direct hnRNP C inhibition.

Despite its contributions, the present study had several limitations. First, the functional experiments in the present study were performed in only two OSCC cell lines (CAL-27 and SCC-15). The selection of these two lines was based on the following rationale: i) The present study initially screened a panel of six OSCC cell lines (CAL-27, SCC-15, SCC-9, SCC-25, HSC-3 and FaDu) by qPCR and identified that CAL-27 and SCC-15 express endogenously high levels of hnRNP C mRNA, providing a physiologically relevant background for loss-of-function experiments. Furthermore, both cell lines have been extensively used as validated models for m⁶A RNA methylation research in OSCC, including functional studies of METTL3, METTL14 and Fat mass and Obesity-associated (37,38). Furthermore, a recent study specifically demonstrated that hnRNP C knockdown in CAL-27 cells significantly impairs proliferation, invasion and malignant transformation (39), directly supporting the suitability of this cell line in investigating hnRNP C-dependent mechanisms. Nevertheless, the present study acknowledges that the number of OSCC models were limited. Validation in additional OSCC cell lines and patient-derived xenograft models, which further recapitulate the heterogeneity of clinical OSCC, would strengthen the translational relevance of the present study findings. Second, formal *in vivo* metabolic intervention studies (for example, using BCAT1 inhibitors or m⁶A modulators) and validation using clinical patient samples remain lacking, to the best of our knowledge. Therefore, the translational and therapeutic implications stated in the present study should be considered preliminary and exploratory. Third, the association between hnRNP C expression and clinical chemoresistance was not validated using patient samples, which is key to translating the present study findings into clinical practice. Future studies should focus on *in vivo* metabolic validation, patient cohort verification and preclinical investigation of combination therapies targeting the hnRNP C/m⁶A/BCAT1 axis to further establish its clinical utility.

In conclusion, the present study revealed a novel epigenetic-metabolic axis in OSCC, demonstrating that hnRNP C promotes BCAA metabolism via m⁶A-dependent stabilization of BCAT1. These findings not only enhance current understanding of OSCC progression but also highlight promising candidate markers and prospective therapeutic targets that warrant further validation toward improvement of OSCC management in the future.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

YA designed the present study. ZZ, XG, TH, FC and MG performed the experiments. ZZ, XG and TH analyzed the data. YA wrote the manuscript. YA and ZZ confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The animal experiments were approved by the Ethics Committee of Zhenjiang Stomatological Hospital (approval no. PJ2023-08-10; Zhenjiang, China) and complied with the National Institutes of Health Guide For The Care And Use Of Laboratory Animals (National Institutes of Health publications no. 8023; revised 1978).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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