RPS15A promotes the proliferation and migration of nasopharyngeal carcinoma

Jin Bian (✉ 85984127@qq.com)

The Central Hospital of Panzhihua

Yan Niu

The Second Affiliated Hospital of Kunming Medical University

Cao Lv

The Second Affiliated Hospital of Kunming Medical University

Research Article

Keywords: Nasopharyngeal carcinoma, RPS15A, proliferation, migration, PI3K/AKT signaling pathway

Posted Date: February 16th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1358846/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: Nasopharyngeal carcinoma (NPC) is an epithelial squamous cell carcinoma arising from the nasopharyngeal mucosal lining. Ribosomal protein S15a (RPS15A) plays vital role in protein translation and was recently reported to be an oncogene in numerous tumor types. However, its biological role in NPC is remain largely unclear.

Methods: In this study, we explored the expression of RPS15A in NPC tissues by immunofluorescence histochemical staining (IHC) staining of human tissue microarray. The C666-1 and CNE-2Z NPC cell lines with RPS15A depletion were used to investigate the effects of RPS15A on NPC cell proliferation, migration and apoptosis. *In vivo* tumor growth of NPC cells was observed by subcutaneous xenograft mice model. The potential mechanism was explored by Human Apoptosis Antibody Array analysis and WB experiments.

Results: RPS15A was significantly up-regulated in NPC and RPS15A knockdown remarkably suppressed NPC cells proliferation, migration and induced cell apoptosis. Moreover, RPS15A silencing also impaired tumor growth of xenograft mice. Further Human Apoptosis Antibody Array analysis indicated that depletion of RPS15A could promote several apoptosis-related proteins expression, and results of WB experiments confirmed the inhibition of PI3K/AKT pathway.

Conclusion: RPS15A knockdown suppressed proliferation, migration and increased apoptosis of NCP cells by inhibiting PI3K/AKT signaling pathway. RPS15A may serve as a promising therapeutic target for NPC patients.

Introduction

Nasopharyngeal carcinoma (NPC) is an epithelial squamous cell carcinoma arising from the nasopharyngeal mucosal lining [1]. Compared to other cancers, NPC is an uncommon tumor type, which is distinctly different from other epithelial head and neck tumors and has unique distribution that mainly focus on East and Southeast Asia [2, 3]. Epidemiological reports suggested that incidence of NPC has declined gradually worldwide in the past decades [4, 5]. It may owe to the significant advancements in imaging diagnosis technology, radiotherapy delivery, and systemic chemotherapy for NPC patients[6]. Moreover, developments of novel therapeutics, such as immune checkpoint therapies and targeted therapies have obviously improved the prognosis of NPC patients with recurrence or metastasis [7-9]. However, several questions about pathogenesis and clinical management on NPC still need to be addressed [2]. A better understanding of biological mechanisms underlying NPC pathogenesis may allow the identification of biomarkers related to NPC progression and management.

Ribosomes is one of organelles of eukaryotes, consisting of a small 40S subunit and a large 60S subunit, responsible for protein synthesis [10]. It's found that regulation of cell proliferation and differentiation by ribosome biogenesis and protein translation is abnormal in tumor cells [11]. The mutations of oncogenes and cancer suppressed genes often stimulate cell growth and proliferation by enhancing ribosome

biogenesis in cancer cells, thereby driving cancer occurrence [12]. Ribosomal protein S15a (RPS15A) encodes a component of 40S ribosomes subunits, plays vital role in protein translation [13]. Recently, accumulating evidences showed the upregulation of RPS15A in a plethora of cancers, including hepatocellular carcinoma [14], pancreatic cancers [15], breast cancer [16], and colorectal cancer [17], *etc.* RPS15A knockdown inhibited proliferation of hepatic cancer cells [18], lung cancer cells [19] and osteosarcoma cells [20] *in vitro*, kidney cancer cells growth *in vitro* and *in vivo* [21], as well as inducing cell apoptosis in glioblastoma [22]. However, the biological functions of RPS15A in NPC is largely unknown.

In this study, we identified the up-regulation of RPS15A in NPC by bioinformatics analysis and IHC staining of human tissues. Moreover, we explored the effects of RPS15A on proliferation, migration and apoptosis of NPC cells by RPS15A stable knockdown cell models. Additionally, we preliminarily investigated the apoptosis-related mechanism of RPS15A-induced malignant phenotypes of NPC cells by Human Apoptosis Antibody Array.

Materials And Methods

Clinical tissue samples and immunofluorescence histochemical staining (IHC) staining

Human tissue chip (Cat No. HNasN132Su01) containing 107 of NPC tissues and 30 of para-carcinoma normal tissues was purchased from Shanghai Outdo Biotech Company (Shanghai, China) and was used to detect the expression of RPS15A. The detailed pathological characters and the informed consents from patients were collected. This study was approved by the Ethics Committee of Kunming Medical University. For the IHC staining of RPS15A, briefly, all the fixed tissues were firstly dewaxed followed by rehydrating. Then the tissues were incubated with primary antibody anti-RPS15A (1:100, Lot No.PA5-51314, Invitrogen) at 4°C overnight following antigen repair and blocking. The secondary HRP-conjugated goat anti-rabbit IgG (1:400, Lot No. ab97080, Abcam) were then incubated with tissue slides at 37°C for 1 h. These slides were finally colored by diaminobenzene (DAB), counterstained by hematoxylin, dehydrated by alcohol, transparent with xylene, and sealed by neutral gum, respectively. The results of IHC staining were reviewed by two independent histopathologists and scored by percentages of positive staining cells (1, 0~24%; 2, 25~49%; 3, 50~74%; 4, 75~100%) and the staining intensity (0, no staining signals; 1, light yellow; 2, pale brown; 3, seal brown). The final IHC scores were determined as product of percentages of positive staining cells and the staining intensity: 0 score (-), 1-4 scores (+), 5-8 scores (++), 9-12 scores (+++) [23].

Cell culture

The human NPC cell lines (CNE-2Z, C666-1, 5-8F, HONE-1) were purchased from the American Type Culture Collection (ATCC) (Manassas, USA). These cell lines were all cultured in 90% RPMI-1640 medium

(Gibco, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco, USA) and 1% Penicillin/Streptomycin (100 U/mL) (Beyotime, China). Cells were maintained in a 5% CO₂ incubator at 37°C.

RNA interference and cell transfection

The RPS15A silencing lentiviral vector (shRPS15A) and its control vector (shCtrl) were constructed in Shanghai YiBR Biosciences Co., Ltd (Shanghai, China). According to the manufacturer's instructions, three RNA interference sequences were firstly determined as follows: shRPS15A-1, 5'-GTGCAACTCAAAGACCTGGAA-3'; shRPS15A-2, 5'-GCGCATGAATGTCCTGGCAGA-3'; shRPS15A-3, 5'-GATGACCACAGAGCTGGGAAA-3'. Then these sequences were synthesized into double stranded DNA oligo followed by connecting it to BR-V108 plasmid (Yibeirui, China) to generate the shRPS15A lentiviral vectors. Subsequently, the connected products were transduced into *Escherichia coli* cells (Cat. #CB104-03, TIANGEN), and positive cloned plasmids were selected for further amplification. Then the validated plasmids were co-transfected with pMD2.G (Qiagen, China) and pSPAX2 (Qiagen, China) for lentivirus generation. For lentivirus transfection, the NPC cell lines were transfected with shRPS15A lentivirus or shCtrl lentivirus using Lipofectamine 2000 (Thermo, USA) at a multiplicity of infection (MOI) of 20. Transfection efficacy was determined by observing the GFP expression.

Real-time quantitative PCR (qPCR)

Total RNA was isolated from the NPC cell samples using TRIzol reagent (Sigma, USA) according to its manufacturer's protocol. The isolated RNA was quantified by Nanodrop 100 (Thermo, USA) and reversely transcribed into cDNA using Hiscript QRT supermix (Vazyme, China). The SYBR Green mastermix Kit (Vazyme, China) and Biosystems 7500 Sequence Detection system were used to amplify targeted genes based on the cDNA template. The targeted genes expression was normalized by GAPDH and calculated according to the $2^{-\Delta\Delta C_t}$ method. Specific primers used in this study were shown as follows: RPS15A-forward: 5'-CGCGCCGCCACAATG-3' and reverse: 5'-CACAGTGAGAAACCGGACGA-3'; GAPDH-forward: 5'-TGACTTCAACAGCGACACCCA-3' and reverse: 5'-CACCTGTTGCTGTAGCCAAA-3'.

Western blotting (WB) analysis

Cell lysates were prepared by radioimmunoprecipitation (RIPA) lysis for total proteins extraction. The protein concentrations were quantified by bicinchoninic acid (BCA) Protein Assay Kit (HyClone-Pierce, USA). 20 µg of proteins were loaded and electrophoresed by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Invitrogen, USA) followed by transferring onto polyvinylidene difluoride (PVDF) membranes (Millipore, USA). The membranes were then blocked by 5% skimmed milk and then incubated with primary antibodies: anti-RPS15A (1:1000, Lot No. PA5-51314, Invitrogen), anti-AKT (1:1000, Lot No. 4685, CST), anti-p-AKT (1:500, Lot No. AF887-sp, R&D), anti-CCND1 (1:1000, Lot

No. 2978, CST), anti-CDK6 (1:1000, Lot No. ab151247, Abcam), anti-PIK3CA (1:1000, Lot No. ab40776, Abcam) and anti-GAPDH (1:3000, Lot No. AP0063, Bioworld) at 4°C overnight. After TBST washing, the membranes were incubated with secondary horseradish peroxidase (HRP)-conjugated goat anti-rabbit (1:3000, Lot No. A0208, Beyotime) at room temperature for 1 h. Protein expression levels were visualized with enhanced chemiluminescence (ECL) kit (Millipore, USA). GAPDH served as an inner control.

Cell proliferation assay

Cell viability was assessed by MTT assays. In brief, the NPC cells were transfected with shCtrl or shRPS15A lentivirus until grew at 85% confluence. Cell were then harvested and plated into 96-well plates at a density of 2×10^3 cells per well. 24 h after inoculation, 20 μ L of MTT solution (5 mg/mL) was added into each well 4 h before termination of culture and incubated for 4 h. After that, 100 μ L of DMSO was added to stop reaction. Finally, the optical density (OD) value at 490 nm was determined by microplate reader (Thermo, USA) and the growth curve was plotted based on the OD value.

Colony formation assay

The NPC cell lines transfected with indicated lentivirus for 5 days were harvested for colony formation assays. The transfected cells were counted and seeded into 6-well plates at a density of 900 cells per well. After 8 days of culture, the cell colonies were fixed by 4% paraformaldehyde for 30 min and stained by GIEMSA for 5 min. Then the colony numbers were determined under microscope.

Cell apoptosis analysis

Cell apoptosis was examined by flow cytometry. Firstly, the NPC cell lines were transfected with indicated lentivirus and cultured until the cell fusion reached 85%. Cells were then collected and re-suspended for Annexin V-APC (eBioscience, USA) staining. 10 min of incubation away from light, cells were washed three times with PBS followed by quantification of positive staining cells by flow cytometer (Millipore, USA).

Cell migration analysis

Cell migration was evaluated by Wound-healing assays and Transwell assays. In brief, the NPC cell lines transfected with indicated lentivirus were harvested when grown at 80% confluence. For wound-healing assays, cells were then re-suspended, counted and plated into 96-well plates at a density of 5×10^4 cells per well. The next day, scratches were formed using a scratch tester to push from bottom center of the 96-well plate to top. After removing of exfoliated cells by serum-free medium washing, cells were then cultured with 0.5% FBS-containing medium until indicated time points. Cellomics (Thermo, USA) was

used to determine migratory distance of cells in each group, and the migration rate was calculated based on the migratory distance.

For transwell assays, the required numbers of chambers were placed into an empty 24-well plate followed by addition of 100 μ L cell suspensions. Besides, 600 μ L of 30% FBS-containing medium was added into the lower chambers. After incubation for 24 h, the non-migrating cells were removed with cotton tip and then the cells were fixed with 4% paraformaldehyde for 30 min and stained with Giemsa for 5 min. The migratory cells were determined under fluorescence microscope (Olympus, Japan).

Mice xenograft Model

10 of BALB/c nude mice (female, four-weeks-old) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd (Beijing, China). Animal procedures were approved by Animal Care Committee of Kunming Medical University (No. kmmu 20211172). The xenograft models were established by subcutaneous injection of C666-1 cells (4×10^6 per mice) transfected with indicated lentivirus. One week after injection, the tumor sizes were monitored twice weekly using a Vernier caliper, and the tumor volumes were calculated as follows: $V = \pi/6 \times L \times W^2$ (L, represents the longest diameter; W, represents the shorter diameter). At the end of this experiments, all mice were sacrificed by injection of pentobarbital sodium as described previously [24] and the tumor were removed for weighting, photographs and Ki-67 staining.

Human Apoptosis Antibody Array

The Human Apoptosis Antibody Array (Cat No. ab134001, Abcam) assay was performed to reveal the expression alterations of apoptotic proteins. Briefly, the C666-1 cell transfected with shCtrl or shRPS15A lentivirus were prepared and lysed by lysis buffer. Cell lysates were then blocked using blocking buffer followed by incubation with the biotin-conjugated anti-cytolines at 4°C overnight, and streptavidin-HRP at room temperature for 2 h, respectively. Finally, the pixel density on the membrane was evaluated by enhanced ECL and quantified by ImageJ software.

Bioinformatics analysis

The independent dataset GSE12452 containing 10 of normal nasopharyngeal tissue and 31 of NPC tissues were available on GEO database (<https://www.ncbi.nlm.nih.gov>). RNA-Seq data were expressed as Fragments Per Kilobase Million (FPKM). The differentially expressed genes (DEGs) between normal tissues and tumor tissues were determined by following criteria: |Fold Change| ≥ 1.5 and p value < 0.05 . RPS15A was identified as a significant upregulated gene in NPC tissues compared to that in normal tissues.

Statistical analysis

Each experiment in this study was performed in triplicate. Graphpad Prism 8.04 software was used to perform statistical analyses and graph. Data were shown as mean $\pm$ SD. The two-tailed Student's *t*-test was used to determine *p* values between two groups. For multiple groups, the *p* values were determined by one-way analysis of variance (ANOVA). A value of $p < 0.05$ was considered statistically significant.

Results

Identification of RPS15A as a critical gene in NPC progression

Based on the gene microarray data of 10 normal nasopharyngeal samples and 31 NPC samples from the GEO database GSE12452 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE12452>), a total of 3334 DEGs were identified, with 1755 up-regulated mRNAs and 1579 down-regulated mRNAs ($|\text{Fold Change}| \geq 1.5$, $\text{FDR} < 0.05$) (Fig. 1A). Among them, RPS15A were significantly up-regulated NPC tissues compared to normal nasopharyngeal tissues ($P = 0.0014$ and $\log |\text{FC}| = 0.68$) (Fig. 1B). Moreover, IHC staining of human tissue chips showed that RPS15A was mainly presented in the NPC tissues, especially expressed strongly in metastatic NPC tissues, while weak staining for RPS15A was observed in normal nasopharyngeal tissues (Fig. 1C, Table 1). We further detected the correlation of RPS15A expression with clinicopathological characteristics of NPC patients. Intriguingly, both Mann-Whitney analysis and Spearman correlation analysis confirmed that RPS15A levels were positively associated with cervical lymph node metastasis (Table 2 and Table 3). Collectively, these results suggested that RPS15A played a vital role in the progression of NPC.

Construction of RPS15A knockdown stable cell lines in NPC cells

In order to explore the specific role of RPS15A in NPC, C666-1 and CNE-2Z cell lines were selected to construct RPS15A knockdown cell model. Briefly, three shRNA sequences targeting RPS15A gene were designed and applied to interfere NPC cells. Among three shRNAs, shRPS15A-1 and shRPS15A-2 demonstrated high efficiency of EIF3B knockdown, especially shRPS15A-1 (Fig. 2A). Moreover, Fig. 2B revealed that RPS15A was smoothly knocked down using lentivirus mediated RPS15A shRNA (shRPS15A-1) in C666-1 and CNE-2Z cells. The mRNA and protein levels of RPS15A in C666-1 and CNE-2Z cells were visibly decreased after infection with shRPS15A (Fig. 2C-D). Collectively, these results indicated that RPS15A knockdown was constructed successfully in C666-1 and CNE-2Z cell lines and shRPS15A-1 could be used to perform further cell function tests.

RPS15A knockdown repressed NPC cell viability

To determine the effect of RPS15A knockdown on cell viability *in vitro*, cell proliferation was performed on C666-1 and CNE-2Z cells by using the MTT assay. Our results showed that RPS15A knockdown significantly suppressed cell proliferation of C666-1 and CNE-2Z cells (Fig. 3A). Moreover, Lv-shRPS15A infected NPC cells formed much fewer colonies compared with those obtained with Lv-shCtrl infected cells (Fig. 3B). Furthermore, flow cytometry with Annexin V-APC single-staining method showed that RPS15A knockdown boosted cell apoptosis in C666-1 and CNE-2Z cells (Fig. 3C). These results demonstrated that RPS15A knockdown suppressed cell viability both in C666-1 and CNE-2Z cells.

RPS15A knockdown repressed NPC cell migration

The role of RPS15A in cell migration of C666-1 and CNE-2Z cells was further investigated by wound healing assay and Transwell assays. In wound healing assays, the C666-1 and CNE-2Z cells transfected with shRPS15A lentivirus displayed impairment of migration ability at 48 h compared to cells transfected with shCtrl lentivirus (Fig. 4A). Moreover, Transwell assays also showed that RPS15A knockdown attenuated the migration ability of C666-1 and CNE-2Z cells (Fig. 4B). Collectively, these results suggested that RPS15A knockdown inhibited the migration capacities of NPC cells *in vitro*.

RPS15A knockdown inhibited tumor growth in nude mice

Given our *in vitro* results showed that RPS15A knockdown could suppress NPC tumor cell phenotypes, we further investigated the effects of down-regulated RPS15A on NPC tumor growth *in vivo* using xenograft mouse model. Briefly, RPS15A knockdown C666-1 cells were subcutaneously injected into nude mice (n=5), and down-regulated RPS15A significantly decreased tumor growth *in vivo* compared with shCtrl group (n=5) (Fig. 5A). 27 days after injection, mice were sacrificed, and tumors were separated to weigh. As shown in Fig. 5B, the size of tumor in shRPS15A group were significantly smaller than those in the shCtrl group. Moreover, RPS15A knockdown diminished tumor weight (Fig. 5C). Consistently, the expression of Ki67 protein was significantly inhibited by shRPS15A, suggesting that the proliferation ability of tumor cells was weakened (Fig. 5D). Taken together, these data indicated that RPS15A knockdown inhibited NPC tumor growth *in vivo*.

RPS15A knockdown regulated the expression levels of proteins related to biological function of NPC cells

To further unveil the mechanisms behind RPS15A regulating the malignant behaviors of NPC cells, we first carried out a Human Apoptosis Antibody Array in C666-1 cells. As shown in Fig. 6A-B, RPS15A knockdown increased the expression of the pro-apoptotic elements CD40, CD40L, cytoC, DR6, FasL, HSP60, IGFBP-3, p21 and TNF- β . These data further imply that RPS15A knockdown contributed to apoptosis of NPC cells. Additionally, we also found that RPS15A knockdown significantly down-regulated

the protein levels of P-AKT, CCND1, CDK6 and PIK3CA (Fig. 6C). Taken together, these results suggested that RPS15A depletion had an inhibitory effect on the progression of NPC.

Discussion

NPC is a high incidence of malignant squamous cell carcinoma in southern China, southeast Asia and north Africa, threatening the health of many people [25]. Despite great progress in radiotherapy and chemoradiotherapy for NPC, the efficacy of locally advanced NPC remains suboptimal [26]. At present, efforts have been made to identify biomarkers that can diagnose the risk of NPC patients or novel targeted therapy that can improve the prognosis of patients [25]. Therefore, it is necessary to explore the mechanics of NPC progression in depth. In recent years, the relationship between ribosomal protein family members and tumor progression has attracted much attention. There is considerable evidence indicated that RPS15A is abnormally high expressed in various types of human cancers, including liver cancer, colorectal cancer, lung adenocarcinoma, breast cancer, and osteosarcoma [16, 18, 20, 27, 28]. In this study, we highlighted that RPS15A is abundantly expressed in NPC and positively correlated with cervical lymph node metastasis. These results suggested that elevated expression of RPS15A predicts the deepening of tumor malignancy, which may be used as a novel diagnostic biomarker for NPC with important clinical significance.

Moreover, the functions of RPS15A in various biological processes have been clarified, such as being responsible for the regulation of cell division and participating in tumor progression [17, 29]. In particular, the role of RPS15A in tumor progression has been increasingly recognized [15, 19, 22, 30-33]. Therefore, RPS15A is currently considered as a promising target for cancer therapy in the design of potent anticancer drugs. Consistently, the present study demonstrates a key driving role of RPS15A in the proliferation and migration of NPC. Moreover, we further revealed that knockdown of RPS15A significantly inhibited the tumorigenic ability of NPC cells in mice. The above findings strongly reveal that RPS15A plays an extremely important role in promoting NPC. Furthermore, our data suggested that knockdown of RPS15A can induce apoptosis in NPC cells. Meanwhile, knockdown of RPS15A resulted in abnormal expression of a series of apoptosis-related factors, such as up-regulation of CD40, CD40L, cytoC, DR6, FasL, HSP60, IGFBP-3, p21 and TNF- β . Therefore, we suspected that RPS15A regulates the progression of NPC by activating these apoptosis-related factors to initiate apoptotic signaling pathways.

As we all known, PI3K/AKT signaling pathway has been widely reported to be one of the most common and important pathways for cancers [34-36]. The pathway includes multiple core components and is controlled at multiple levels. In this study, we found that RPS15A knockdown could down-regulated the expression of p-AKT and PIK3CA. Additionally, knockdown of RPS15A significantly reduced the expression of CCND1/CDK6 at the protein level. CCND1 and CDK6 are cell cycle promoter that induces cell proliferation in tumors [37]. Taken together, these results suggested that RPS15A can induce NPC cell progression through the PI3K/AKT signaling pathway and CCND1/CDK6. Of course, the specific molecular mechanism is worthy of our further study.

Conclusion

In this study, we revealed the expression level and role of RPS15A in NPC. Our data showed that RPS15A is significantly up-regulated in NPC. Moreover, knockdown of RPS15A inhibited the proliferation, migration and tumorigenic capacity of NPC cells. In summary, RPS15A may enhance NPC progression by PI3K/AKT signaling pathway, which may be a potential therapeutic target to prevent NPC progression.

Abbreviations

NPC: Nasopharyngeal carcinoma

RPS15A: Ribosomal protein S15a

IHC: Immunofluorescence histochemical

DAB: Diaminobenzene

MOI: Multiplicity of infection

qPCR: Real-time quantitative PCR

RIPA: Radioimmunoprecipitation

HRP: Horseradish peroxidase

ECL: Enhanced chemiluminescence

OD: Optical density

FPKM: Fragments Per Kilobase Million

DEGs: Differentially expressed genes

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Kunming Medical University.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no conflict of interest.

Funding

None.

Authors' contributions

Jin Bian designed this research. Yan Niu operated the cell and animal experiments. Yan Niu and Cao Lv conducted the data processing and analysis. Yan Niu drafted the manuscript which was reviewed by Jin Bian. All the authors have confirmed the submission of this manuscript.

Acknowledgements

None.

References

1. Chen YP, Chan ATC, Le QT, Blanchard P, Sun Y, Ma J. Nasopharyngeal carcinoma. *Lancet*. 2019; 394: 64-80. doi: 10.1016/S0140-6736(19)30956-0
2. Lee HM, Okuda KS, Gonzalez FE, Patel V. Current Perspectives on Nasopharyngeal Carcinoma. *Adv Exp Med Biol*. 2019; 1164: 11-34. doi: 10.1007/978-3-030-22254-3_2
3. Guo R, Mao YP, Tang LL, Chen L, Sun Y, Ma J. The evolution of nasopharyngeal carcinoma staging. *Br J Radiol*. 2019; 92: 20190244. doi: 10.1259/bjr.20190244
4. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018; 68: 394-424. doi: 10.3322/caac.21492
5. Tang LL, Chen WQ, Xue WQ, He YQ, Zheng RS, Zeng YX, et al. Global trends in incidence and mortality of nasopharyngeal carcinoma. *Cancer Lett*. 2016; 374: 22-30. doi: 10.1016/j.canlet.2016.01.040

6. Lam WKJ, Chan JYK. Recent advances in the management of nasopharyngeal carcinoma. *F1000Res*. 2018; 7. doi: 10.12688/f1000research.15066.1
7. Masterson L, Howard J, Gonzalez-Cruz J, Jackson C, Barnett C, Overton L, et al. Immune checkpoint inhibitors in advanced nasopharyngeal carcinoma: Beyond an era of chemoradiation? *Int J Cancer*. 2020; 146: 2305-2314. doi: 10.1002/ijc.32869
8. Chow JC, Ngan RK, Cheung KM, Cho WC. Immunotherapeutic approaches in nasopharyngeal carcinoma. *Expert Opin Biol Ther*. 2019; 19: 1165-1172. doi: 10.1080/14712598.2019.1650910
9. Kaidar-Person O, Gil Z, Billan S. Precision medicine in head and neck cancer. *Drug Resist Updat*. 2018; 40: 13-16. doi: 10.1016/j.drug.2018.09.001
10. Fatica A, Tollervey D. Making ribosomes. *Curr Opin Cell Biol*. 2002; 14: 313-318. doi: 10.1016/s0955-0674(02)00336-8
11. Ruggero D, Pandolfi PP. Does the ribosome translate cancer? *Nat Rev Cancer*. 2003; 3: 179-192. doi: 10.1038/nrc1015
12. Barna M, Pusic A, Zollo O, Costa M, Kondrashov N, Rego E, et al. Suppression of Myc oncogenic activity by ribosomal protein haploinsufficiency. *Nature*. 2008; 456: 971-975. doi: 10.1038/nature07449
13. Jimenez L, Becerra A, Landa A. Cloning, expression and partial characterization of a gene encoding the S15a ribosomal protein of *Taenia solium*. *Parasitol Res*. 2004; 92: 414-420. doi: 10.1007/s00436-003-1021-4
14. Guo P, Wang Y, Dai C, Tao C, Wu F, Xie X, et al. Ribosomal protein S15a promotes tumor angiogenesis via enhancing Wnt/beta-catenin-induced FGF18 expression in hepatocellular carcinoma. *Oncogene*. 2018; 37: 1220-1236. doi: 10.1038/s41388-017-0017-y
15. Liang J, Liu Y, Zhang L, Tan J, Li E, Li F. Overexpression of microRNA-519d-3p suppressed the growth of pancreatic cancer cells by inhibiting ribosomal protein S15A-mediated Wnt/beta-catenin signaling. *Chem Biol Interact*. 2019; 304: 1-9. doi: 10.1016/j.cbi.2019.02.026
16. Feng W, Liang C, Wang C, Yu X, Li Q, Yang H. Knockdown of ribosomal protein S15A inhibits proliferation of breast cancer cells through induction of apoptosis in vitro. *Cytotechnology*. 2018; 70: 1315-1323. doi: 10.1007/s10616-018-0221-9
17. Wan BS, Wang XY, Tiang J, Zhou C, Lin J, Wang Z. Ribosomal protein RPS15A augments proliferation of colorectal cancer RKO cells via regulation of BIRC3, p38 MAPK and Chk1. *Eur Rev Med Pharmacol Sci*. 2021; 25: 3967-3980. doi: 10.26355/eurev_202106_26037
18. Xu M, Wang Y, Chen L, Pan B, Chen F, Fang Y, et al. Down-regulation of ribosomal protein S15A mRNA with a short hairpin RNA inhibits human hepatic cancer cell growth in vitro. *Gene*. 2014; 536: 84-89. doi: 10.1016/j.gene.2013.11.075
19. Zhao X, Shen L, Feng Y, Yu H, Wu X, Chang J, et al. Decreased expression of RPS15A suppresses proliferation of lung cancer cells. *Tumour Biol*. 2015; 36: 6733-6740. doi: 10.1007/s13277-015-3371-9

20. Zhang C, Zhang T, Song E, Himaya SW, Chen X, Zheng L. Ribosomal protein S15A augments human osteosarcoma cell proliferation in vitro. *Cancer Biother Radiopharm.* 2014; 29: 451-456. doi: 10.1089/cbr.2014.1698
21. Liang J, Liu Z, Zou Z, Wang X, Tang Y, Zhou C, et al. Knockdown of ribosomal protein S15A inhibits human kidney cancer cell growth in vitro and in vivo. *Mol Med Rep.* 2019; 19: 1117-1127. doi: 10.3892/mmr.2018.9751
22. Zhang C, Fu J, Xue F, Ryu B, Zhang T, Zhang S, et al. Knockdown of ribosomal protein S15A induces human glioblastoma cell apoptosis. *World J Surg Oncol.* 2016; 14: 129. doi: 10.1186/s12957-016-0891-8
23. Ma Y, Ma L, Guo Q, Zhang S. Expression of bone morphogenetic protein-2 and its receptors in epithelial ovarian cancer and their influence on the prognosis of ovarian cancer patients. *J Exp Clin Cancer Res.* 2010; 29: 85. doi: 10.1186/1756-9966-29-85
24. Haage V, Semtner M, Vidal RO, Hernandez DP, Pong WW, Chen Z, et al. Comprehensive gene expression meta-analysis identifies signature genes that distinguish microglia from peripheral monocytes/macrophages in health and glioma. *Acta Neuropathol Commun.* 2019; 7: 20. doi: 10.1186/s40478-019-0665-y
25. Wong KCW, Hui EP, Lo KW, Lam WKJ, Johnson D, Li L, et al. Nasopharyngeal carcinoma: an evolving paradigm. *Nat Rev Clin Oncol.* 2021; 18: 679-695. doi: 10.1038/s41571-021-00524-x
26. Mane M, Benkhaled S, Dragan T, Paesmans M, Beauvois S, Lalami Y, et al. Meta-Analysis on Induction Chemotherapy in Locally Advanced Nasopharyngeal Carcinoma. *Oncologist.* 2021; 26: e130-e141. doi: 10.1002/ONCO.13520
27. Zhang Y, Zhang G, Li X, Li B, Zhang X. The effect of ribosomal protein S15a in lung adenocarcinoma. *PeerJ.* 2016; 4: e1792. doi: 10.7717/peerj.1792
28. Chen J, Wei Y, Feng Q, Ren L, He G, Chang W, et al. Ribosomal protein S15A promotes malignant transformation and predicts poor outcome in colorectal cancer through misregulation of p53 signaling pathway. *Int J Oncol.* 2016; 48: 1628-1638. doi: 10.3892/ijo.2016.3366
29. Li G, Zhang L, Liu J, Xiao T, Liu G, Wang J, et al. shRNA-mediated RPS15A silencing inhibits U937 acute myeloid leukemia cell proliferation and enhances apoptosis. *Mol Med Rep.* 2016; 13: 4400-4406. doi: 10.3892/mmr.2016.5064
30. Yao Y, Liu Y, Lv X, Dong B, Wang F, Li J, et al. Down-regulation of ribosomal protein S15A inhibits proliferation of human glioblastoma cells in vivo and in vitro via AKT pathway. *Tumour Biol.* 2016; 37: 4979-4990. doi: 10.1007/s13277-015-4323-0
31. Liu C, He X, Liu X, Yu J, Zhang M, Yu F, et al. RPS15A promotes gastric cancer progression via activation of the Akt/IKK-beta/NF-kappaB signalling pathway. *J Cell Mol Med.* 2019; 23: 2207-2218. doi: 10.1111/jcmm.14141
32. Shi D, Liu J. RPS15a Silencing Suppresses Cell Proliferation and Migration of Gastric Cancer. *Yonsei Med J.* 2018; 59: 1166-1173. doi: 10.3349/ymj.2018.59.10.1166

33. Ning Q, Pang Y, Shao S, Luo M, Zhao L, Hu T, et al. MicroRNA-147b suppresses the proliferation and invasion of non-small-cell lung cancer cells through downregulation of Wnt/beta-catenin signalling via targeting of RPS15A. *Clin Exp Pharmacol Physiol*. 2020; 47: 449-458. doi: 10.1111/1440-1681.13203
34. Iksen, Pothongsrisit S, Pongrakhananon V. Targeting the PI3K/AKT/mTOR Signaling Pathway in Lung Cancer: An Update Regarding Potential Drugs and Natural Products. *Molecules*. 2021; 26. doi: 10.3390/molecules26134100
35. Pungsrinont T, Kallenbach J, Baniahmad A. Role of PI3K-AKT-mTOR Pathway as a Pro-Survival Signaling and Resistance-Mediating Mechanism to Therapy of Prostate Cancer. *Int J Mol Sci*. 2021; 22. doi: 10.3390/ijms222011088
36. Wu S, Chen M, Huang J, Zhang F, Lv Z, Jia Y, et al. ORAI2 Promotes Gastric Cancer Tumorigenicity and Metastasis through PI3K/Akt Signaling and MAPK-Dependent Focal Adhesion Disassembly. *Cancer Res*. 2021; 81: 986-1000. doi: 10.1158/0008-5472.CAN-20-0049
37. Li N, Zeng J, Sun F, Tong X, Meng G, Wu C, et al. p27 inhibits CDK6/CCND1 complex formation resulting in cell cycle arrest and inhibition of cell proliferation. *Cell Cycle*. 2018; 17: 2335-2348. doi: 10.1080/15384101.2018.1526598

Tables

Table 1

Expression patterns in nasopharyngeal carcinoma tissues and para-carcinoma tissues revealed in immunohistochemistry analysis

RPS15A expression	Tumor tissue		Para-carcinoma tissue		p value
	Cases	Percentage	Cases	Percentage	<0.001
Low	42	39.3%	30	100%	
High	65	60.7%	0	-	

Table 2

Relationship between RPS15A expression and tumor characteristics in patients with nasopharyngeal carcinoma

Features	No. of patients	RPS15A expression		p value
		low	high	
All patients	107	42	65	
Age (years)				0.871
≤48	55	22	33	
>48	52	20	32	
Gender				0.855
Male	80	31	49	
Female	27	11	16	
Tumor size				0.518
≤1.2cm	52	22	30	
>1.2cm	42	15	27	
Stage				0.220
1	15	8	7	
2	48	19	29	
3	28	10	18	
4	16	5	11	
Recurrence				0.306
no	57	20	37	
yes	49	22	27	
Cervical lymph node metastasis				<0.001
no	35	22	13	
yes	72	20	52	

Table 3

Relationship between RPS15A expression and tumor characteristics in patients with nasopharyngeal carcinoma

		RPS15A
Cervical lymph node metastasis	Spearman correlation	0.337
	Signification (double-tailed)	<0.000
	N	107

Figures

Figure 1

RPS15A was up-regulated in NPC

(A) The clustered heatmap showed DEGs between normal nasopharyngeal tissues and NPC tissues, red represents relative up-regulation, and green represents relative down-regulation, black neutral, and gray scale indicates that the signal strength is not high enough to detect. (B) The correlation analysis of RPS15A expression in NPC tissues and normal nasopharyngeal tissues. (C) The expression levels of RPS15A in normal nasopharyngeal tissues and NPC tissues were determined by immunohistochemical staining.

Figure 2

Construction of RPS15A knockdown stable cell lines in NPC cells

(A) The knockdown efficiency of 3 lentiviruses that interfered with RPS15A expression was determined by qPCR. (B) The expression of green fluorescence in C666-1 and CNE-2Z cells infected with shRPS15A lentivirus was observed to evaluate the efficiency of infection. Lentiviruses carried the green fluorescence

protein gene. (C) The mRNA levels of RPS15A in C666-1 and CNE-2Z cells was detected by qPCR to assess the knockdown efficiency. (D) The protein levels of RPS15A in C666-1 and CNE-2Z cells was detected by western blot to assess the knockdown efficiency. Results were presented as mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 3

RPS15A knockdown repressed NPC cell viability

(A) MTT assay was conducted to assess the effect of RPS15A silencing on cell proliferation. (B) Plate colony assay was employed to evaluate the ability of colony-formation of C666-1 and CNE-2Z cells affected by RPS15A knockdown. (C) The effects of RPS15A knockdown on C666-1 and CNE-2Z cell apoptosis were examined by flow cytometry. Results were presented as mean $\pm$ SD. *** $P < 0.001$.

Figure 4

RPS15A knockdown repressed NPC cell migration

(A) The effects of RPS15A knockdown on the migration of C666-1 and CNE-2Z cells were detected using Wound healing. (B) The effects of RPS15A knockdown on the migration of C666-1 and CNE-2Z cells were detected using Transwell. Results were presented as mean $\pm$ SD. *** $P < 0.001$.

Figure 5

RPS15A knockdown suppressed tumor growth

(A) The growth curve of tumor volume was measured in nude mice. (B) The photograph of tumors was taken after removing tumors. (C) Measurement of tumor weight in nude mice. (D) The expression of Ki67 was detected by immunohistochemistry. Results were presented as mean $\pm$ SD. ** $P < 0.01$, *** $P < 0.001$.

Figure 6

The effects of RPS15A knockdown on the proteins related to biological function of NPC cells

(A) The levels of apoptosis-related proteins in C666-1 cells infected with shCtrl and shRPS15A were measured using a human apoptosis antibody array. (B) Protein expression was visualized by R studio and presented in gray value. (C) Several cancer-related protein was detected by WB. GAPDH was used as the loading control. Results were presented as mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$.