Molecular Mechanisms Underlying the Role of RPS4X in Promoting Gastric Cancer Progression Based on Bioinformatics

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Abstract [**Objectives**] To investigate the expression patterns of RPS4X in gastric cancer and the related molecular mechanisms promoting the progression of gastric cancer based on bioinformatics analysis, and identify potential targets for the early diagnosis and clinical management of gastric cancer. [**Methods**] Gene expression data for gastric cancer were obtained from the TCGA database. GEPIA2 and UALCAN were used to analyze differential expression of RPS4X and its relationship with patient survival prognosis. The protein-protein interaction (PPI) network was constructed using STRING. GO and KEGG enrichment analyses were performed using DAVID. GSEA was conducted with the clusterProfiler package in R language. The expression of RPS4X in normal gastric mucosal epithelial cells and gastric cancer cells was measured by RT-qPCR assay. [**Results**] The expression of RPS4X was significantly increased in gastric cancer tissues and correlated with poor patient prognosis ($P<0.05$). PPI network analysis indicated that RPS4X may interact with proteins such as RPL13, RPS23, RPS3, and RPS12. GO and KEGG enrichment analysis suggested that RPS4X may be involved in biological functions such as cytoskeletal remodeling and signaling pathways related to cornified envelope formation. These findings were further verified by GSEA. RT-qPCR results showed that RPS4X expression levels in gastric cancer cells were significantly higher than those in normal gastric mucosal epithelial cells ($P<0.05$). [**Conclusions**] RPS4X is highly expressed in gastric cancer and may promote the occurrence and progression of the disease by regulating biological processes such as cytoskeletal remodeling and related signaling pathways, and has the potential to serve as a target for the early diagnosis and treatment of gastric cancer.

Key words Gastric cancer, RPS4X, Bioinformatics, RT-qPCR

1 Introduction

Gastric cancer is one of the most prevalent malignant tumors worldwide and ranks third in global mortality rates. Its high incidence and mortality are mainly associated with tumor invasion and metastasis[1-3]. The pathogenesis of gastric cancer is a complex, multistage process influenced by multiple factors. Due to the nonspecific nature of early clinical symptoms, most patients are diagnosed at intermediate or advanced stages, resulting in limited treatment efficacy and poor prognosis[4]. Therefore, a comprehensive investigation into the molecular mechanisms underlying the occurrence and progression of gastric cancer, along with the identification of novel diagnostic biomarkers and potential therapeutic targets, is of significant clinical importance for improving diagnostic accuracy, treatment outcomes, and patient prognosis.

Ribosomal proteins (RPs), as fundamental components of ribosomes, have traditionally been regarded as key factors involved in protein synthesis. Nevertheless, studies have shown that many RPs possess "extra-ribosomal" functions and participate in various biological processes such as cell proliferation, apoptosis, differentiation, and tumorigenesis[5]. RPS4X is a homolog of the ribosomal small subunit protein S4 located on the X chromosome[6]. Previous studies have shown that SLFN11 inhibits the occurrence and metastasis of hepatocellular carcinoma by targeting and regulating the mTOR pathway via RPS4X[1]. The down-regulation of RPS4X significantly inhibits the stemness of tumor epithelial cells, thereby suppressing liver metastasis of colorectal cancer[7]. These findings suggest that that RPS4X plays multiple roles in tumor biology.

This study employed bioinformatics to screen and verify the differential expression of RPS4X in gastric cancer and its correlation with the poor prognosis of patients, and systematically analyzed the potential molecular mechanisms facilitating gastric cancer progression, thereby providing a new theoretical foundation for the diagnosis and treatment of gastric cancer.

2 Materials and methods

2.1 Screening differentially expressed genes in gastric cancer The transcriptome expression dataset for gastric cancer (STAD) was obtained from TCGA database (https://portal.gdc.cancer.gov/). Differential expression analysis was performed using the limma package. The screening threshold were set as the absolute value of fold change ($|\log_2 FC|>1$), with statistical significance determined by a Benjamini-Hochberg adjusted P-value of less than 0.05. Gene expression differences across groups were visually displayed using heat maps generated by the pheatmap package.

2.2 Expression differences of *RPS4X* across various levels and age groups Using the UALCAN network analysis tool (http://ualcan.path.uab.edu/index.html), differences in *RPS4X* gene expression among gastric cancer patients were examined across various cancer stages, age groups, and genders. The screening criteria were established as $|\log_2 FC|>1$ and $P<0.01$.

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Subsequently, box plots were drawn for visual analysis.

2.3 Relationship between *RPS4X* expression and overall survival in patients with gastric cancer Survival analysis was performed using the GEPIA2 database (http://gepia2.cancer-pku.cn/). Gastric cancer patients were divided into high-expression and low-expression groups according to the median expression level of the *RPS4X* gene. Kaplan-Meier survival curves were plotted, and the Log-rank test was used to assess differences in survival between the two groups of patients. A *P*-value of less than 0.05 was considered statistically significant.

2.4 Construction of the RPS4X protein-protein interaction (PPI) network Using the STRING database (https://string-db.org/), the gene name "*RPS4X*" was entered, and the species "*Homo sapiens*" was selected. The minimum interaction score was set to greater than 0.7 to ensure a high confidence level, and free nodes were hidden. Subsequently, potential interacting proteins of RPS4X were identified, and a PPI network was constructed. The network data were then exported.

2.5 GO enrichment analysis and KEGG pathway enrichment analysis GO enrichment analysis of the identified key factors was conducted using the Metascape online platform (https://metascape.org/), including three categories: biological processes (BP), cellular components (CC), and molecular functions (MF). Meanwhile, KEGG pathway enrichment analysis was conducted. A significance threshold of $P<0.05$ was applied to identify significantly enriched functional terms and signaling pathways, facilitating a systematic investigation of the biological functions and potential molecular regulatory mechanisms associated with the target genes.

2.6 Gene set enrichment analysis (GSEA) The clusterProfiler package in R language was used to conduct GSEA. The species parameter was set to *Homo sapiens*, and the gene set size was constrained to a range of 15 to 500 genes. Statistical significance was assessed using 1 000 permutation tests, with a false discovery rate (FDR) threshold of less than 0.25, adjusted by the Benjamini-Hochberg (BH) method. Enrichment results were visualized using the plotEnrichment function from the fgsea package, and the extent and significance of enrichment were quantitatively presented by the normalized enrichment score (NES) and FDR values.

2.7 Verification by *in vitro* experiments

2.7.1 Cell culture. Human normal gastric mucosal epithelial cell line GS-1 and human gastric cancer cell lines HGC-27, MGC-803, SGC-7901, and MKN45 were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. The cells were maintained under the conditions of 37 ℃ and 5% CO_2. When the cell density reached 80% – 90%, subculturing was performed at a ratio of 1∶3, and the culture medium was replaced 2 to 3 times per week.

2.7.2 RT-qPCR assay. Human gastric cancer cell lines (HGC-27, MGC-803, SGC-7901, and MKN45) and human normal gastric mucosal epithelial cells (GS-1) were collected. Total RNA was extracted using a centrifugation column method, and the RNA concentration and the A260/A280 ratio were measured with a nucleic acid quantification analyzer. The extracted RNA was then reverse transcribed into cDNA, which served as the template for subsequent RT-qPCR assays. *GAPDH* was employed as the internal reference gene, and the relative expression levels of the target gene were quantified using the $2^{-\Delta\Delta CT}$ method (Table 1).

Table 1 Primer sequence

Gene	Primer sequence
RPS4X	F primer: GATGGCAAGGTCCGAACTGA
	R primer: AAAGCGACCCTTGGTGTCAT
GAPDH	F primer: CTCCTCCTGTTCGACAGTCAGC
	R primer: CCCAATACGACCAAATCCGTT

2.8 Statistical analysis All statistical analyses were performed using GraphPad Prism 8 software. Experimental data were expressed as mean ± standard deviation. Differences between the normal group and the gastric cancer group were assessed using the *t*-test. A significance level of $\alpha=0.05$ was applied, with *P*-values less than 0.05 considered statistically significant.

3 Results and analysis

3.1 Screening differentially expressed genes in gastric cancer The differences in gene expression between gastric cancer tissue samples and normal tissue samples were presented using a heat map (Fig. 1). The analysis identified a total of 4 851 significantly differentially expressed genes, including 74 upregulated genes and 4 777 downregulated genes.

3.2 Expression differences of *RPS4X* across various levels and age groups Using the UALCAN network analysis tool, a total of 34 normal gastric tissue samples and 415 gastric adenocarcinoma tissue samples were selected for statistical analysis. The analysis showed that the expression level of *RPS4X* was significantly higher in gastric adenocarcinoma tissues compared to normal gastric tissues (Fig. 2A). Further statistical analysis was performed on 18 stage I, 123 stage II, 169 stage III, and 41 stage IV gastric adenocarcinoma tissue samples. The findings indicated that *RPS4X* expression was markedly increased in gastric adenocarcinoma tissues from stage II onward (Fig. 2B). An analysis of tumor tissues from 268 male and 147 female patients with gastric adenocarcinoma revealed that the expression level of *RPS4X* was significantly higher in the female group compared to the male group (Fig. 2C). Furthermore, an examination of patients divided by age—4 patients aged 21 to 40, 128 patients aged 41 to 60, 253 patients aged 61 to 80, and 25 patients aged 81 to 100—indicated that *RPS4X* expression was higher in tumor tissues of gastric adenocarcinoma patients over 40 years of age (Fig. 2D).

3.3 Relationship between *RPS4X* expression and overall survival in patients with gastric cancer The Kaplan-Meier survival analysis method was used to explore the correlation between *RPS4X* gene expression levels and overall survival in patients with gastric adenocarcinoma. Prognostic survival curves for gastric adenocarcinoma patients divided by high and low *RPS4X* expression

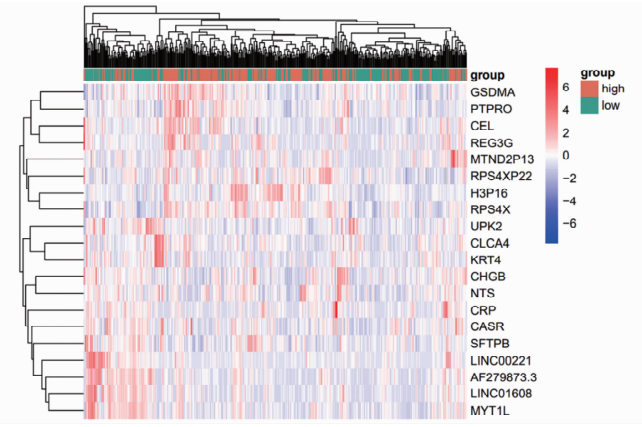


Fig. 1 Heat map of differentially expressed genes in gastric cancer

were plotted (Fig. 3). The results showed that patients with low *RPS4X* expression exhibited significantly prolonged overall survival compared to those with high expression, with the difference reaching statistical significance ($P<0.05$).

3.4 Construction of the RPS4X PPI network The PPI network was constructed using the STRING database (Fig. 4). The analysis revealed that RPS4X had direct interaction relationships with proteins including RPS23, RPL13, RPS3, and RPS12. Meanwhile, the network included other functional proteins such as RPS27A, RPL19, NACA, and PELO. These interacting proteins were primarily involved in biological processes such as ribosome assembly, protein translation, and cytoskeletal remodeling. This network provides a foundational reference for subsequent investigations into the molecular regulatory mechanisms underlying gastric cancer.

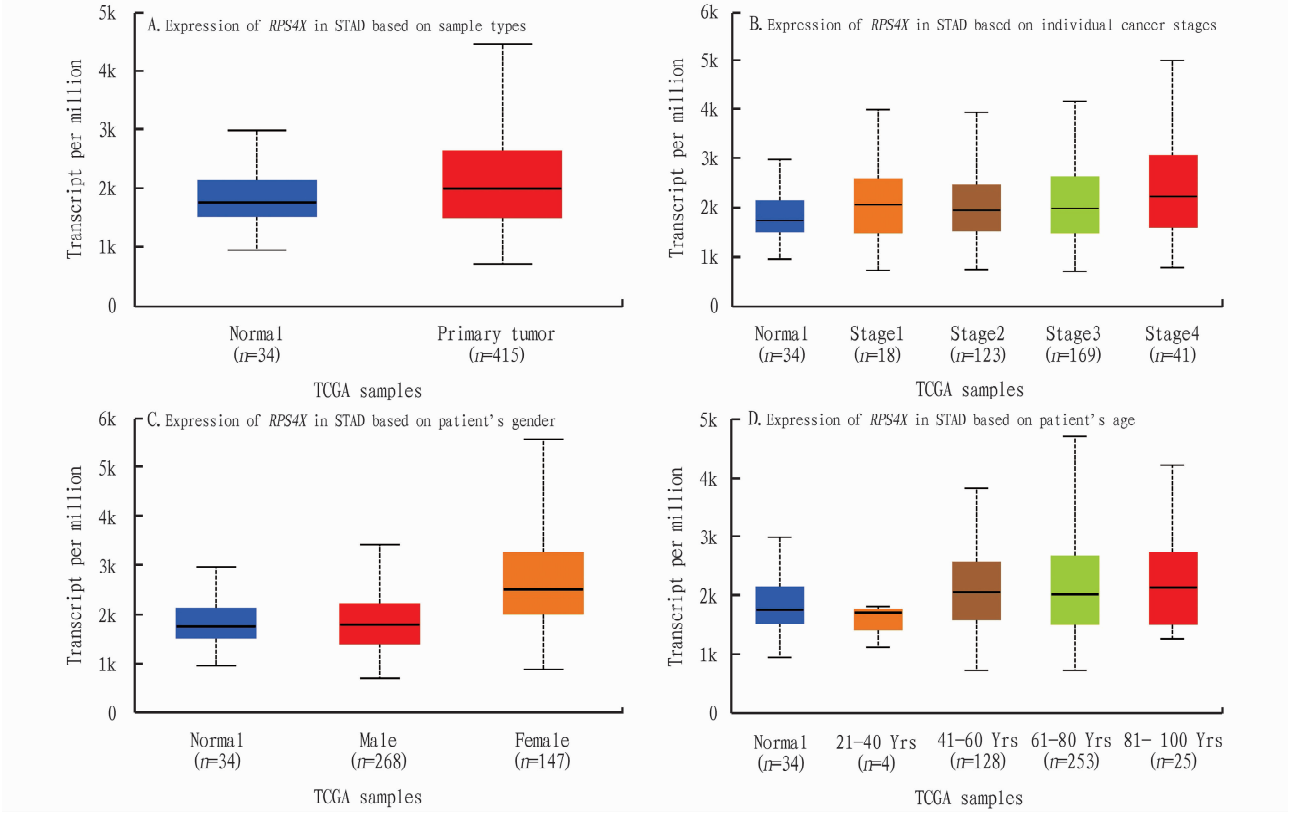


Fig. 2 Expression differences of *RPS4X* across various groups

3.5 GO enrichment analysis and KEGG pathway enrichment analysis GO enrichment analysis was conducted on the above targets. The *P*-values were ranked in ascending order, and the top 10 items were selected for visual analysis (Fig. 5A). At the biological process (BP) level, the genes were significantly enriched in processes related to keratinocytes and intermediate filament tissues. At the cellular component (CC) level, the enrichment items were highly concentrated in the intermediate filament cytoskeleton and myosin complex structures. At the molecular function (MF) level, significant enrichment was observed in activities such as G protein-coupled amine receptor activity. The above results indicate that the relevant genes may regulate cell proliferation and differentiation through the regulation of cellular structures, including the intermediate filament cytoskeleton and myosin complex, the mediation of signal transduction associated with G protein-coupled amine receptors, and the participation in biological processes such as keratinization and intermediate filament tissue formation. KEGG pathway enrichment analysis (Fig. 5B) showed that the core factors were mainly enriched in pathways related to cornified envelope formation and neuroactive ligand-receptor interaction. These findings suggest that these genes may contribute to tumorigenesis and tumor progression by

modulating the remodeling of the tumor cytoskeleton.

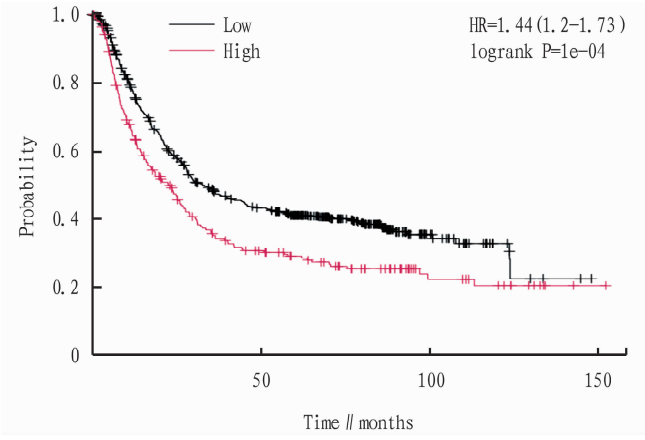


Fig. 3 Overall survival rate of gastric cancer patients with different *RPS4X* expression states

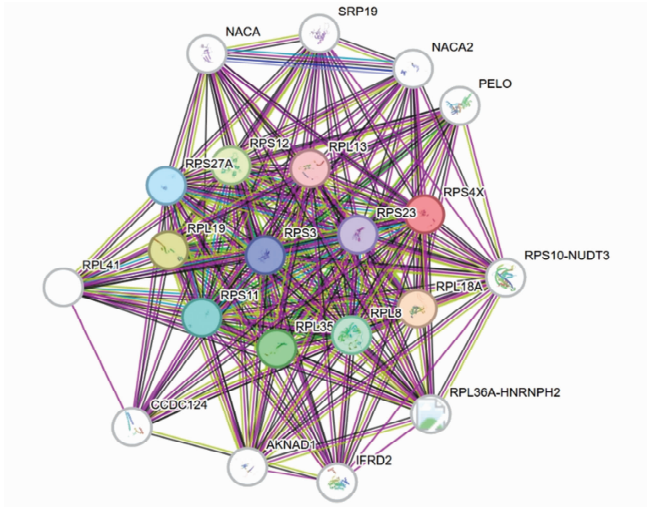
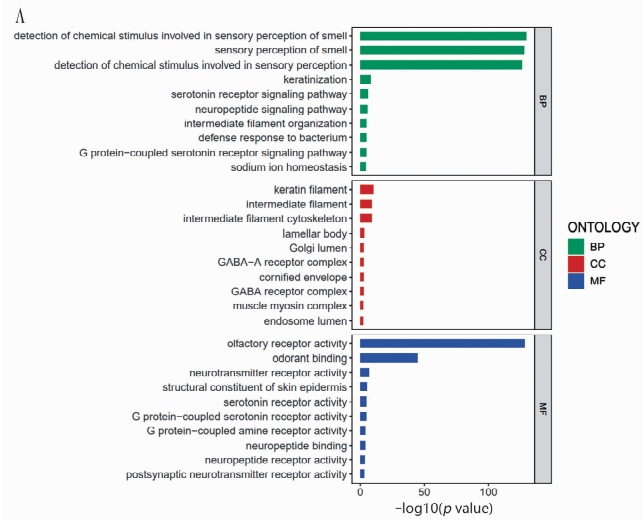


Fig. 4 PPI network of RPS4X



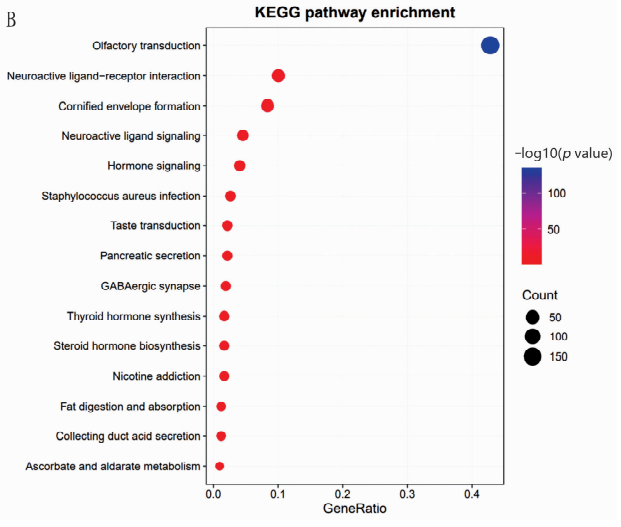


Fig. 5 GO and KEGG pathway enrichment analysis

3.6 Gene set enrichment analysis (GSEA) The results of GSEA showed that in the *RPS4X* high-expression group, several pathways were significantly enriched, including centromere tissue ($NES = 2.45$, $P < 0.001$), intermediate filament cytoskeleton ($NES = -1.28$, $P < 0.001$), cytoskeleton biosynthesis ($NES = 1.76$, $P < 0.01$), and mismatch repair ($NES = 2.59$, $P < 0.01$). These findings are consistent with the previously reported GO, KEGG and PPI analysis results (Fig. 6).

3.7 Verification by RT-qPCR assays To further clarify the expression of *RPS4X* in gastric cancer, the mRNA expression levels of *RPS4X* were assessed in normal gastric mucosal epithelial cells (GES-1) and gastric cancer cell lines (HGC-27, MGC-803, SGC-7901, and MKN45) using RT-qPCR assays (Fig. 7). The results showed that, compared to GES-1 cells, the mRNA expression levels of *RPS4X* were significantly increased in HGC-27, MGC-803, SGC-7901, and MKN45 cells, consistent with the findings obtained from bioinformatics analysis.

4 Discussion

According to the *2022 Global Cancer Statistics Report* released by the World Health Organization, both the incidence and mortality rates of gastric cancer rank among the highest worldwide[8]. In China, more than 60% of gastric cancer patients are diagnosed with advanced or locally advanced disease at their initial diagnosis. The incidence and mortality rates of advanced gastric cancer in China are significantly higher than those observed in other regions globally, and the proportion of patients under 50 years of age is increasing rapidly[9-10]. Although early diagnostic techniques and clinical treatment strategies for gastric cancer have been continuously improved in recent years, the long-term prognosis for patients remains unsatisfactory. Therefore, investigating the molecular mechanisms underlying gastric cancer progression is of great significance for the development of novel targeted therapies.

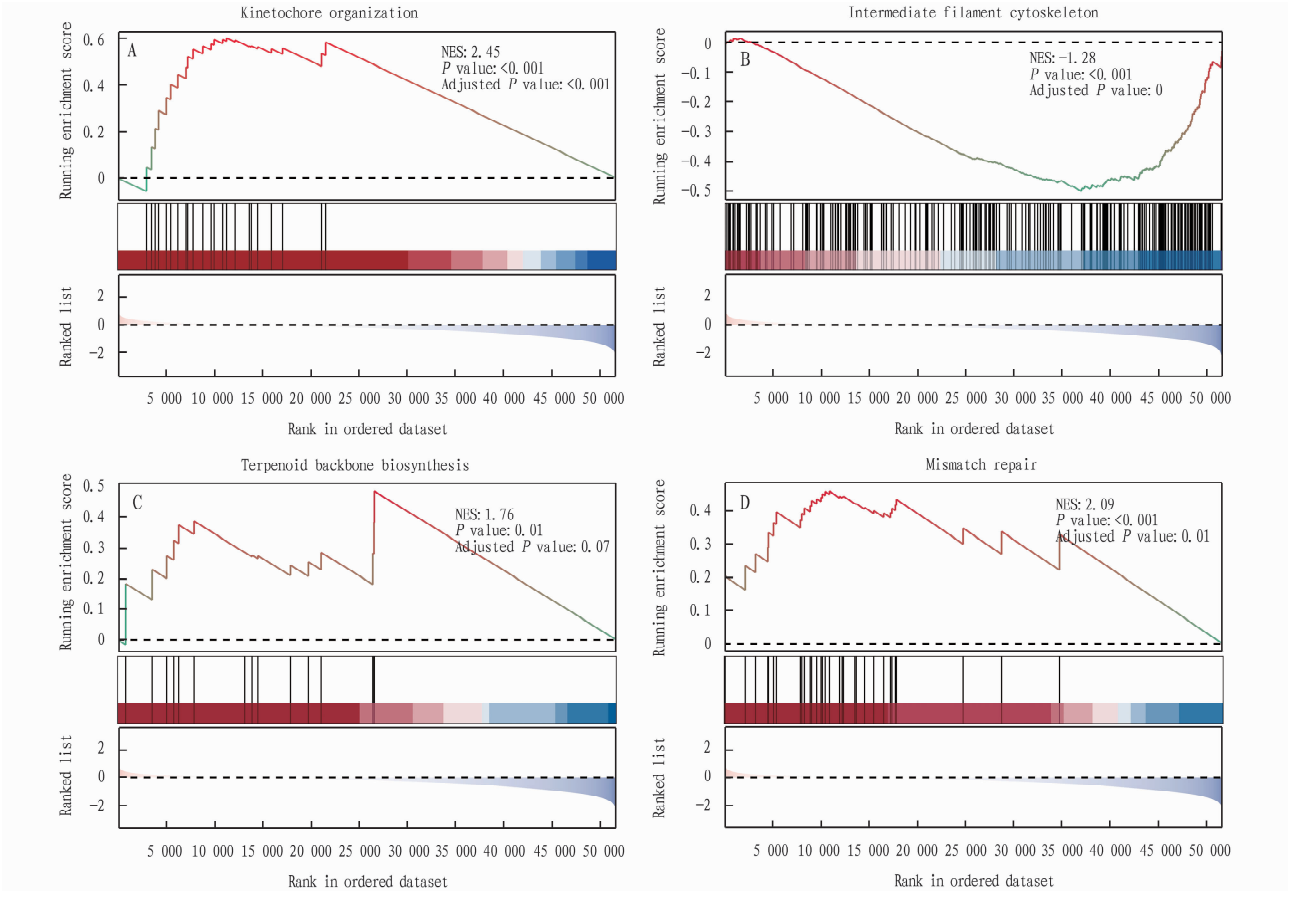


Fig. 6 GSEA maps

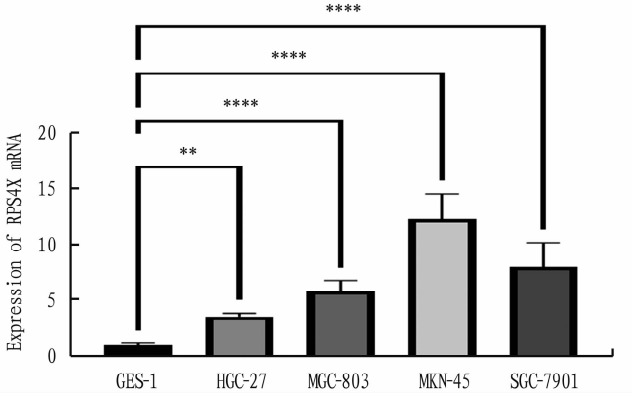


Fig. 7 mRNA expression levels of *RPS4X* across various groups of cells

RPS4X, a homolog of the ribosomal small subunit protein S4 located on the X chromosome, participates in mRNA recognition and the initiation of translation[6]. Previous studies have showed that RPS4X is significantly overexpressed in lung adenocarcinoma and is negatively correlated with patient overall survival[11]. In breast cancer cells, RPS4X interacts with YB-1 to form the RPS4X/YB-1 complex, which inhibits DNA synthesis and thereby induces the development of cisplatin resistance[12]. At the molecular level, RPS4X associates with the Cullin1 protein, thereby inhibiting the ubiquitination activity of the MDM2 and SCF complexes. This inhibition leads to the stabilization of anti-apoptotic proteins such as MCL1 and HAX1, which maintain mitochondrial inner membrane potential and induce cell survival[5]. SLFN11 binds to RPS4X to block the mTOR signaling pathway, thereby inhibiting the progression of hepatocellular carcinoma[13]. Nevertheless, the expression of RPS4X in gastric cancer and its regulatory mechanisms in gastric cancer progression remain unreported.

Through bioinformatics analysis, this study showed that RPS4X was significantly upregulated in gastric cancer tissues and was closely associated with poor patient prognosis. Using the UALCAN network analysis tool, it was observed that RPS4X expression levels were markedly higher in female gastric cancer patients compared to male patients and exhibited a positive correlation with increasing age. RT-qPCR assays further confirmed that RPS4X expression in gastric cancer cells was significantly elevated relative to normal gastric mucosal epithelial cells. To understand the functional role of RPS4X in gastric cancer, a PPI network was constructed, followed by functional and pathway enrichment analysis. The findings revealed that RPS4X interacted with proteins including RPL13, RPS23, RPS3, and RPS12, and may participate in biological processes such as cytoskeletal remodeling and G protein-coupled amine receptor activity, thereby contributing to the progression of gastric cancer.

In conclusion, RPS4X is expected to become a potential mo-

lecular target for the early clinical diagnosis and treatment of gastric cancer, providing novel insights for precise therapeutic strategies. However, the specific molecular regulatory network and downstream target genes associated with RPS4X remain to be clarified. Future research will involve validation through cellular phenotypic assays and *in vivo* experiments to comprehensively investigate the mechanisms by which RPS4X influences the progression of gastric cancer.

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ping standardized training and quality control protocols for scoring, and integrating nursing information systems, Internet of Things (IoT) monitoring, and machine learning methods to further optimize model performance and clinical applicability.

5 Conclusion

The occurrence of ICU artificial airway-related MDRPI is influenced by multiple factors, including the duration of airway placement, use of SSD tubes, type of fixation device, and the moisture, mobility, and friction/shear subscale scores of the Braden Scale. The nomogram model developed through meta-analysis and a prospective cohort demonstrates favorable predictive performance and clinical net benefit. It provides an evidence-based tool to support nursing staff in early identification, stratified management, and precision prevention of MDRPI. To enhance the consistency and operability of model implementation in clinical practice, we recommend accompanying its rollout with standardized assessment forms, quality control checklists for airway fixation, and clearly defined processes for dynamic reassessment.

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