



OPEN OTX2 expression contributes progression of gastric cancer in young adults

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Gastric cancer of young adults is poorly differentiated and has a poor prognosis. However, there are few reports regarding the genetic alteration in gastric cancer of young adults. Bioinformatics methods were used to screen the key genes and signaling pathways of gastric cancer in young adults, and molecular biology techniques were used to verify the key proteins involved in the occurrence and development of gastric cancer in young adults. RNA expression profile microarray data of gastric cancer patients ≤ 45 years old and > 45 years old were downloaded from the TCGA database, and differentially expressed genes were screened by Limma package. GO analysis and KEGG enrichment analysis of DEG were performed in the Gene Function annotation database (DAVID). CytoHubba is used to construct protein interaction networks (PPI) and perform visual analysis to screen out core genes. We obtained 10 hub molecules, including FBXO44, FBXO6, HERC5, FBXL13, FBXO41, NT5E, BMP4, TRIM36, ACAN, ALPL by PPI network with MCODE. Ingenuity Pathway Analysis predicts TBX1, DFNB31, TGFBR3, FBXO44, SLC7A8, DNMT1, KITLG, MSH5, MLLT3, DUSP5, ADAMTSL4, ACPP and TBX1 as the transcription factors directly regulated by OTX2. OTX2 had the highest positive expression rate in gastric cancer of young adults by immunohistochemistry. Interference with OTX2 expression inhibits proliferation, migration, invasion and promotes differentiation, apoptosis of NUGC-4 cells (from 35 year old female). Moreover, after interfering OTX2 expression, the downstream proteins and signaling Pathways of OTX2 in NUGC-4 were further analyzed by Transcriptome sequencing and Ingenuity Pathways Analysis. We found interference with OTX2 expression inhibits CEBPB expression and activates calcitriol by IPA analysis, thereby promoting differentiation of NUGC-4. Therefore, OTX2 plays important roles in restraining the differentiation and promoting progression of gastric cancer cells in young adults. Moreover, OTX2/CEBPB signal axis is likely to be a key molecular event in regulating the differentiation of gastric cancer cells in young adults.

Keywords Gastric cancer, Young adults, OTX2, Differentiation, Progression

Gastric cancer is one of the most common malignant tumors of the digestive system, ranking second among the causes of cancer death, and its highest incidence is generally in middle-aged and elderly people (over 50 years old)^{1–4}. In recent years, the incidence and mortality of stomach cancer have gradually decreased, but gastric cancer in young adults (the latest definition of WHO < 45 years old) has been increasing since the 1990s. Gastric cancer of young adults has unique clinical and pathological features: ① Poorly differentiated adenocarcinoma is more common; ② Strong infiltration ability, more likely to metastasize, high malignancy; ③ The disease progresses rapidly and the prognosis is poor^{5–12}. Considering that young adults under the age of 45 are the main spiritual and economic pillar of the family and society, the impact of young gastric cancer patients on the family and society cannot be ignored. Given that little is known about the molecular mechanism of gastric cancer in young adults, and the gene expression is time-specific, the molecular mechanism driving gastric cancer in young adults must be quite different from that in middle-aged and elderly people. Therefore, it is of great clinical and social significance to carry out basic research targeting this characteristic population and explore the specific molecular mechanism of the pathogenesis of gastric cancer in young adults.

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In this study, biological information analysis was used to obtain significantly differentially expressed genes in ≤ 45 years old and > 45 years old gastric cancer samples from the TCGA data. GO function annotation and KEGG pathway enrichment analysis were performed on the differentially expressed genes to screen out the differentially expressed genes involved in the occurrence and development mechanism of gastric cancer in young adults. The core genes were selected by constructing protein interaction network, and the key genes and signaling pathways driving the pathogenesis of gastric cancer in young adults were further screened by immunohistochemistry, molecular biology experiments, transcriptional sequencing and IPA analysis.

Materials and methods

Data source, differential expression gene screening and functional enrichment analysis

RNA sequencing data and clinical information of Stomach adenocarcinoma (STAD) project were downloaded from TCGA database by using R package “biolinks”. Patients were divided into two groups, ≤ 45 years old and > 45 years old. “limma” package in the R was used to identify the differentially expressed genes (DEGs) between the two group samples, with adjusted P value < 0.05 and $|\log_2\text{-fold change (FC)}| > 2$ as the selection criteria. The volcano maps and heatmaps of DEGs were drawn using the “ggplot2” package and the “pheatmap” package in R respectively.

The “clusterProfiler”, “enrichplot” and “ggplot2” packages in R were used to perform GO (including Biological Process, Molecular Function, and Cellular Component) and KEGG (including key related pathways). $P < 0.05$ and adjusted $P < 0.05$ were set as the thresholds for GO analysis and KEGG analyses. The protein–protein interaction network (PPI) of 10 genes was constructed and the interactions among 10 genes were analyzed by cytoscape software. Important subnetworks were screened for the overall PPI network through Cytoscape function. After sequencing the importance of the genes in the important subnetworks, the 10 most critical hub genes were finally obtained. Core subnetwork analysis using the MCODE plugin and hub gene analysis using the cytohubba plugin. The R package “GSVA” was employed to conduct GO annotation and KEGG analysis. P -value < 0.05 and q -value < 0.05 were selected as criteria for identifying significant enrichment pathways. GSVA was used to assess differences in biological pathways between subtypes. The R package “GSVA” was employed to conduct GO annotation and KEGG analysis. P -value < 0.05 and q -value < 0.05 were selected as criteria for identifying significant enrichment pathways. GSVA was used to assess differences in biological pathways between subtypes.

Patients and samples

We acquired 40 cases of gastric cancer (≤ 45 years old) between January 2019 to December 2023 from Department of Pathology of the Eighth Affiliated Hospital, Sun Yat-sen University. The diagnoses were conducted by three professional pathologists.

Cell lines and cell culture

We purchased the human gastric cancer cell line NUGC-4 (from 35 year old female) from the China Center for Type Collection (CCTCC) (Wuhan, China). Cell line was cultured in DMEM (Gibco, CA, USA) medium containing 10% fetal bovine serum (FBS, Sera Gld, Amarica), 100 U/mL of penicillin, and 100 U/ mL of streptomycin. All of the cells were incubated in a humidified incubator in 5% CO₂ in compressed air at 37 °C.

Transfection with small interfering RNA (siRNA)

Homo sapiens OTX2 siRNA was obtained from Guangzhou RiboBio Biological Technology (Guangzhou, China). NUGC-4 cells were seeded at 2×10^5 cells/well in six-well plates. We transfected HeLa and SiHa cells with CD36 siRNA-1 (sense strand, 5'- CAUGACCUAUACUCAGGCUTT-3'; antisense, 5'- AGCCUGAGUAUAGGUCAU G TT-3'), CD36 siRNA-2 (sense strand, 5'- GCUUGGAUUAUAAAGGACCATT-3'; antisense, 5'- UGGUC CU UAUAAUCCAAGCTT-3'), CD36 siRNA-3 (sense strand, 5'-CUGCUUGGAUUAUAA AGAUTT-3'; antisense, 5'-AUCUUUUAUAAUCCAAGCAGT T-3') or control siRNA (sense strand, 5'- UU CUCCGAACGUGUCA CGUTT-3'; antisense, 5'-ACGUG ACACGUUC GGAGA ATT-3') with Lipo3000 at a final concentration of 100 nM and incubated the cells at room temperature for 15 min. The complex was then added to the culture medium for subsequent experiments.

Transcriptome sequencing and Ingenuity Pathways Analysis

After transfected with OTX2 siRNA in NUGC-4 cells, we further used transcriptome sequencing to investigate the differentially expressed genes and signaling pathways in NUGC-4 cells after OTX2 down-regulation. Transcriptome sequencing is based on Illumina sequencing platform to study all mRNA transcribed by specific tissues or cells in a certain period, which is the basis of gene function and structure research, and plays an important role in understanding the occurrence of gastric cancer in young adults. Ingenuity Pathways Analysis (IPA) could predict upstream drivers of gene expression as well as downstream response effects and identify novel markers associated with the development of gastric cancer in young adults.

Immunohistochemistry

Paraffin blocks were cut into 4- μ m sections and treated routinely following the reagent instructions. After microwaving in citrate buffer for 5 min, the slides were incubated with anti-OTX2 (Proteintech, 13497-1-AP, 1:150 dilution), NT5E (Proteintech, 12231-1-AP, 1:100 dilution), CXCL17 (Proteintech, 18108-1-AP, 1:200 dilution) and FBXO44 (Proteintech, 10626-1-AP, 1:150 dilution) at room temperature. The sections were then incubated with a secondary antibody (Maxim Bio Company, Fuzhou, China), labeling was monitored using diaminobenzidine (Maxim-Bio Company, Fuzhou, China), and hematoxylin was used to stain the sections. We scored expression in accordance with the intensity (0, no staining; 1, weak staining; 2, moderate staining;

3, strong staining), and the percentage of cervical cancer cells that were stained (0, none stained; 1, < 10% stained; 2, 10–50% stained; 3, > 50% stained; 4, > 75% of all of the cervical cancer cells stained). If the product of multiplying staining intensity by the percentage of positively stained cervical cancer cells was ≥ 2 , it was regarded as positive (+)

Wound healing assay and trans-well assay

The NUGC-4, NUGC-4/nc-siRNA (control) and NUGC-4/siRNA-OTX2 cells were seeded into 12-well plates at a density of 1×10^5 cells/well. The cells were then scraped with a 200- μ L sterile pipette tip when they formed monolayers. After washing the cells three times with PBS, we used serum-free medium for culture, and photographed the cells at 0 and 48 h. We performed the invasion assay using transwell plates (Costar, USA). The NUGC-4, NUGC-4/NC-siRNA (control) and NUGC-4/siRNA-OTX2 cells (each at a density of 1×10^5 cells/well) were added to the upper chamber with 0.2 mL of serum-free RPMI-1640. Then added 0.5 mL of 10% FBS medium to the lower chamber. The cells were allowed to invade for 48 h at 37 °C. After removing the cells on the upper surface of the membrane, we stained cells on the lower aspect with trypan blue.

Colony formation assay and evaluation of cellular apoptosis by Hoechst33258

We adjusted the concentrations of NUGC-4, NUGC-4/nc-siRNA (control) and NUGC-4/siRNA-OTX2 cells to appropriate densities, and then inoculated each culture dish with 200 cells at 37 °C, changing the medium every 4 days. After 2 weeks, cells were stained with trypan blue, and numbers of cell colonies were counted using a light microscope.

Samples of each group after treatment were taken, fixed at room temperature for 20 min with 1 mL 4% paraformaldehyde, then the fixing solution was discarded, and washed twice with 1 mL PBS for 3 min each time, then PBS was discarded. Add 1 mL Hoechst 33,258 dyeing solution and stain at room temperature for 5 min. Discard the dyeing solution and wash it with PBS five times. Then we observed under fluorescence microscope.

Ethical clearance

The study was approved by the Institutional Research Ethics Board of the Eighth Affiliated Hospital, Sun Yat-sen University and all methods were performed by relevant guidelines and regulations.

Informed consent

Informed consent was obtained from all subjects and/or their legal guardians involved in the study.

Statistical analysis

All statistical analyses were conducted using SPSS 17.0 (SPSS, Inc., Chicago, IL, USA). We performed experiments in triplicate, and data are presented as mean $\pm$ SEM. Data from two groups were analyzed by unpaired t tests; and, if more than two groups, by oneway ANOVA. A *P* value of < 0.05 was considered statistically significant.

Results

Significantly mutated genes in gastric cancer of young adults

We performed differential analysis of genes with young patients (≤ 45 years old, $n = 16$) versus those aged (> 45 years old, $n = 355$) based on TCGA data sets and plotted volcano. The volcano plot (Fig. 1A) showing differentially expressed 282 genes (includes 69 upregulated genes and 213 downregulated genes) in number identified in the gene expression profiling of the young patient group compared with the older group. The top 10 up-regulated genes were PIK3C2G, CXCL17, VSIG1, OLFM4, GATA5, SLC7A8, SUS4, SPTSSB, IGFBP2 and FCGBP. Then, a total of 69 differentially expressed genes were used to construct the protein–protein interaction (PPI) network to identify hub genes. Further analysis of the PPI network with MCODE, we obtain 10 hub molecules, including FBXO44, FBXO6, HERC5, FBXL13, FBXO41, NT5E, BMP4, TRIM36, ACAN, ALPL (Fig. 1E).

CytoHubba (a Cytoscape plugin that ranks nodes in a network based on network characteristics) was used to analyze the core nodes of the PPI network, and maximal clique centrality (MCC) algorithm was used to obtain the interaction network of top10 core genes, including TRIM36, FBXO41, FBXL13, HERC5, FBXO6, FBXO44, NT5E, ACAN, ALPL, BMP4 (Fig. 1F).

Altered pathways in gastric cancer of young adults

The functions of these differentially expressed genes were annotated and predicted by the analysis of Gene Ontology (GO) based on three parts: Biological Process (BP), Cellular Component (CC) and Molecular Function (MF). Of them, the significant BPs were mainly involved in regulation of cell morphogenesis and extracellular structure and so on (Fig. 1B). The significant CCs were mainly related to extracellular matrix, axon part and transmembrane transporter complex. The significant MFs were mainly involved in metal ion transmembrane transporter and potassium ion transmembrane transporter activity. GO Chord plot displays the relationship between a list of pathway and corresponding genes. The GO Chord plot (Fig. 1D) shows 45 DEGs related to the 8 pathways associated exhibiting enrichment in the presented order level. KEGG analysis (Fig. 1C) showed that several functional pathways were enriched, and the top 15 functionally enriched KEGG pathways were analysis. Pathways highly correlated with the aberrantly expressed proteins included phospholipase D signaling pathway, synaptic vesicle cycle, protein digestion and absorption, Neurotrophin signaling pathway and so on. In order to identify the common regulon (a transcription factor (TF) and its direct transcriptional targets, which contain common TF binding sites), we use the iRegulon cytoscape plugin. Ingenuity Pathway Analysis predicts the transcription factors and co-factors that may regulate a list of genes. This analysis revealed TBX1, DFNB31, TGFBR3, FBXO44, SLC7A8, DNMT1, KITLG, MSH5, MLLT3, DUSP5, ADAMTSL4 and ACP as the transcription factors directly regulated by OTX2 (Fig. 1G). We use sequence logo to predicted the OTX2 binding

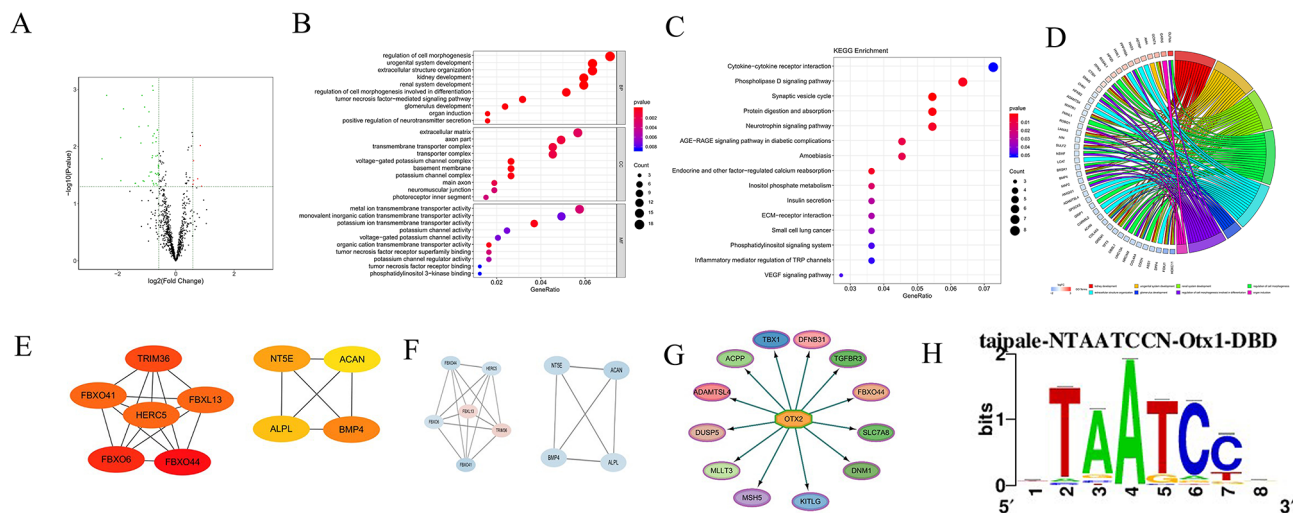


Fig. 1. (A) The volcano plot identified in the gene expression profiling of the young patient group compared with the older group; (B) The significant biological process of these differentially expressed genes involved in regulation of cell morphogenesis and extracellular structure; (C) The top 15 functionally enriched pathways were analysis by KEGG; (D) GO Chord plot displays the relationship between a list of pathway and corresponding genes; (E) Ten hub molecules were found by MCODE analysis; (F) Using Cytoscape, we also found 10 hub molecules; (G) Ingenuity Pathway Analysis predicts TBX1, DFNB31, TGFBR3, FBXO44, SLC7A8, DNMI1, KITLG, MSH5, MLLT3, DUSP5, ADAMTSL4, ACPP and TBX1 as the transcription factors directly regulated by OTX2; (H) Using sequence logo to predicted the OTX2 binding sites with DBD.

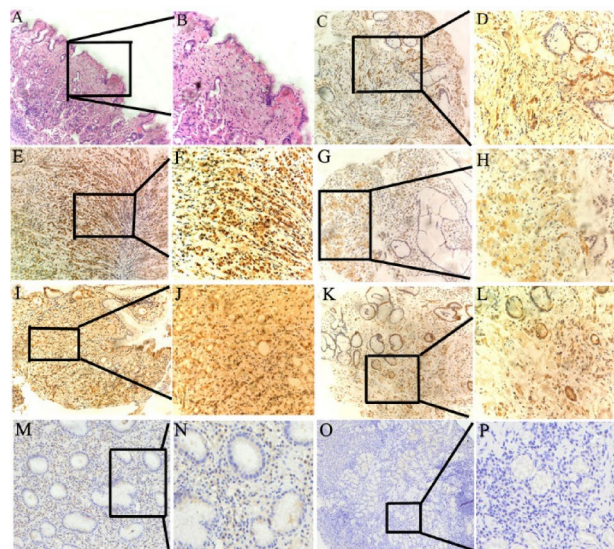


Fig. 2. (A–B) Signet-ring cell carcinoma is common in gastric cancer of young adults; (C–D, E–F) OTX2 expression was present in the cytoplasm and nucleus of gastric cancer cells of young adults; (G–H) NTSE expression was present in the cytoplasm of gastric cancer cells; (I–J) CXCL17 expression was present in the cytoplasm of gastric cancer cells; (K–L) FBXO44 was present in the cytoplasm of gastric cancer cells. (M–N, O–P) OTX2 expression was negative in the normal gastric tissues of young adults.

sites with DBD (Fig. 1H). The height of the stack indicates the sequence conservation at that position, while the height of symbols within the stack indicates the relative frequency of each amino acid at that position.

Immunohistochemistry determined the expressions of OTX2, NTSE, CXCL17 and FBXO44

Signet-ring cell carcinoma is more common in gastric cancer of young adults (Fig. 2A–B). Immunohistochemistry was performed to determine whether a correlation exists between the expressions of OTX2, NTSE, CXCL17, FBXO44 and gastric cancer of young adults in 40 paraffin-embedded samples. Results indicated that OTX2 expression was present in the cytoplasm and nucleus of gastric cancer cells (Fig. 2C–F). However, normal

gastric mucosa epithelial cells adjacent to tumor tissues did not show OTX2 immunoreactivity (Fig. 2 M–P). Among the 40 samples in our study, immunoreactivity of OTX2 was detected in 92.5% (37/40) cases. NT5E immunoreactivity was detected in 35.0% (14/40) of the samples, of which five samples had strong NT5E labeling. NT5E expression was present in the cytoplasm of gastric cancer cells (Fig. 2G–H). In addition, CXCL17 and FBXO44 expression was observed in the cytoplasm of gastric cancer cells. However, in 40 samples, only nine and twelve cases showed CXCL17 (Fig. 2I–J) and FBXO44 (Fig. 2K–L) immunoreactivity respectively.

Interference with OTX2 expression inhibits proliferation, migration, invasion and promotes differentiation, apoptosis of NUGC-4 cells

To further investigate the effects of OTX2 expression on biologic processes in gastric cancer cells of young adults, we used a series of molecular biology experiments after downregulated OTX2 expression in NUGC-4 cells. As shown in Fig. 3A–C, we found that the morphology of some NUGC-4 cells changed from round to spindle cells after interference with OTX2 expression, suggesting OTX2 may be correlated with promoting differentiation of NUGC-4 cells. Compared with SiRNA1 and SiRNA3, SiRNA2 had the best effect on interfering OTX2 expression (Fig. 3S). Colony formation assays and Hoechst 33258 were used to examine the proliferative and apoptotic effects of OTX2 in NUGC-4 cells. Our results revealed that interference of NUGC-4 expression attenuated colony formation in NUGC-4 cells (Fig. 3D–F, T). Knocking-down expression of OTX2 increased the rate of apoptosis of NUGC-4 cells (Fig. 3G–I). NUGC-4 cells-transfected OTX2 siRNA-2 were used to

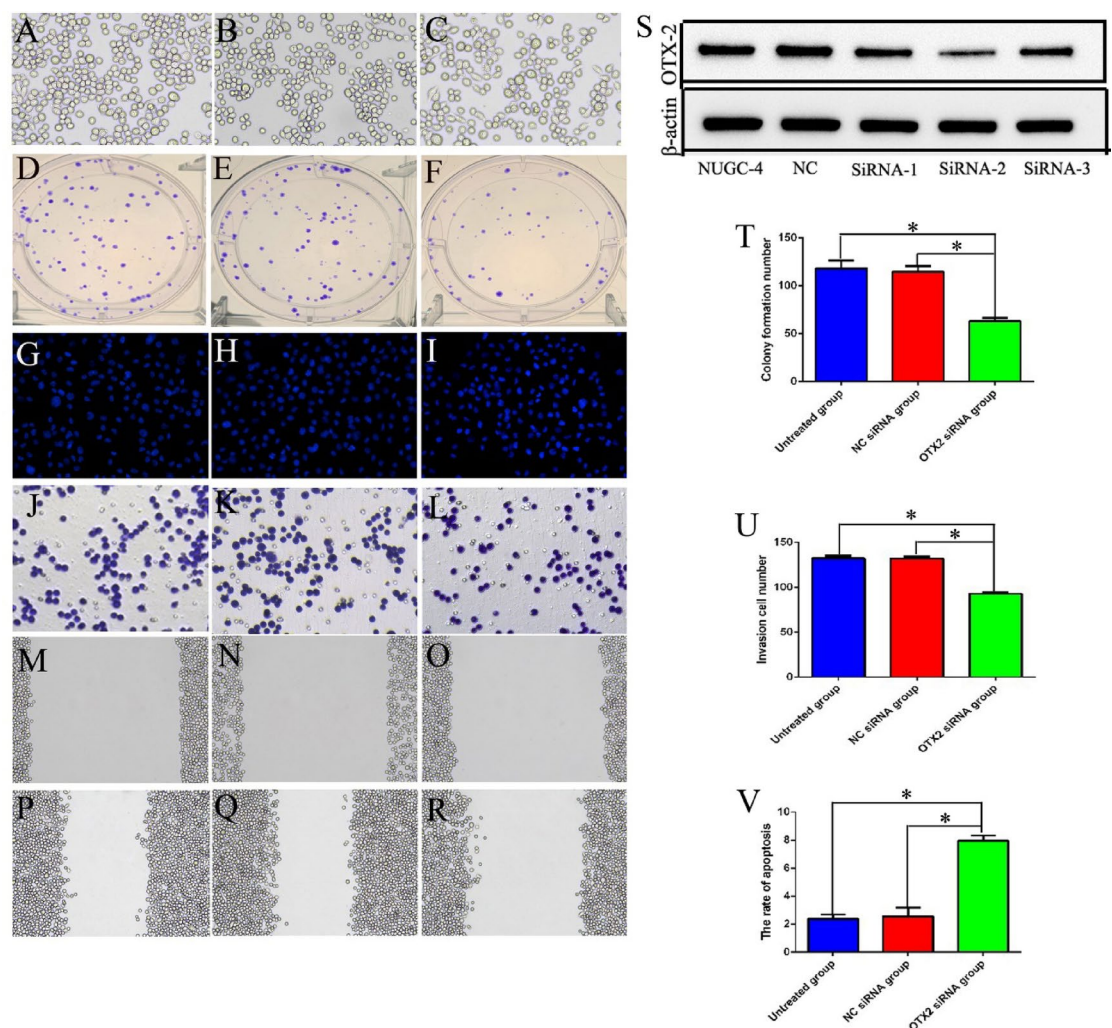


Fig. 3. The effects of OTX2 expression on biologic processes in NUGC-4 cells after downregulated OTX2 expression. (A–C) The morphology of some NUGC-4 cells changed from round to spindle cells after interference with OTX2 expression; (D–F, T) Interference of NUGC-4 expression attenuated colony formation in NUGC-4 cells. (* $P < 0.05$); (S) Compared with SiRNA1 and siRNA3, siRNA2 had the best effect on interfering OTX2 expression. The upper bands represent OTX2 expression levels. The bands below represent β-actin expression levels. (G–I, V) Knocking-down expression of OTX2 increased the rate of apoptosis of NUGC-4 cells (* $P < 0.05$); (J–L, U) Compared with cells transfected with control siRNA (NC-siRNA), interference of OTX2 expression markedly inhibited the invasion of NUGC-4 cells; (M–R) Interference of OTX2 expression by siRNA inhibited migration of NUGC-4 cells.

ascertain the effects of OTX2 on invasion and migration of NUGC-4 cells by trans-well and wound-healing assays. Compared with cells transfected with control siRNA (NC-siRNA), interference of OTX2 expression markedly inhibited the invasion of NUGC-4 cells (Fig. 3J–L, U). Similarly, interference of OTX2 expression by siRNA inhibited migration of NUGC-4 cells (Fig. 3M–R, V). Therefore, our evidence verified the important role for OTX2 in the promotion of NUGC-4 cells migration and invasion in vitro.

Interference with OTX2 expression inhibits CEBPB and activates calcitriol might promotes differentiation of gastric cancer cells of young adults by IPA analysis

To further study the effects of OTX2 on gastric cancer cells of young adults, We performed transcriptome sequencing of NUGC-4/siRNA-OTX2 cells and control cells using the Illumina sequencing platform and Ingenuity Pathways Analysis (IPA). Do (Disease Ontology) enrichment analysis showed that the signaling pathways associated with germ cell cancer and embryonal cancer were significantly correlated with the down-regulation of OTX2 expression (Fig. 4A). KEGG (Kyoto Encyclopedia of Genes and Genomes) enrichment analysis showed that signaling pathways related to DNA replication and cell cycle were associated with down-regulation of OTX2 expression (Fig. 4B). Classical pathway analysis based on IPA showed that the signaling pathway of Cell Cycle Control of Chromosomal Replication was significantly inhibited after inhibition of OTX2 expression (z-score: -4.123) (Fig. 4C). Graphic summary based on IPA also showed that CEBPB was significantly inhibited (Fig. 4D). Upstream regulatory factor analysis suggested that CEBPB was predicted to be strongly repressed, and there were 52 consistently repressed genes after inhibition of OTX2 expression (Fig. 4E). Moreover, we confirmed that CEBPB expression was decreased in NUGC-4/siRNA-OTX2 cells by using Western blot (Fig. 4F). The above results suggest OTX2/CEBPB signal axis may be involved in the development of gastric cancer in young adults.

Discussion

As a special type of gastric cancer, the incidence of gastric cancer in young adults had gradually increased in recent years, which had brought great negative impact on society and family. Blair et al. reported that gastric cancer in young adults had obvious genetic heterogeneity¹³. Part of gastric cancer in young adults is hereditary diffuse gastric cancer, which is closely related to germline mutations. The common molecular types of gastric cancer in young adults are EBV positive type and genome stable type¹⁴. CDH1 and CTNNA1 remain as the main genes for hereditary gastric cancer¹⁵. However, they only explain a small fraction of gastric cancer cases in young adults with suspected inherited basis. It can be seen that gastric cancer in young adults is more malignant and has unique clinical and molecular pathological characteristics.

In recent years, with the rapid development of gene chip technology, bioinformatics analysis has been increasingly used to find new therapeutic targets for various cancers to achieve prognosis evaluation and accurate diagnosis and treatment of malignant tumors^{16–21}. This study analyzed the expression profiles of gastric cancer gene chip data of ≤ 45 years old and > 45 years old gastric cancer samples from the TCGA database to obtain significant differentially expressed genes between them, including 69 up-regulated genes and 213 down-regulated genes. In addition, the enrichment of GO and KEGG pathways of the two differential genes was also analyzed to elucidate the main functions and modes of action of the differential genes.

Using PPI network, we obtained 10 hub genes: FBXO44, FBXO6, HERC5, FBXL13, FBXO41, NT5E, BMP4, TRIM36, ACAN, ALPL. Using Cytoscape, we obtained another 10 hub genes: TRIM36, FBXO41, FBXL13, HERC5, FBXO6, FBXO44, NT5E, ACAN, ALPL, BMP4. Enrichment analysis of KEGG pathway found that the above genes were involved in phospholipase D signaling pathway, synaptic vesicle cycle, protein digestion and absorption, Neurotrophin signaling pathway is highly enriched. IPA (Ingenuity Pathway Analysis) predicts transcription factors: TBX1, DFNB31, TGFB3, FBXO44, SLC7A8, DNM1, KITLG, MSH5, MLLT3, DUSP5, ADAMTSL4, ACPP and TBX1 directly regulated by OTX2.

We selected OTX2, NT5E, CXCL13 and FBXO44 as the study objects. Through immunohistochemistry, OTX2 protein with the highest positive expression rate in young gastric cancer specimens was selected as the study objects for subsequent experiments. The results of cytological function experiments showed that OTX2 can promote the morphological differentiation of NUGC-4 cells. Moreover, OTX2 could promote the proliferation, invasion, migration and inhibit apoptosis of NUGC-4 cells. Similar to the results of this study, OTX2 is also an oncogene that promotes medulloblastoma growth²². OTX2 silencing modulates the repressive chromatin landscape, decreases levels of PRC2 complex genes and increases the expression of neurodevelopmental transcription factors²². The common feature of medulloblastoma and young gastric cancer is the relatively young age of patients, OTX2 may also play a key role in the occurrence and development of young gastric cancer. Peng et al.^{23,24} found that methylation of the CGI Shore in the p16 non-promoter region can hamper the transcriptional activity of OTX2, leading to a reduction in the expression of p16, which might contribute to the development of lung cancer. Zheng et al.²⁵ also found that OTX2 expression was an independent prognostic factor for lung squamous cell carcinoma. These findings demonstrated that OTX2 plays an important role in the occurrence and development of a variety of tumors.

In order to further study the signaling pathway downstream of OTX2, this study took NUGC-4 gastric cancer cells as the research object by using Illumina sequencing platform and Ingenuity Pathways Analysis (IPA). After interfering the expression of OTX2, we explore the downstream genes and signaling pathways regulated by OTX2. Our study found that the Chromosomal Replication and Cell Cycle Control signaling pathways were significantly inhibited after inhibition of OTX2 expression. Graphic summary based on IPA also showed that CEBPB was significantly inhibited. Western blot further confirmed that silencing OTX2 expression in NUGC-4 cells could also reduce CEBPB expression. It has been reported that CEBPB can induce differentiation of acute promyelocytic leukemia cells²⁶. GALM, ITPR2 and ORM2 may be downstream target genes of the transcription factor CEBPB²⁶. One of the characteristics of gastric cancer in young adults is poor differentiation of cancer cells.

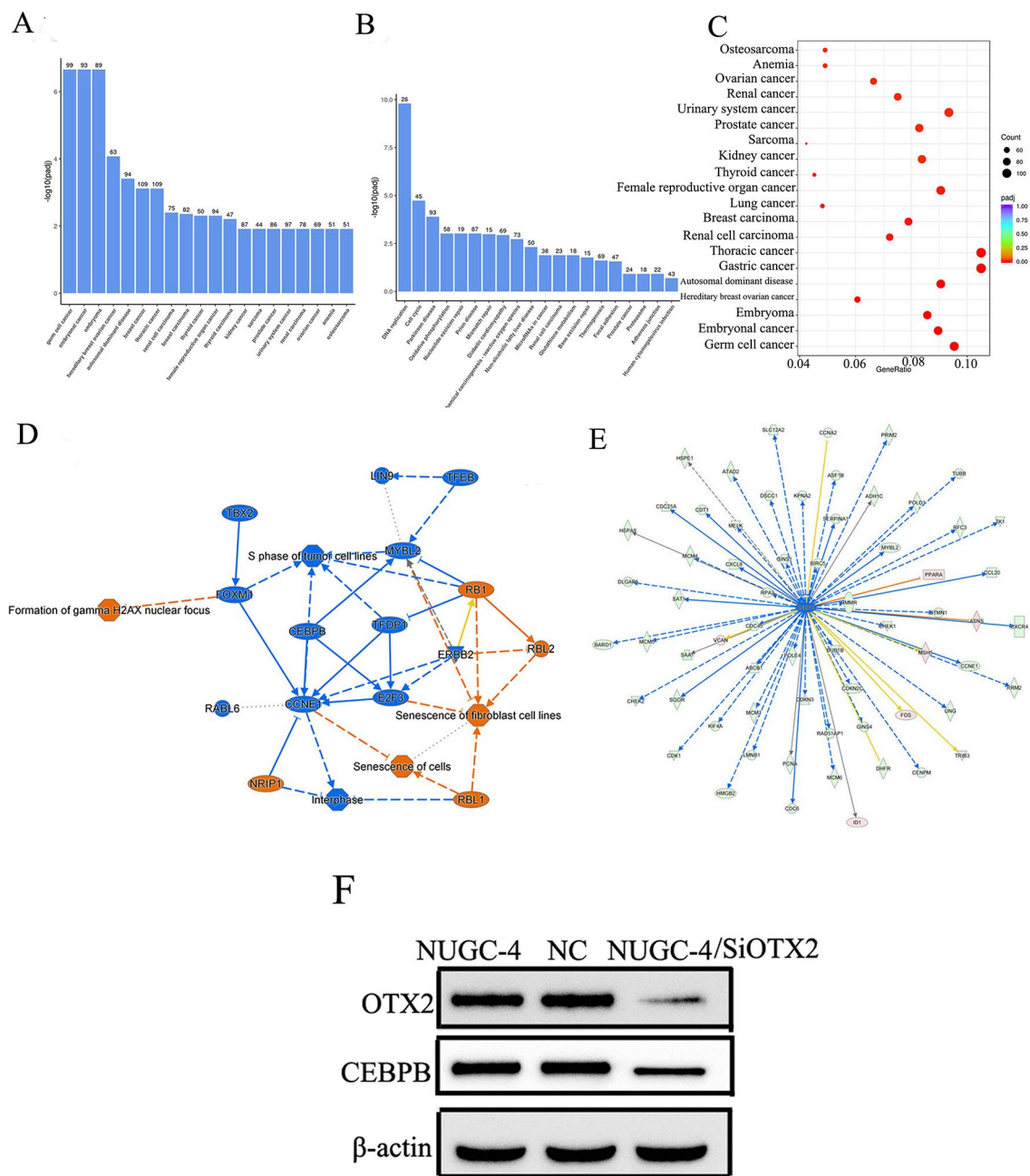


Fig. 4. (A) Disease Ontology enrichment analysis showed that the signaling pathways associated with germ cell cancer and embryonal cancer were correlated with the down-regulation of OTX2 expression; (B) KEGG enrichment analysis showed that signaling pathways related to DNA replication and cell cycle were associated with down-regulation of OTX2 expression; (C) Cell cycle Control of Chromosomal Replication was significantly inhibited after inhibition of OTX2 expression; (D) IPA showed that CEBPB was significantly inhibited; (E) CEBPB was predicted to be strongly repressed, and there were 52 consistently repressed genes after inhibition of OTX2 expression; (F) CEBPB expression was decreased in NUGC-4/SiRNA-OTX2 cells.

Therefore, this study suggests that OTX2 inhibits the differentiation and promotes progression of NUGC-4 cells by regulating CEBPB and its downstream factors.

In conclusion, through bioinformatics analysis, molecular biology experiments, transcriptional sequencing and IPA analysis, this study found that OTX2 plays an important role in restraining the differentiation and promoting progression of gastric cancer cells in young adults. Moreover, OTX2/CEBPB signal axis is likely to be a key molecular event in regulating the differentiation of gastric cancer cells in young adults. This study providing an important molecular target and experimental basis for clinical prevention and treatment of gastric cancer in young adults.

Data availability

The datasets generated and analysed during the current study are not publicly available due the data contains patient information but are available from the corresponding author on reasonable request.

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Author contributions

J.Z. and C.Z. conceptualization. J.Z. and T.L. data curation and original draft writing. Y.G. and L.X., statistical analysis. T.L., Y.G., L.X., Y.A. and Y.X. performed the experiments. J.Z., Y.X. and C.Z. manuscript review and editing. All authors contributed to the article and approved the submitted version.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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