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Article

Knockdown of DNAJC12 slows tumor progression and affects tumor radiosensitivity in esophageal squamous cell carcinoma

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Abstract: Purpose: To look into the influence of DNAJC12 knockdown on the progression and radio-sensitivity of esophageal squamous cell carcinoma (ESCC), with a focus on cellular mechanics and tumor microenvironment interactions. **Methods:** The TCGA database combined with immunohistochemical staining was used to validate the DNAJC12 expression in ESCC patients from the perspective of the clinic. DNAJC12 knockdown was performed in TE-1 and KYSE-150 cell lines to assess changes in proliferation, migration, invasion, apoptosis, and cellular mechanical properties (e.g., stiffness, adhesion, and contractility). The downstream molecule regulated by DNAJC12 was explored using Western blotting and biomechanical assays. The effect of DNAJC12 knockdown on tumor radiosensitivity was evaluated in vivo, with a focus on tumor stiffness and extracellular matrix (ECM) remodeling under irradiated conditions. **Results:** Upon analyzing the TCGA database and examining tumor tissue samples from patients, it was discovered that DNAJC12 exhibited high expression levels in tissues of ESCC. Vitro experiments showed that DNAJC12 knockdown significantly decreased cellular proliferation and migration ($P < 0.05$). Biomechanical assays revealed that DNAJC12 knockdown decreased cellular stiffness and contractility, suggesting a role in regulating cytoskeletal dynamics. Molecular analysis showed downregulation of P-ERK, MMP-2, N-Cadherin, P-P38, Snail, Vimentin, β-Catenin, Fibronectin, and Twist alongside upregulation of E-Cadherin ($P < 0.05$). Overexpression of SNAI1 could restore the proliferative and migratory capabilities of cells with downregulated DNAJC12. In vivo experiments, knockdown of DNAJC12 resulted in faster tumor growth under irradiated conditions ($P < 0.05$). **Conclusion:** DNAJC12 knockdown slows ESCC progression by modulating cellular biomechanical properties and molecular pathways. However, it enhances tumor growth post-radiotherapy, potentially due to altered mechanosensitive signaling and ECM remodeling. These findings highlight the interplay between molecular biology and biomechanics in ESCC progression and treatment response.

Keywords: DNAJC12; esophageal squamous cell carcinoma; SNAI1; tumor progression; radiotherapy; biomechanics

1. Introduction

Considered the sixth leading cause of cancer-related mortality worldwide [1], esophageal squamous cell carcinoma (ESCC) is characterized by high aggressiveness, poor survival rates, and dismal prognosis, even with advancements in therapeutic

approaches such as multimodal therapy [2,3]. The persistent challenges in treating ESCC underscore the urgent need for novel therapeutic targets and a more profound comprehension of the disease's underlying molecular and biomechanical mechanisms.

The HSP40/DNAJ family of proteins that stimulate the ATPase activity of HSP70 have the capacity to exert crucial roles in protein-associated processes, including folding, unfolding, translation, transport, and degradation. These proteins are categorized into three subclasses: DNAJA subclass, DNAJB subclass, and DNAJC subclass [4]. It has been observed that several members of the HSP40/DNAJ family serve as potential biomarkers for tumor diagnosis, prognosis evaluation, and treatment sensitivity prediction [5]. Among them, the mRNA level of DNAJ heat shock protein family member C12 (DNAJC12) has garnered attention due to its elevated mRNA levels in clinical gastric cancer tissues [6–9]. Furthermore, DNAJC12 has been implicated in the progression of various cancers like colorectal cancer, prostate cancer, breast cancer, and gastric cancer. DNAJC12 is closely correlated with the poor prognosis, invasion, and metastasis [6–10]. Despite these findings, the role of DNAJC12 in ESCC development remains unexplored, leaving a significant gap in our understanding of its potential contributions to this aggressive cancer type.

Snail, a zinc-finger transcription factor, is a well-known regulator of epithelial-mesenchymal transition (EMT), a critical process in cancer metastasis. Snail promotes EMT by repressing the expression of E-cadherin, a key epithelial marker. In ESCC, reduced E-cadherin levels or positive Snail expression, clinical outcomes tend to be less favorable, underscoring Snail as a critical factor in the disease progression [11,12]. Nevertheless, the regulatory mechanisms governing Snail expression in ESCC remain poorly understood, particularly in the context of how DNAJC12 might influence these pathways. Given the established role of EMT in cancer metastasis and the potential involvement of DNAJC12 in tumor progression, it is plausible that DNAJC12 may interact with Snail-mediated pathways to drive ESCC aggressiveness. In the present study, we aimed to bridge these gaps by investigating the expression and functional role of DNAJC12 in ESCC. We first explored the expression levels of DNAJC12 in ESCC samples and investigated its correlation with the clinical characteristics of ESCC. We then delved into the role of DNAJC12 in cellular proliferation, invasion, and EMT, with a particular focus on its potential molecular mechanisms in modulating the expression of SNAI1, a key regulator of EMT. To further elucidate the biomechanical implications, we evaluated how DNAJC12 influences tumor stiffness and ECM remodeling, which are critical factors in cancer progression and therapy resistance. Finally, we assessed the impact of DNAJC12 on tumor growth and radiotherapy sensitivity using a nude mouse model. This study will provide significant theoretical backing for the ESCC diagnosis and treatment.

2. Materials and methods

2.1. Clinical samples

Between 2019 and 2020 at the Cancer Hospital affiliated with Shandong First Medical University, surgical specimens comprising ESCC tissues and their respective adjacent tissues (postoperative gross pathological residual tissues) were obtained from 50 patients who underwent radical surgery. Participants who had undergone

preoperative chemotherapy or radiotherapy were strictly excluded. Each participant offered the written informed consent, and the Ethics Committee of the Cancer Hospital affiliated with Shandong First Medical University approved this research (The approval number: No. 2021-023-02). The study methodologies adhered to the Declaration of Helsinki standards.

2.2. DNAJC12 expression in TCGA database

To take the DNAJC12 expressions in ESCC into measurement, the Gene Expression Profiling Interactive Analysis (GEPIA, http://gepia.cancer-pku.cn/) was utilized to provide access to RNA sequencing expression data harvested from The Cancer Genome Atlas (TCGA) that could offer customizable and interactive functionalities for analyzing gene expression in various cancer types and Genotype-Tissue Expression (GTEx) projects. Specifically, we investigated and compared the expression levels of DNAJC12 between ESCC tissues and adjacent non-cancerous tissues, utilizing the TCGA database as integrated within GEPIA. The analysis was conducted by choosing "ESCC" as the cancer type and "DNAJC12" as the gene of interest. The resulting data were depicted using box plots to illustrate the differential expression in tumor versus paracancerous tissues. Statistical significance was evaluated through the utilization of the GEPIA platform's proprietary algorithms, which employ one-way ANOVA to compare expression levels between tumor and normal tissues, with the corresponding p-values being reported.

2.3. Immunohistochemical staining

The surgical specimens underwent fixation in 10% neutral buffered formalin, followed by selection, dehydration, embedding in paraffin, and were subsequently sectioned into 4-micron-thick slices. Briefly, sections were bound to an anti-DNAJC12 rabbit polyclonal antibody diluted with 1:100 (Proteintech, 12338-1-AP) via antigen-antibody interactions and then washed via phosphate-buffered saline, reacted sequentially with reaction enhancers and highly sensitive enzyme-labeled anti-mouse-rabbit IgG polymers (Elivision Super detection reagent; Kit-9921; MXB biotechnology, China), stained with 3,3-diaminobenzidine (DAB-0031; MXB biotechnology, China), and the nuclei were dehydrated, rendered transparent, and then sealed following counterstaining with hematoxylin. The data and semi-quantitatively scored each slide by computing the percentage and intensity of staining at each representative site on the slide, which all were assessed by two experienced clinical researchers.

2.4. The selection, culture and lentiviral transfection of cell

TE-1 and KYSE-150 cell lines were procured from the Chinese Academy of Sciences in Shanghai, China. These cells were subsequently cultured in the 10% FBS-containing (VS 500T, Australia) and 100 IU/mL Ampicillin-containing (A100339-0025, Genebase) RPMI-1640 medium, under controlled conditions of 37 ℃ and 5% CO_2.

GV115 plasmids (Shanghai Genechem Co., Ltd., China) were used to clone the short hairpin RNA targeting DNAJC12 (shDNAJC12) and control (shctrl), and the

sequences of shDNAJC12 and shctrl were 5'GGATGTGATGAACTATCTT, 5'TTCTCCGAACGTGTCACGT respectively. The coding sequences of cTNNB1 (NM_001904.4), p65 (NM_021975.4), and VIM (NM_003380.5) were cloned into GV610 vectors (Shanghai Genechem co., Ltd.) for overexpression. Subsequently, employing Lipofectamine® 3000 (from Invitrogen, a division of Thermo Fisher Scientific, Inc.), the GV115 or GV610 plasmids (each at a dose of 20 μg) were co-transfected into cells in conjunction with the previously specified pHelper1.0 at a dose of 15 μg and 10 μg pHelper2.0 packaging vectors (22). After 48 hours of transfection with the lentiviruses (multiplicity of infection = 10), the cells undergoing transfection were selected with 5 g/ml Sigma-Aldrich puromycin (Merck KGaA).

Transfection efficiency was initially assessed by fluorescence microscopic observation of GFP expression and quantified by flow cytometry (FACS). Specifically, 48 h after transfection, cells were collected and the percentage of GFP-positive cells was analyzed using flow cytometry to determine transfection efficiency. In addition, to ensure that the transfection process had minimal impact on cell viability, we assessed cell viability at 24 and 48 h post-transfection using the CCK-8 assay. The results showed that there was no significant difference in cell viability between the transfected group and the untransfected control group, indicating that the transfection process caused less damage to the cells.

2.5. The mRNA assay via Real-time quantitative polymerase chain reaction (qPCR)

The RNA extraction agent, SPARKeasy Superpure Total RNA Kit, was employed to isolate the total RNA from cells. The DNAJC12 mRNA levels were quantified in ESCC cell lines via qPCR utilizing the SYBR Green Premix Ex TaqTM from Takara Bio Inc. in Japan. Using a Roche LightCycler480 (Roche Diagnostics, Penzberg, Germany), each qPCR reaction was carried out in triplicate. The relative gene expression levels were determined by normalizing against GAPDH expression. The $2^{-\Delta\Delta}$Ct formula was to conduct the calculation of DNAJC12 and GAPDH. The primers were listed as follows:

DNAJC12: Forward, 5′-AATGGTTGGCACCTTCGTTTC-3′ and Reverse, 5′-GTTGGCAGCATAGGGGACAG-3′

GAPDH: Forward, 5′-TGACTTCAACAGCGACACCCA-3′ and Reverse, 5′-CACCCTGTTGCTGTAGCCAAA-3′.

2.6. High-content screening (HCS) assay

In 96-well plates, 2000 KYSE-150 cells and 1000 TE-1 cells were cultivated. HCS assays were utilized to set a fixed amount of the GFP-expressing cells, thereby estimating the cell population. The proliferation of cells in each well was monitored every 24 h over a period of five days using Nexcelom's Celigo HCS software.

2.7. MTT assay

Utilizing an MTT test kit from Genview Scientific, Inc., the proliferation of cells at a density of 2×10^3 per well seeded into 96-well plates was assessed. Subsequently, each well was infused with MTT solution and incubated for a duration of 4 h at a

temperature of 37 ℃. Upon substituting the solution with DMSO, the optical density was evaluated using a microplate spectrophotometer (Invitrogen; Thermo Fisher Scientific) at a wavelength of 490 nm and then shaken for 5 min.

2.8. Apoptosis assay

Apoptosis of KYSE-150 and TE-1 cells was detected through employing the Annexin V-allophycocyanin (APC) Apoptosis Detection Kit purchased from Thermo Fisher Scientific. These cells introduced with lentiviruses encoding sh-control or sh-DNAJC12 were harvested through centrifugation speed with 1000 g for 5 min from ambient temperature and then rinsed with PBS, subsequently they were resuspended with the staining buffer to attain a final concentration of 1×10^6 cells/ml, and 100 μl of which was taken out and cultivated with Annexin V-APC (5 μl) for 15 min at ambient temperature. Finally, cells were detected by flow cytometry after staining.

2.9. The detection of clone formation

Whether or not DNAJC12 was knocked down, 1×10^3 cells were cultured for a period of two weeks following their seeding into six-well plates. Colonies became visible following staining with the crystal violet from Sangon Biotech Co., Ltd. In Switzerland. The size and quantity of the colony were subsequently evaluated.

2.10. Migration and transwell invasion assay

For the migration measurement, the cell culture was seeded into the upper chamber, with media supplemented with 30% FBS being provided to the lower chamber. After 24 h, the migratory cells were subjected to fixation using 4% paraformaldehyde, stained with hematoxylin, and examined under a DM2500 bright-field microscope at a magnification of 200X. For invasion testing, the cells were put in the upper chamber coated with Matrigel, and medium supplemented with 30% FBS was introduced into the lower chamber. The invading cells were preserved in 95% ethanol, stained with hematoxylin, and examined 24 h later using a DM2500 bright-field microscope at a magnification of 200X.

2.11. Western Blotting (WB) analysis

Total protein from tissues of ESCC as well as from A549 and NCI-H1975 cells was extracted by utilizing RIPA lysis buffer provided by the Beyotime Institute of Biotechnology and supplied with a protease inhibitor cocktail. Cell samples were received and washed twice with PBS, and the moderately pre-cooled 2 × Lysis Buffer [100 mM 1MTris-HCl (pH 6.8), 2% mercaptoethanol, 20% gly-cerol, 4% SDS] was lysed. Denaturing 10% polyacrylamide gel electrophoresis was employed to fractionate the protein samples, which were then transferred onto Hybond-ECL nitrocellulose membranes in equal protein quantities. The PVDF membrane was subsequently sealed at room temperature for 1 hour using a 5% skim milk solution in TBST. The primary antibodies were diluted in a blocking solution and incubated with the blocked PVDF membrane lasting for 2 h at room temperature, followed by four times of washing with TBST, each lasting 8 min. The appropriate two-string antibodies were diluted with blocking solution, and the membranes made of PVDF

were incubated for 1.5 h at ambient temperature before being washed four times for 8 min each with TBST. Following that, an X-ray exposure analysis was performed.

2.12. Vivo experiments

25 female BALB/c nude mice (8-week-old) obtained from Taconic Biosciences were kept in pathogen-free environments with 12-hour light/dark cycles at 50%–60% humidity and 22 °C–26 °C. They were divided into the KD group (n = 5), the NC group (n = 5), and the Blank group (n = 5). Upon receiving appropriate treatment, the KYSE150 cells were inoculated into the dorsum of these animals. The changes in volume of nude mice were recorded daily, and growth curves were plotted. All mice were administered radiation therapy following inoculation, once the tumor diameter had reached approximately 5 mm, at a dose of 6 Gy. The radiation dose of 6 Gy was chosen based on the results of our previous pre-experiments. This dose has been shown to be effective in inhibiting tumor growth with low systemic effects on the animals, ensuring experimental feasibility and animal well-being. To ensure uniformity and consistency of radiation, we use specialized X-ray irradiators for radiation. The equipment was calibrated before use to ensure the accuracy and uniformity of the radiation dose. During the radiation process, mice were fixed in special molds to ensure accurate positioning of the tumor site and uniform radiation. In addition, we periodically verify the radiation dose using a dosimeter to ensure consistency of experimental conditions.

Tumor progression was monitored bi-daily until the 35-day mark was attained. Upon the sacrifice of the mice, the tumors were excised and weighed, followed by their analysis using Western Blot and qPCR techniques. The equation was used to calculate the tumor volume, $V = ab^2/2$ (a represented the longest axis; b represented the shortest axis). The animal experiments were strictly conducted in compliance with the NIH Guideline for the Care and Use of Laboratory Animals.

2.13. Statistical analysis

The generated data was processed utilizing SPSS 25.0 software (SPSS, Inc., Chicago, USA), and the outcomes were presented as the mean ± SEM. The ANOVA test or the Student's t-test was utilized to take differences between groups into analysis. A P-value of less than 0.05 was considered the statistical variance.

3. Results

3.1. DNAJC12 expressions were up-regulated in ESCC tissues

The workflow chart depicted the design framework of this study (see Supplementary **Figure 1**). An analysis of the TCGA database indicated that DNAJC12 was highly expressed in tissues associated with the ESCC (**Figure 1A**). Immunohistochemical analysis further substantiated that DNAJC12 exhibited a higher level of expression in tissues of ESCC when compared with DNAJC12 level in adjacent non-cancerous tissues. Additionally, it was observed that elevated expression of DNAJC12 correlated with the advanced T and N stages (**Figure 1B–E**, Appendix **Table A1**). To assess the clinical significance of DNAJC12 expression, we collected

clinicopathological data from patients, including tumour stage, lymph node metastasis, and patient survival data. Using Kaplan-Meier survival analysis, we found that the overall survival of patients in the DNAJC12 high-expression group was significantly shorter than that in the low-expression group ($P < 0.05$, log-rank test). In addition, Spearman correlation analysis showed that DNAJC12 expression level was positively correlated with tumour stage ($r = 0.45$, $P < 0.01$) and lymph node metastasis ($r = 0.38$, $P < 0.05$).

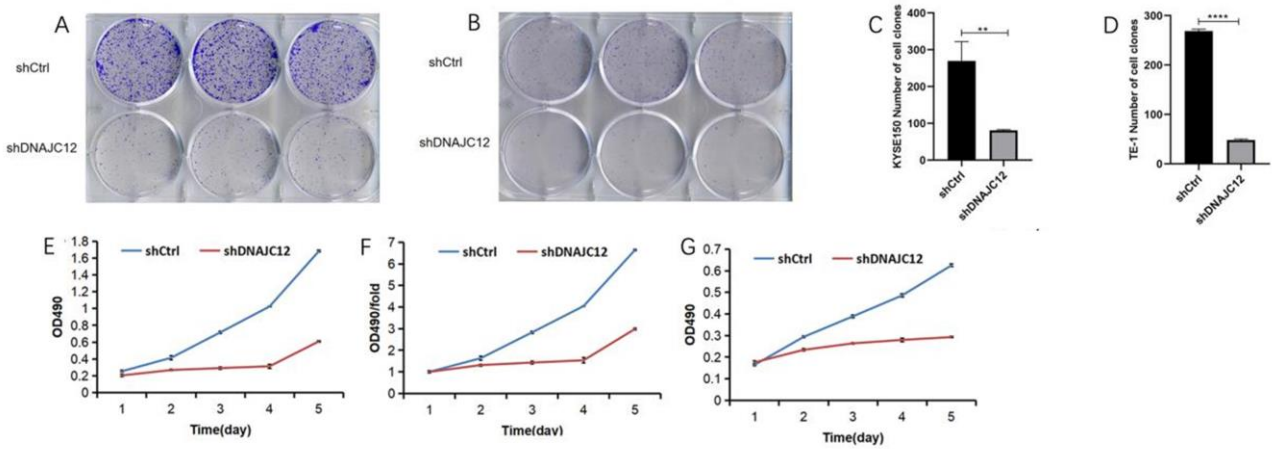

Figure 1. DNAJC12 exhibited a high level of expression in esophageal squamous cell carcinoma. **(A)** TCGA database analysis of DNAJC12 differential expression in ESCC tissues and paracancerous tissues; **(B–E)** DNAJC12 negative (paracancerous tissues); DNAJC12 low expression; DNAJC12 moderate expression; DNAJC12 high expression; **(F,G)** qPCR assay was performed to detect the efficiency of target gene knockdown at mRNA level in KYSE150 cell and TE-1 cell.

$^{**}P < 0.01$, statistically different.

3.2. Knockdown of DNAJC12 suppressed the proliferation, migration, and invasion of ESCC, while promoting apoptosis

As shown in **Figure 1F,G**, the sh-DNAJC12 and sh-control lentivirus was successfully infected into KYSE-150 and TE-1 cell lines. The HCS and MTT assays demonstrated that silencing DNAJC12 markedly compromised the proliferative ability of the cells, as illustrated in **Figure 2**. The clones formation assay demonstrated that the reduction of DNAJC12 reduced the clones' number and size, as shown in **Figure 3A–D**. The proliferative capacity of cells was found to be significantly correlated with the DNAJC12 gene in **Figure 3E–H**. Else, DNAJC12 knockdown would significantly diminish the migratory and invasive capabilities of ESCC cells, as shown in Supplementary **Figures 2** and **3**. The flow cytometry detection showed that the down-regulation of DNAJC12 would promote apoptosis of ESCC cells (**Figure 4**). In conclusion, IN ESCC, the silence of DNAJC12 may inhibit the proliferation, migration, and invasion, while facilitate the apoptosis of cells.

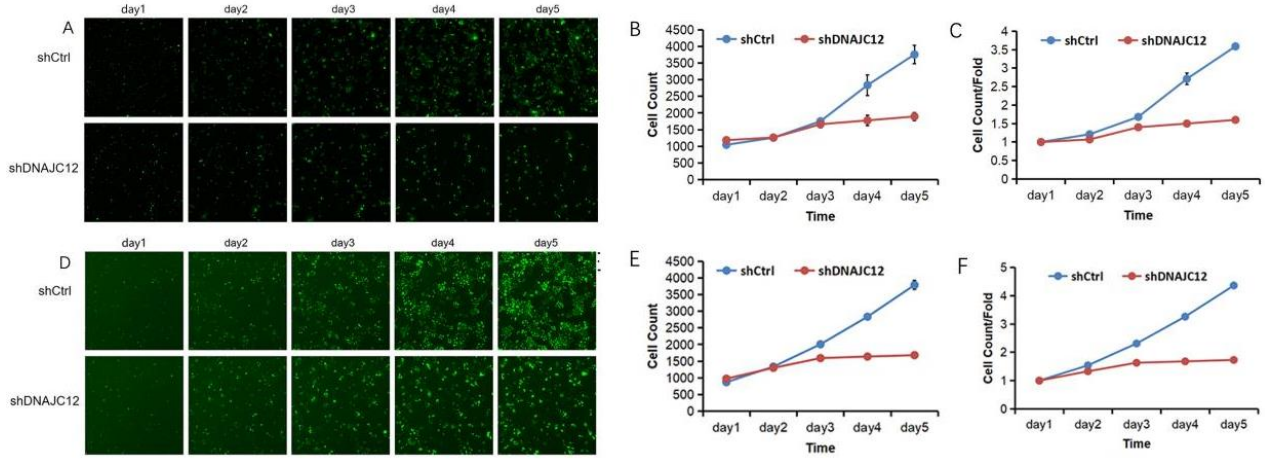

Figure 2. The decreased proliferation of KYSE150 and TE-1 cells was found after knockdown of DNAJC12. **(A)** Celigo assay growth of KYSE150 cells (200px); **(B)** Curve of shRNA lentivirus infected KYSE-150 cells, 3 days later, in the shDNAJC12 group versus the control group (shCtrl); **(C)** shRNA lentivirus infection of KYSE-150 cells, 3 days later, the fold change in cell number of the shDNAJC12 group versus the control group (shCtrl); **(D)** Celigo assay growth of TE-1 cells (200px); **(E)** Curve of shRNA lentivirus infected TE-1 cells, 3 days later, shDNAJC12 group versus control group (shCtrl); **(F)** shRNA lentivirus infection of TE-1 cells, 3 days later, the fold change in cell number of the sh-DNAJC12 group versus the control group (shCtrl).

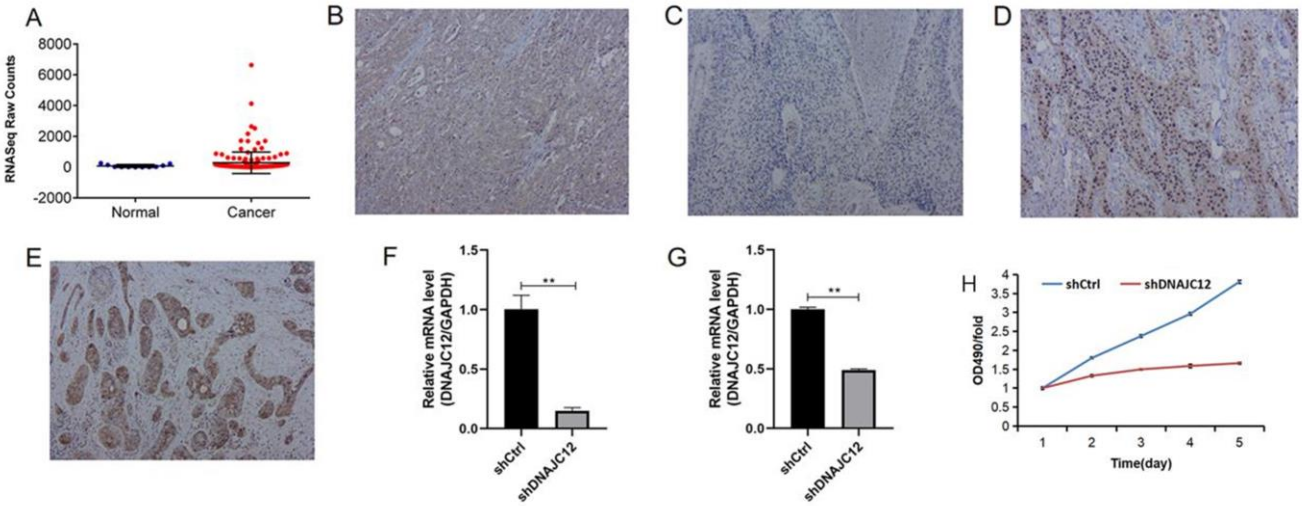

Figure 3. Cellular clone formation assay and MTT assay. **(A,C)** The number of clones in KYSE150 cells and TE-1 cells after knockdown of DNAJC12; **(B,D)** Comparison of the clones' numbers in the DNAJC12 knockdown group and the control group after shRNA lentivirus infection of KYSE-150 cells and TE-1 cells; **(E,F)** Comparison of the absorbance of light at a wavelength of 490 nm in KYSE150 cells in the shDNAJC12 group versus the control group (shCtrl) in an enzyme marker and an enzyme labeling instrument; **(G,H)** Comparison of the change in absorbance of light at a wavelength of 490 nm in TE-1 cells between the shDNAJC12 group and the control group (shCtrl) in an enzyme labeling instrument and in the zymograph. OD_{490} reflects the number of viable cells.

$^{**}P < 0.01$, $^{****}P < 0.0001$, statistically different.

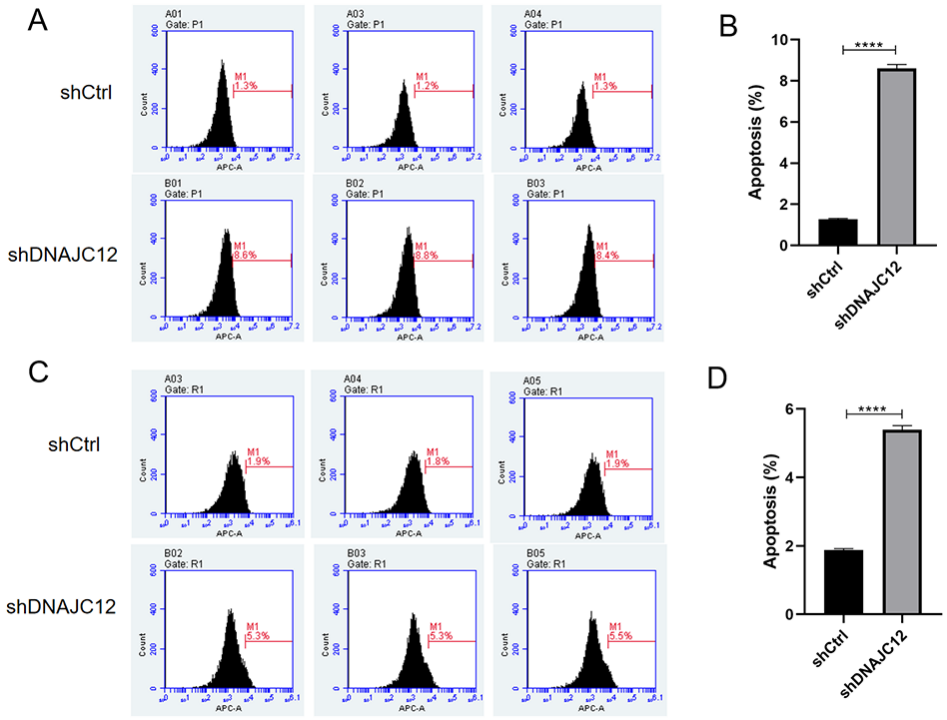

Figure 4. FACS apoptosis assay showed the increased the numbers of KYSE150 apoptotic cells and TE-1 apoptotic cells after knockdown of DNAJC12.
****$P < 0.0001$, statistically different.

3.3. Regulation of downstream pathway molecules by knockdown of DNAJC12

Through western blot, it was revealed that the expressions of P-ERK, MMP-2, N-Cadherin, P-P38, Snail, Vimentin, β-Catenin, Fibronectin and Twist were down-regulated and E-Cadherin was up-regulated following the silencing of DNAJC12 (**Figure 5**). Five genes including SNAI1, Fibronectin, Vimentin, MMP2 and N-Cadherin with significant differences were selected for inclusion in the downstream response HCS experiment (**Figure 5**). The HCS proliferation screening analysis revealed that interference with the DNAJC12 gene and the over-expression of the SNAI1 gene could most notably decelerate the propensity for replication (Supplementary **Figure 4A**). Consequently, lentiviruses overexpressing SNAI1 and those designed to knock down the target gene were chosen for co-infection of the cells, to proceed with the validation experiments for functional recovery. To determine if SNAI1 plays a role in the impact of DNAJC12 on ESCC, we overexpressed SNAI1 in KYSE-150 cells that had DNAJC12 expression suppressed. It was confirmed by MTT and transwell experiments in Supplementary **Figure 4B–D** that the SNAI1 over-expression could reverse the proliferative and metastatic capabilities in cells that the DNAJC12 was knockdown. Taken together, these data indicated that DNAJC12 might control cellular proliferation and invasion via SNAI1 in ESCC.

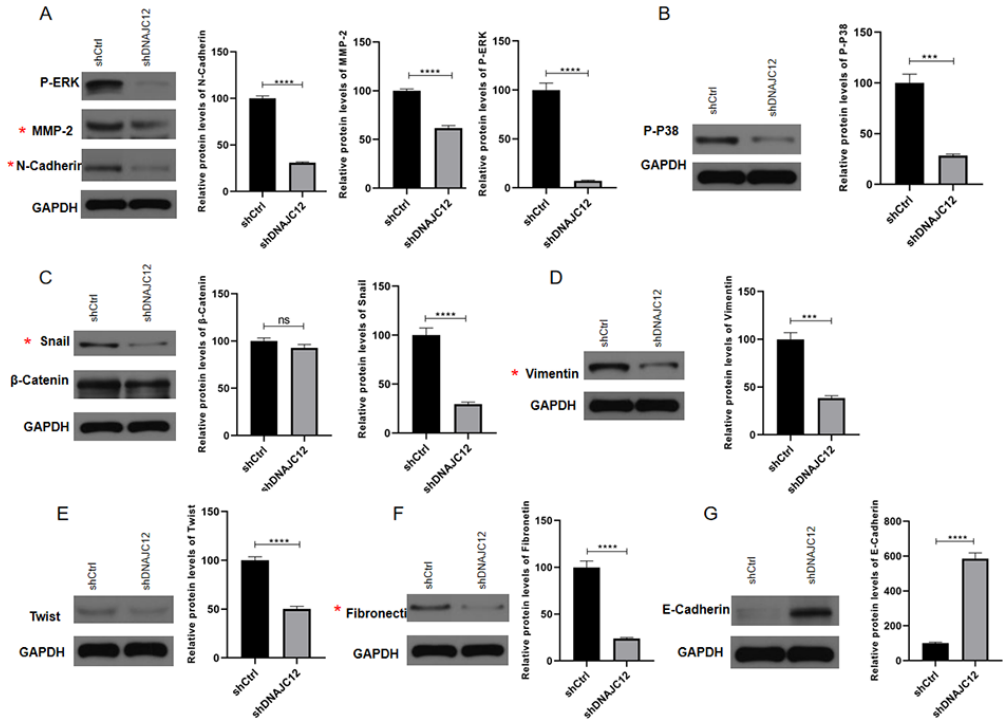

Figure 5. Changes in intracellular expression levels of P-ERK, MMP-2, P-P38, Snail, β-Catenin, Vimentin, Twist, Fibronectin, E-Cadherin proteins detected by Western blot after knockdown of DNAJC12.

3.4. Effect of transplanted tumors on radiation susceptibility in nude mice

In vivo, the changes of tumor volume in three groups were shown in **Figure 6A** and the suppression of DNAJC12 expression hastened tumor progression. Furthermore, the DNAJC12 expression was notably reduced in the KD group than that in the NC group following DNAJC knockdown and radiotherapy (**Figure 6B**). Knockdown of DNAJC12 could potentially influence the growth rate of tumors post-irradiation. Differences in PARP-1, γH2AX, SNAIl, and E-cadherin protein levels detected by WB showed the down-regulation of PARP-1 and SNAIl and the elevated levels of E-Cadherin and γH2AX expression (**Figure 6C–G**).

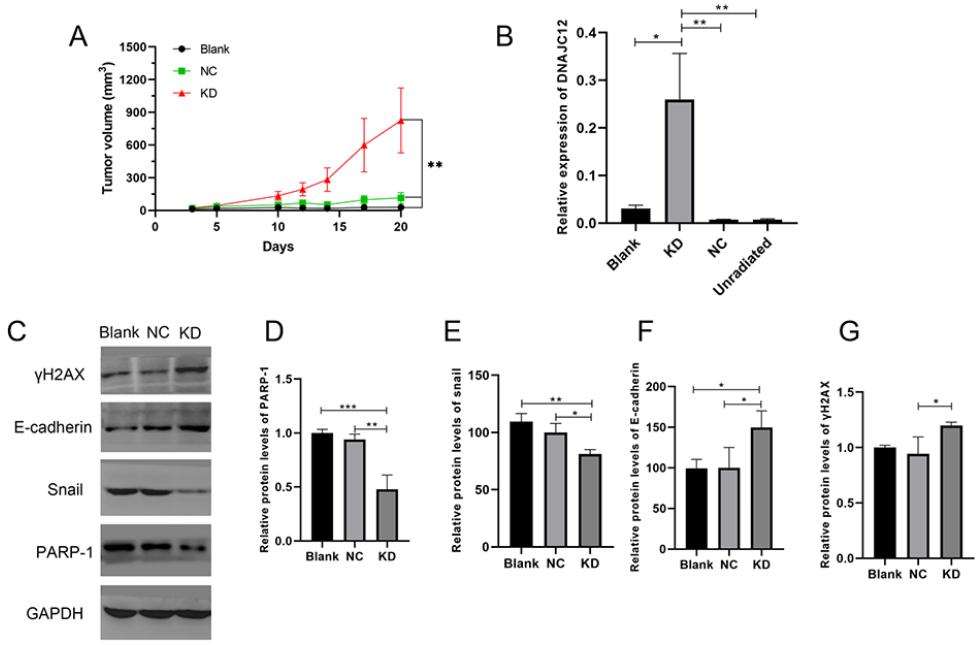

Figure 6. Efficacy of Irradiation Treatment on Transplanted Tumors in Nude Mice Following DNAJC12 Knockdown; **(A)** Tumor volume changes after irradiation; **(B)** qPCR detection of DNAJC12 expression in transplanted tumors of nude mice after DNAJC12 knockdown after irradiation; **(C–G)** WB detection showed that PARP-1 and snail were down-regulated, the expression level of E-cadherin and γH2AX was up-regulated.

*$P < 0.05$, **$P < 0.01$, statistically different. Data were analyzed using a one-way ANOVA.

4. Discussion

In the current research, DNAJC12 could promote the multiplication, invasion, and migration while simultaneously inducing apoptosis in ESCC, likely via its interaction with SNAI1. Combined with other investigations, these data revealed that DNAJC12 was markedly increased in cancerous cells in comparison to those in non-cancerous tissues [12]. Rescue experiments indicate that SNAI1 influences the functional role of DNAJC12 in controlling the biology of ESCC cells. DNAJC12, a member of the DNAJ gene family, is classified as a heat shock protein; some of the family members are known for their oncogenic properties in various types of cancer [13]. It is essential to elucidate the potential pathogenesis and functional roles of HSP40/DNAJ in cancer, as it may contribute to carcinogenesis by acting as an oncogene or by promoting tumorigenesis through its function as a co-chaperone for various oncogenes and tumor suppressors [14]. DNAJC12 promotes cell proliferation and drug resistance by activating β-catenin through HNF1α. β-catenin, as a key protein in the Wnt signaling pathway, is regulated by a variety of factors for its stability. Studies have shown that increased stability of β-catenin promotes the expression of SNAI1, which in turn activates the EMT process. Therefore, DNAJC12 may influence the expression of SNAI1 by regulating the stability of β-catenin and thus participate in the EMT process.

SNAI1 is a core transcription factor in the EMT process that induces the transition from epithelial to mesenchymal cells. In addition to the direct upregulation of SNAI1 expression, other signaling pathways such as the Wnt/β-catenin pathway also play

important roles in EMT. In addition, SNAI1 may also be regulated by non-coding RNAs such as miR-153 and lncRNA XIST. These complex regulatory networks suggest that the role of SNAI1 in EMT goes far beyond a single signaling pathway.

Although current studies have focused on the activation of β-catenin by DNAJC12 through HNF1α to affect cell proliferation and migration, future studies could further explore whether DNAJC12 affects SNAI1 expression through other pathways (e.g., direct or indirect regulation of β-catenin). In addition, considering the multiple regulatory mechanisms of SNAI1 in EMT, researchers can explore whether DNAJC12 interacts with other signaling pathways (e.g., the Wnt/β-catenin pathway) to participate in the EMT process.

Uno et al. [8] identified DNAJC12 as an underlying candidate gene with the aid of transcriptome analysis and revealed that DNAJC12 was notably induced in tissues from gastric cancer individuals, as opposed to those in adjacent normal tissues. Furthermore, patients with high DNAJC12 amounts correlated with the significantly reduced overall survival rates. In gastric cancer, the over-expression of DNAJC12 over-expression was closely associated with lymphatic metastasis, invasive tumor growth patterns, lymph node involvement, and advanced disease staging. Notably, DNAJC12 had been identified as an independent prognostic factor for overall survival through multivariate analyses. The suppression of DNAJC12 expression markedly diminished the proliferation and invasive capabilities of gastric cancer cells, lending support to the notion that DNAJC12 is related to the aggressive phenotype of gastric cancer as a latent candidate gene [8]. A pivotal trait of cancer cells is their ability to form colonies [15]. This study further revealed that DNAJC12 influenced not only the quantity of colonies produced by the cells but also their dimensions. Interestingly, vivo studies proved that the knockdown of DNAJC12 suppressed the proliferation of ESCC cells. In conclusion, our findings indicate that DNAJC12 exerts a substantial influence on the proliferation and growth of ESCC cells, whether in vitro or in vivo. From the available data, the role of DNAJC12 in a variety of cancers has been extensively studied. For example, knockdown of DNAJC12 in lung cancer cells significantly induced apoptosis in A549 and NCI-H470 cells and decreased the phosphorylation level of NF-κB p65, as well as down-regulated the expression of β-catenin and vimentin in lung cancer cells. This suggests that DNAJC12 may affect the proliferation, migration, and invasion of lung cancer cells by regulating the NF-κB and β-catenin signaling pathways.

DNAJC12 also inhibited iron death and apoptosis by activating AKT, which enhanced the cytotoxicity of the chemotherapeutic drug doxorubicin. This further suggests the important role of DNAJC12 in apoptosis and iron death.

From a proteomic perspective, DNAJC12 is a member of the Hsp70 family of proteins, which is widely involved in a variety of metabolic activities in the cell, such as protein synthesis, folding, transport and degradation. These functions suggest that DNAJC12 may regulate cell survival and death by affecting cellular stress responses and protein homeostasis.

In prostate cancer, FGL1 was positively correlated with DNAJC12 expression, and silencing of FGL1 promoted apoptosis in prostate cancer cells. This suggests that DNAJC12 may regulate apoptosis through interaction with other proteins.

Presently, the cause of ESCC metastasis remains to be controversial [16]. This study assessed cellular invasion and migration through in vitro Migration and Transwell Invasion assays observed that the restraint of DNAJC12 could markedly diminish the invasive and migratory capacities of KYSE-150 and TE-1 cells, implying that one probable mechanism underlying the metastasis of ESCC may involve the up-regulated expression of DNAJC12 in tumor cells. Following therapeutic pharmacological treatment, the death of cancer cells inhibits tumor growth and spread [17]. In KYSE-150 and TE-1 cells, silencing DNAJC12 significantly enhances apoptosis. All in all, DNAJC12 could govern the proliferation, metastasis, apoptosis capabilities of ESCC cells. According to the available information, DNAJC12 is a member of the HSP70 family of proteins, which is widely involved in protein folding, degradation and transport. In tumour cells, the up-regulation of DNAJC12 expression is closely related to the development of various cancers. In addition, DNAJC12 was positively correlated with the expression level of FGL1 in prostate cancer, and the high expression of FGL1 was associated with poorer survival of prostate cancer patients. This suggests that DNAJC12 may influence the behavior of tumor cells by affecting the expression or function of FGL1.

However, current studies have focused on the role of DNAJC12 within tumor cells and have not yet clarified its specific mechanism in the tumor microenvironment. The tumor microenvironment is a complex ecosystem that includes components such as tumor cells, immune cells, fibroblasts, and extracellular matrix (ECM).

SNAI1 serves as a driver of cancer progression, playing a role in cell invasion, survival, immune regulation, stem cell characterization, and metabolic regulation [18]. SNAI1 has the ability to induce the loss of epithelial cell characteristics and lead to the acquisition of more aggressive, metastatic mesenchymal cell traits [19]. Vimentin acts as a mesenchymal marker and is involved in the remodeling of the extracellular matrix, facilitating the dissemination of cancers like ESCC [20]. Here, the objective was to probe into the impact of DNAJC12 on the vimentin expression. Further MTT assays indicated that SNAI1, rather than vimentin, might come into play in the participation of DNAJC12 in the proliferation, invasion, and migration of ESCC cells. SNAI1 gene overexpression was chosen for the knockdown of DNAJC12 to conduct functional recovery experiments, and it was found that the over-expression of SNAI1 restored the metastatic and proliferative capabilities that were diminished by DNAJC1 knockdown.

We demonstrated that radiation therapy is an effective strategy to aggressively target DNAJC12-related progression of ESCC [21]. During radiation therapy, cancer cells that have sustained irreparable DNA damage proliferate at a reduced rate and ultimately stop multiplying, resulting in a decrease in tumor size as these damaged cells undergo cell death [22]. Notably, the depletion of DNAJC12 exacerbates inefficiencies in DNA repair, thereby increasing the cells' susceptibility to radiation-induced damage [23,24]. This study delineates several pivotal advantages that markedly enhance our comprehension and the potential therapeutic approaches for ESCC. Firstly, by focusing on DNAJC12, a gene that had not been extensively studied in ESCC, our work identifies a novel therapeutic target. The findings that DNAJC12 down-regulation hinders cell proliferation, migration, and invasion in vitro, while also enhancing radiosensitivity in vivo, provide crucial insights into the molecular

mechanisms driving ESCC progression and treatment response. Secondly, our comprehensive approach, which integrates the vitro cell line experiments, vivo animal studies and data analysis from the TCGA database, offers robust validation of the results, thus strengthening the reliability and applicability of our conclusions. Finally, this study not only clarifies the role of DNAJC12 in ESCC but also lays the groundwork for future research into targeted therapies, which could drive more valid and personalized therapy strategies in ESCC patients. Furthermore, our examination of DNAJC12 expression under irradiation revealed that DNAJC12 down-regulation promotes cell proliferation, ultimately contributing to the increase in tumor volume.

To explore the expression pattern of DNAJC12 in different clinical stages and metastatic status, we analyzed ESCC samples from the TCGA database using GSEA. Specifically, we grouped the samples according to clinical stage (e.g., stage I vs. stage III-IV) and metastatic status (with metastasis vs. without metastasis) and analyzed the differences in DNAJC12 expression levels between the groups. The GSEA results revealed a trend of high expression of DNAJC12 in highly staged and metastatic samples, suggesting its potential role in ESCC progression.

Combined with the clinical prognostic data of patients, we evaluated the clinical value of DNAJC12 as a potential prognostic marker. Specifically, we used Kaplan-Meier survival analysis to assess the overall survival (OS) and disease-free survival (DFS) of patients in the DNAJC12 high-expression and low-expression groups. The results showed that patients in the DNAJC12 high-expression group had significantly lower OS and DFS than those in the low-expression group ($P < 0.05$), suggesting that DNAJC12 may be an independent risk factor for the prognosis of ESCC patients.

Nevertheless, the current study has several limitations. Initially, the regulatory mechanisms by which DNAJC12 exerts its influence on ESCC remain to be fully clarified. Secondly, the precise mechanisms through which DNAJC12 influences SNAI1 have yet to be elucidated. The mechanisms through which DNAJC12 augments tumor radiosensitivity remain unclear. Future research that addresses these questions will greatly improve our comprehension of DNAJC12 in esophageal cancer.

5. Conclusion

In summary, the suppression of DNAJC12 decelerates the progression of ESCC, and the expression of DNAJC12 enhances radiosensitivity, DNAJC12 could act as a prospective prognostic marker and therapeutic target for ESCC.

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Availability of data: All data generated or analyzed are included herein during this study. For further inquiries, please contact the corresponding author.

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Ethical approval: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Cancer Hospital affiliated with Shandong First Medical University (protocol code: No. 2021-023-02)" for studies involving humans. Written informed consent has been obtained from the patient(s) to publish this paper.

Conflict of interest: The authors declare no conflict of interest.

Abbreviations

ESCC	esophageal squamous cell carcinoma
DNAJC12	DNAJ heat shock protein family member C12
EMT	epithelial-mesenchymal transition
GEPIA	Gene Expression Profiling Interactive Analysis
TCGA	The Cancer Genome Atlas
GTEx	Genotype-Tissue Expression
FBS	fetal bovine serum
shRNA/sh	Short hairpin RNA
HCS	High-content screening
APC	Annexin V-allophycocyanin

References

1. Cao W, Chen HD, Yu YW, et al. Changing profiles of cancer burden worldwide and in China: a secondary analysis of the global cancer statistics 2020. Chinese Medical Journal. 2021; 134(7): 783-791. doi: 10.1097/cm9.0000000000001474
2. Procopio F, Marano S, Gentile D, et al. Management of Liver Oligometastatic Esophageal Cancer: Overview and Critical Analysis of the Different Loco-Regional Treatments. Cancers. 2019; 12(1): 20. doi: 10.3390/cancers12010020
3. Puhr HC, Prager GW, Ilhan-Mutlu A. How we treat esophageal squamous cell carcinoma. ESMO Open. 2023; 8(1): 100789. doi: 10.1016/j.esmoop.2023.100789
4. Macario AJ, Conway DME. Molecular chaperones: Multiple functions, pathologies, and potential applications. Frontiers in Bioscience. 2007; 12(1): 2588. doi: 10.2741/2257
5. Liu T, Daniels CK, Cao S. Comprehensive review on the HSC70 functions, interactions with related molecules and involvement in clinical diseases and therapeutic potential. Pharmacology & Therapeutics. 2012; 136(3): 354-374. doi: 10.1016/j.pharmthera.2012.08.014
6. Euhus D, Bu D, Xie XJ, et al. Tamoxifen DownregulatesEtsOncogene Family Members ETV4 and ETV5 in Benign Breast Tissue: Implications for Durable Risk Reduction. Cancer Prevention Research. 2011; 4(11): 1852-1862. doi: 10.1158/1940-6207.capr-11-0186
7. He HL, Lee YE, Chen HP, et al. Overexpression of DNAJC12 predicts poor response to neoadjuvant concurrent chemoradiotherapy in patients with rectal cancer. Experimental and Molecular Pathology. 2015; 98(3): 338-345. doi: 10.1016/j.yexmp.2015.03.029
8. Uno Y, Kanda M, Miwa T, et al. Increased Expression of DNAJC12 is Associated with Aggressive Phenotype of Gastric Cancer. Annals of Surgical Oncology. 2019; 26(3): 836-844. doi: 10.1245/s10434-018-07149-y
9. Cajigas-Du Ross CK, Martinez SR, Woods-Burnham L, et al. RNA sequencing reveals upregulation of a transcriptomic program associated with stemness in metastatic prostate cancer cells selected for taxane resistance. Oncotarget. 2018; 9(54): 30363-30384. doi: 10.18632/oncotarget.25744
10. Lee J, Hahn Y, Yun JH, et al. Characterization of JDP genes, an evolutionarily conserved J domain-only protein family, from human and moths. Biochim Biophys Acta. 2000; 1491(1-3): 355-363. doi: 10.1016/S0167-4781(00)00047-6
11. Choi J, Djebbar S, Fournier A, et al. The co-chaperone DNAJC12 binds to Hsc70 and is upregulated by endoplasmic reticulum stress. Cell Stress and Chaperones. 2014; 19(3): 439-446. doi: 10.1007/s12192-013-0471-6

12. Li Y, Li M, Jin F, et al. DNAJC12 promotes lung cancer growth by regulating the activation of β-catenin. International Journal of Molecular Medicine. 2021; 47(6). doi: 10.3892/ijmm.2021.4938
13. Fan YC, Meng ZY, Zhang CS, et al. DNAJ heat shock protein family member C1 can regulate proliferation and migration in hepatocellular carcinoma. Peerj; 2023.
14. Zhong BX, Shen FM, Chen JK. The role of HSP40 in cancer: Recent advances. Histol Histopathol. 2024; 39(7): 845-851. doi: 10.14670/HH-18-693
15. Hanahan D, Weinberg RA. Hallmarks of Cancer: The Next Generation. Cell. 2011; 144(5): 646-674. doi: 10.1016/j.cell.2011.02.013
16. Liu Y, Xiong Z, Beasley A, et al. Personalized and targeted therapy of esophageal squamous cell carcinoma: an update. Annals of the New York Academy of Sciences. 2016; 1381(1): 66-73. doi: 10.1111/nyas.13144
17. Olson MF. Mechanisms of tumour cell invasion and metastasis. Journal of Molecular Medicine. 2007; 85(6): 543-544. doi: 10.1007/s00109-007-0189-y
18. Dong B, Wu Y. Epigenetic Regulation and Post-Translational Modifications of SNAI1 in Cancer Metastasis. International Journal of Molecular Sciences. 2021; 22(20): 11062. doi: 10.3390/ijms222011062
19. Ma T, Zhao Y, Lu Q, et al. MicroRNA-30c functions as a tumor suppressor via targeting SNAI1 in esophageal squamous cell carcinoma. Biomedicine & Pharmacotherapy. 2018; 98: 680-686. doi: 10.1016/j.biopha.2017.12.095
20. Abudureheman A, Ainiwaer J, Hou Z, et al. High MLL2 expression predicts poor prognosis and promotes tumor progression by inducing EMT in esophageal squamous cell carcinoma. Journal of Cancer Research and Clinical Oncology. 2018; 144(6): 1025-1035. doi: 10.1007/s00432-018-2625-5
21. Li F, Lv JH, Liang L, et al. Downregulation of microRNA-21 inhibited radiation-resistance of esophageal squamous cell carcinoma. Cancer Cell International. 2018; 18(1). doi: 10.1186/s12935-018-0502-6
22. Cho EJ, Kim JK, Baek HJ, et al. Preclinical evaluation of radiation therapy of BRCA1-associated mammary tumors using a mouse model. International Journal of Biological Sciences. 2021; 17(3): 689-701. doi: 10.7150/ijbs.53667
23. Jing S, Guo H, Qi Y, et al. A portable fast neutron irradiation system for tumor therapy. Applied Radiation and Isotopes. 2020; 160: 109138. doi: 10.1016/j.apradiso.2020.109138
24. van Spronsen FJ, Himmelreich N, Rüfenacht V, et al. Heterogeneous clinical spectrum of DNAJC12-deficient hyperphenylalaninemia: from attention deficit to severe dystonia and intellectual disability. Journal of Medical Genetics. 2017; 55(4): 249-253. doi: 10.1136/jmedgenet-2017-104875

Appendix

Table A1. DNAJC12 expression in 100 patients with ESCC.

		N	DNAJC12 expression level		*P*-value
			Low expression	High expression	
Sex	Male	68	35	33	0.752
	Female	32	17	15	
Age (year)	<70	79	40	39	0.531
	≥70	21	11	10	
T staging (T)	T1-T2	12	8	4	<0.001
	T3-T4	88	31	57	
N staging (N)	N0-N1	69	47	22	<0.001
	N2-N3	31	10	21	
Neurological violation	Yes	95	51	44	0.015
	No	5	0	5	
Vascular invasion	Yes	91	48	43	0.023
	No	9	1	8	

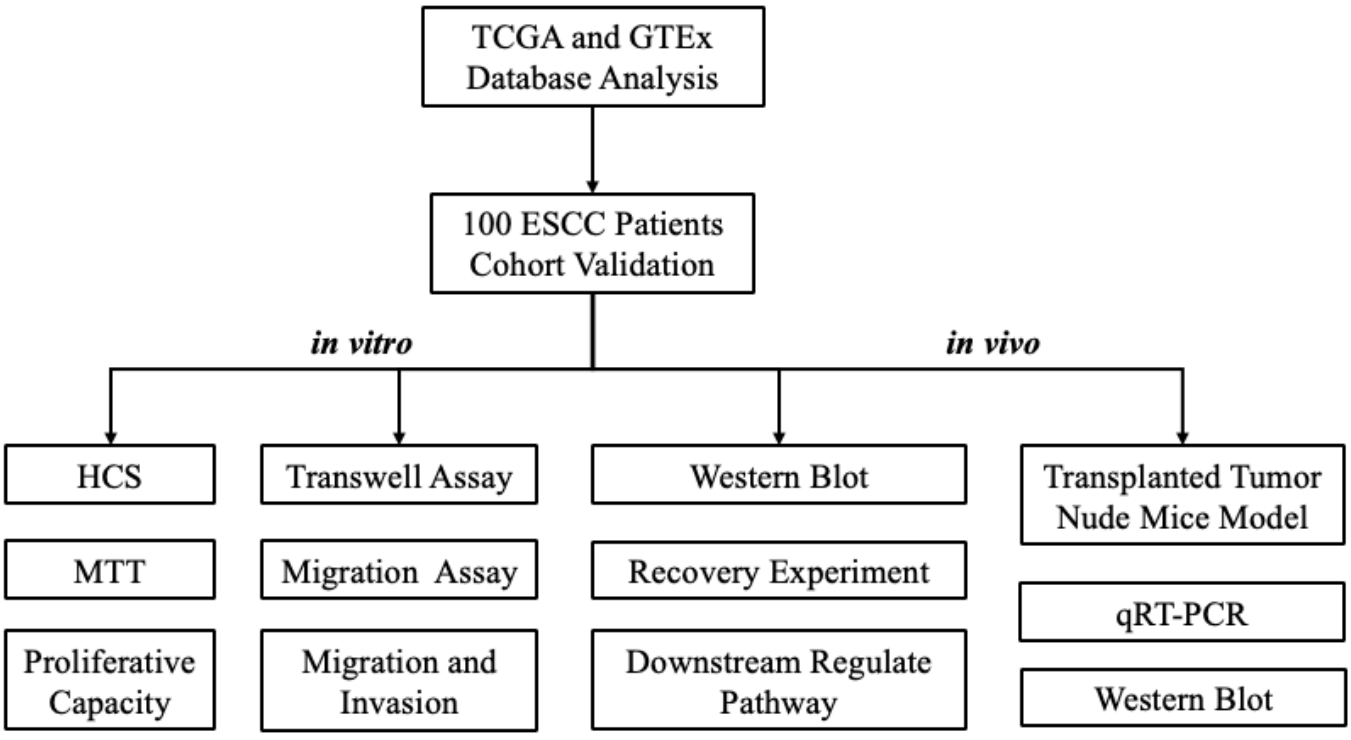

Figure A1. The workflow chart of this study.

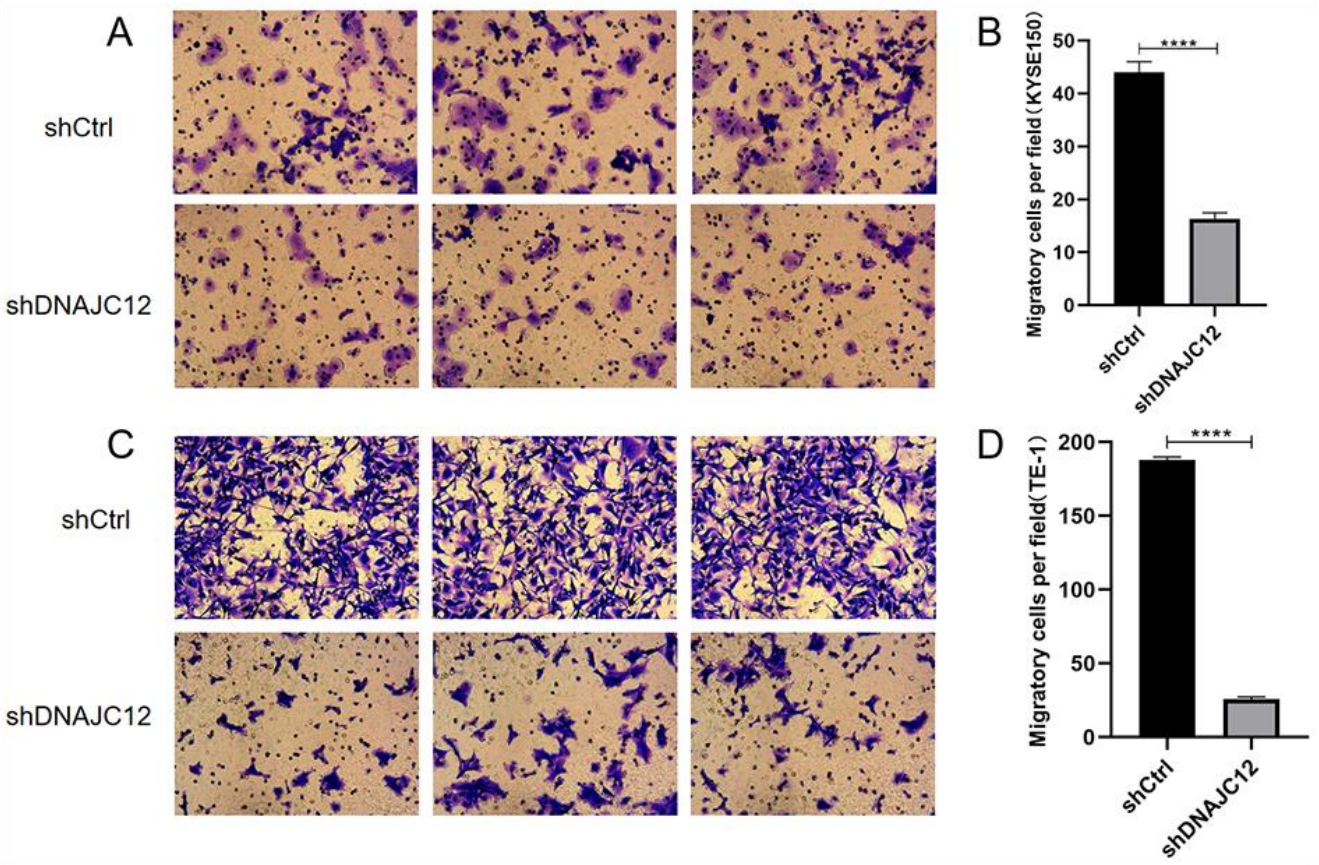

Figure A2. Transwell assay knockdown of DNAJC12 resulted in decreased migration ability of KYSE150 cells and TE-1 cells. **(A)** Micrographs of KYSE150 cells (200px). **(B)** Comparison of the number of transferred cells of KYSE-150 cells in each experimental group after 48h incubation in transwell chambers. **(C)** Micrographs of TE-1 cells (200px). **(D)** Comparison of the number of metastatic cells of TE-1 cells in each experimental group after 48h incubation in transwell chambers.

****$P < 0.0001$, statistically different. Data were analyzed using a Student's t-test. DNAJC12, DnaJ heat shock protein family (Hsp40) member C12; short hairpin RNA; Ctrl, control.

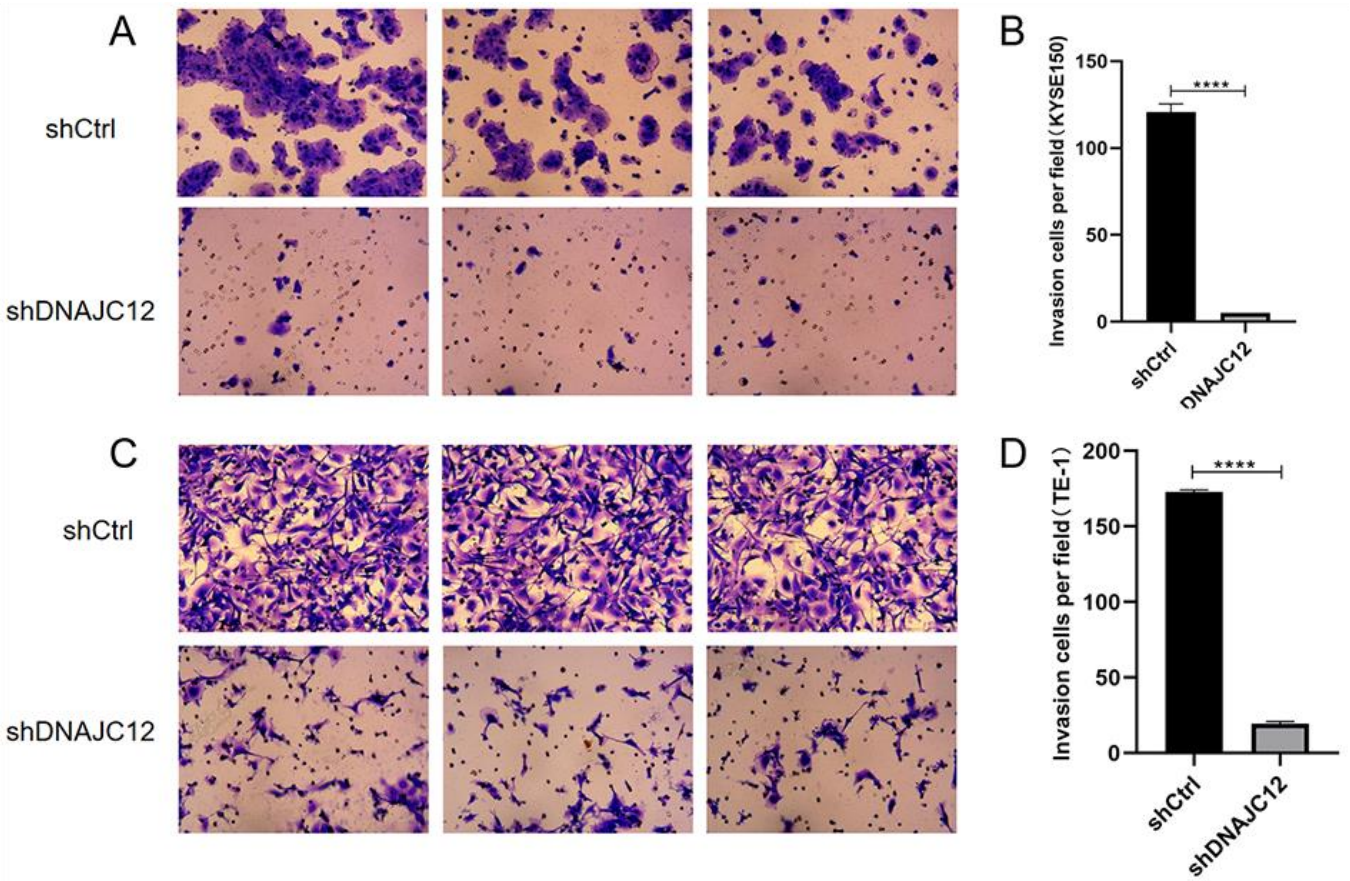

Figure A3. Decreased invasion ability of KYSE150 cells and TE-1 cells after knockdown of DNAJC12 by invasion assay. **(A)** Micrographs of KYSE150 cells (200px). **(B)** Comparison of the number of transferred cells in each experimental group of KYSE-150 cells after 48h incubation in the invasion chamber. **(C)** Micrographs of TE-1 cells (200px). **(D)** Comparison of the number of metastatic cells of TE-1 cells in each experimental group after 48h of incubation in the invasion chamber.

****$P < 0.0001$, statistically different. Data were analyzed using a Student's t-test. DNAJC12, DnaJ heat shock protein family (Hsp40) member C12; short hairpin RNA; Ctrl, control.

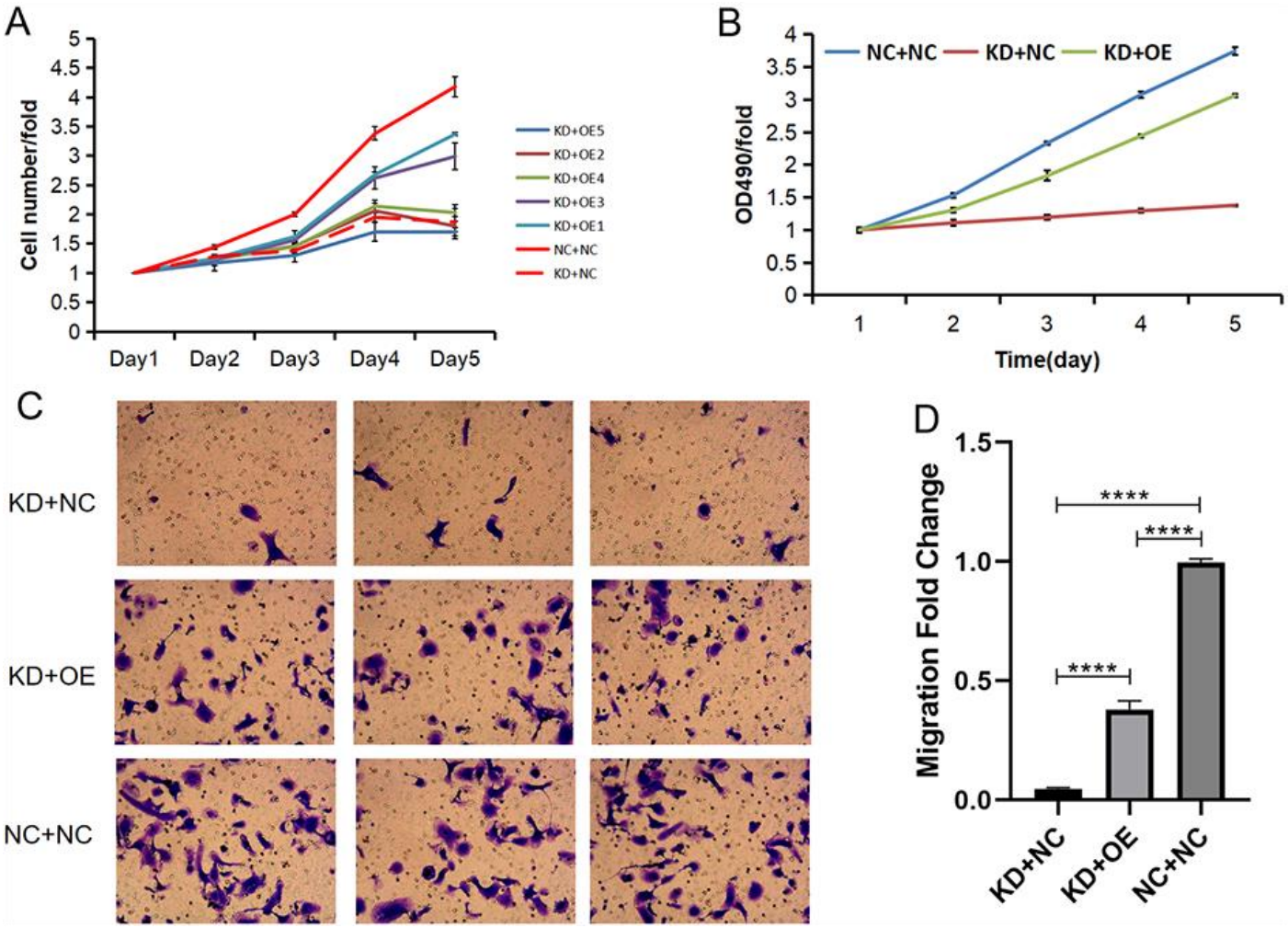

Figure A4. Confirmation that the target gene DNAJC12 interference can regulate the expression of SNAI1, and the downstream gene SNAI1 has a functional reply to the target gene DNAJC12. **(A)** Results of HCS proliferation screening analysis, NC+NC: normal target cells, plus negative control virus-infected cell groups; KD+NC: normal target cells, plus DNAJC12 gene-interfering virus and negative control virus-infected cell groups; KD+OE1: normal target cells, plus DNAJC12 gene-interfering virus and SNAI1 gene overexpression virus-infected cell groups KD+OE2: normal target cells, plus DNAJC12 interfering virus and FN1 overexpression virus infected cell group; KD+OE3: normal target cells, plus DNAJC12 interfering virus and VIM overexpression virus infected cell group; KD+OE4: normal target cells, plus DNAJC12 interfering virus and MMP2 overexpression virus infected cell group. OE4: normal target cells, plus DNAJC12 gene-interfering virus and MMP2 gene overexpression virus infected cell group; KD+OE5: normal target cells, plus DNAJC12 gene-interfering virus and CDH2 gene overexpression virus infected cell group. **(B)** MTT assay results of SNAI1 gene overexpression on the proliferative function of DNAJC12 knockdown group. **(C)** Micrographs (200px) of three groups of YSE150 cells in transwell experiments. **(D)** transwell experiments showed that SNAI1 gene overexpression had a reply on the metastatic function of the target gene knockdown group.

****$P < 0.0001$, statistically different. Data were analyzed using a one-way ANOVA. DNAJC12, DnaJ heat shock protein family (Hsp40) member C12; HCS, high -content screening.