

Original Research DDX60 Promotes Migration and Invasion of Head and Neck Squamous Cell Carcinoma Cell through the NF-κB/IFI27 Signaling Pathway

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Abstract

Background: Despite its significance in multiple cancer types. the function and mechanism of DEXD/H box helicase 60 (DDX60) in head and neck squamous cell carcinoma (HNSCC) remain unreported. **Methods**: Thirty paired HNSCC tissues and adjoining normal tissues and human normal oral epithelial keratinocytes (HOK) and four HNSCC cells (CAL27, SAS, CAL33, and SCC25) were analyzed for DDX60 expression by Semi-quantitative real-time PCR (SQ RT-PCR) and western blot. To investigate how DDX60 affects HNSCC cell migration and invasion, transwell experiments were performed. The western blot was implemented to understand the interaction among DDX60, Interferon Alpha Inducible Protein 27 (IFI27), and the NF- κ B pathway. **Results**: Results revealed the upregulation of DDX60 in HNSCC cell lines and tissues. Additionally, patients with upregulated DDX60 expression exhibited a dismal prognosis relative to those with downregulated expression. DDX60 enhanced the migration, invasion, and epithelial to mesenchymal transition (EMT) in HNSCC cells. The results from mechanistic studies revealed that DDX60 could promote the IFI27 expression following the activation of NF- κ B pathway. **Conclusion**: DDX60 promoted the migratory and invasive capacities of HNSCC cells via the NF- κ B/IFI27 axis.

Keywords: head and neck squamous cell carcinoma; DDX60; EMT; NF-*k*B pathway; IFI27

1. Introduction

Head and neck cancer (HNC) is among the most prevalent malignant tumors that cause cancer-related deaths, and both its incidence and mortality rates have been rising over the last several years [1,2]. Head and neck squamous cell carcinomas (HNSCCs) stem from the squamous epithelium cells of the oral cavity, oropharynx, larynx, and hypopharynx and account for the vast majority of HNC [3]. Surgery, radiotherapy, and chemotherapy are the preferred treatment regimens [4]. The recurrence and metastasis of HNSCC can affect treatment effects, prognosis, and survival time of patients [5,6]. In addition, those diagnosed with HNSCC have a dismal chance of surviving in the long term, although the field of HNSCC treatment and prevention has advanced considerably [7-10]. Hence, conducting in-depth studies is important to explore the mechanisms (from the molecular viewpoint) implicated in the onset and progression of HNSCC. Research on molecular targets should be conducted to achieve targeted HNSCC treatment.

The DEAD-box (DDX) protein family is the largest family of RNA helicases, with 37 members, including DDX5, DDX21, DDX23, and DDX60, all of which have a conserved amino acid sequence ASP-GLUALA-ASP [11]. Via their ATP-dependent RNA helicase activity, DDX proteins are capable of interacting with both ribosomal RNAs (rRNAs) and messenger RNAs (mRNAs) and perform important functions in cell life, such as participating in the initiation of translation, the production of mRNA, the splicing and modification of RNA, and the assembly of ribosomes and spliceosomes [12–15]. It is also implicated in the modulation and transcription of genes related to DNA repair, proliferation, cell cycle arrest, apoptosis, and other cell life activities, suggesting that the DDX protein is potentially involved in carcinogenesis [16,17]. DEXD/H box helicase 60 (DDX60) is a newly discovered DEAD-box RNA helicase that is upregulated following viral infection and performs an important function in antiviral immunity by promoting the retinoic acid-inducible gene I (RIG-I) receptormediated signaling pathway [18]. Recent research has illustrated that DDX60 is highly expressed in oral squamous cell carcinoma (OSCC) and is significantly associated with early recurrence of OSCC, indicating DDX60's potential involvement in tumorigenesis [19]. Recently, DDX60 has been implicated in the development of numerous tumors as well as in immunotherapy. However, the function of DDX60 and the mechanism via which it acts in HNSCC remain unreported. Therefore, conducting studies is important to understand the mechanism of HNSCC progression.

In this experiment, we explored the transcription and expression level of DDX60 and its function and mechanism in HNSCC. We also investigated that Interferon Al-



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pha Inducible Protein 27 (IFI27), which was positively regulated by DDX60, was also regulated by DDX60-mediated activation of NF- κ B in HNSCC. Our data indicated that DDX60 shows great promise as a potential HNSCC diagnostic marker or therapeutic target.

2. Materials and Methods

2.1 Clinical Samples

From January 2015 to December 2022, the samples (30 pairs of HNSCC tissues and matched normal tissues) were obtained from The Third Central Hospital of Tianjin. According to clinical diagnosis, 30 cases of HNSCC were determined, and 30 cases of adjacent normal tissue (more than 2 cm away from cancer tissue) were selected as control group. Inclusion criteria: first-episode patients who had not received other treatment before this surgery; the patient did not receive preoperative radiotherapy, chemotherapy, and intervention, as well as all disease-related management; postoperative pathological diagnosis of HNSCC; patients who have not been diagnosed and treated in other hospitals. Exclusion criteria: previous chemoradiotherapy; patients with multiple primary tumors; liver, kidney, and heart dysfunction combined with other serious diseases; secondary HNSCC.

2.2 Cell Culture

ATCC (USA) provided the human normal oral epithelial keratinocyte (HOK). CAL27 and SAS were purchased from Bohui Biotech (Guangzhou, Cina). CAL33 and SCC25 cells were purchased from Wanwu Biotech (Hefei, China). DMEM (CAS # 9001331600, Beijing Solarbio Science & Technology Co., Ltd., Beijing, China), with 10% fetal bovine serum (FBS, CAS # 10099-141, Gibco, Thermo Fisher Scientific, Waltham, MA, USA), streptomycin (100 mg/mL, CAS # S8290, Beijing Solarbio Science & Technology Co., Ltd., Beijing, China), and penicillin (100 U/mL, CAS # IA0340, Beijing Solarbio Science & Technology Co., Ltd., Beijing, China), was utilized to culture all cells. The cells were cultured in T75 cell culture flasks (CAS # 708013, Wuxi Nesi Life Technology Co., Ltd., Wuxi, China) at 37 °C and 5% CO₂ (v/v) for about 48 h, and then the cells were digested with 0.25% trypsin (CAS # T1320, Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) and passed in a ratio of 1:3. All cell lines were validated by STR profiling and tested negative for mycoplasma. Cells were all cultured in a humidified incubator at 37 °C and 5% CO₂.

2.3 Cell Transfection

One day before transfection studies, six-well plates were used for HNSCC cell culturing (1.2×10^6) . The cells were transfected when the cell fusion degree reached approximately 80% on the second day. pcDNA3 vector (NC) was purchased from Youbao Bio (CAS # VT2051, Hunan, China). pcDNA3-DDX60

(DDX60) was constructed by General Biology (Anhui) Co., Ltd. (Anhui, China). Small interfering RNA control (si-NC, 5'-UUCUCCGAACGUGUCACGUTT-3') and small interfering RNA-DDX60 (si-DDX60, 5'-CCACUUUCCUACGAAUUGUTT-3') were synthesized by General Biology (Anhui) Co., Ltd. (Anhui, China). Lipofectamine 2000 (CAS # 11668019, Invitrogen, Waltham, MA, USA) was employed to transfect the HNSCC cells with the plasmids or siRNAs, following the instructions outlined by the manufacturer. Specific transfection steps were implemented as per the direction specified for Lipofectamine 2000.

2.4 Semi-Quantitative Real-Time PCR (SQ RT-PCR)

Total RNA was isolated from cancer tissues and cells in each group utilizing TRIzol Reagent (CAS # R1100, Beijing Solarbio Science & Technology Co., Ltd., Beijing, China). cDNA was reverse-transcribed with an EasyScript® One-Step gDNA Removal and cDNA Synthesis Super Mix (CAS # AW311-02, TransGen Biotech, Beijing, China). An SYBR Green qPCR Mix Kit (CAS # BL697A, Biosharp, Beijing, China) was employed to perform SQ RT-PCR. The $2^{-\Delta\Delta Ct}$ method was applied to calculate the relative expression. β -actin was used as the internal reference for DDX60, IFI27, Vimentin, E-cadherin and N-cadherin. **Supplementary Table 1** summarizes the primers used for SQ RT-PCR.

2.5 Transwell Experiment

The Transwell experiment was conducted following a previously reported method [20]. In brief, for Transwell migration experiments, we resuspended the transfected cells (2×10^4) in serum-free DMEM (200 µL) and inoculated them in each chamber of the upper chamber (CAS # 3470, Corning Incorporated, Corning, NY, USA). For the Transwell invasion experiment, Matrigel (40 µL, 1 mg/mL, CAS # 356234, Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) was added in the upper chamber, and then DMEM without serum (200 µL) was used to resuspend the transfected cells (5 \times 10⁴), followed by inoculating them in each chamber of the upper chamber. In the bottom chamber, 24-well plates were filled with DMEM complete medium (600 µL) supplemented with 20% FBS. Then, 4% paraformaldehyde (PFA) was used to fix cells in the bottom chamber for 20 min after 48 h of incubation. Cell staining was performed with 0.1% crystal violet for 5 min. Transwell cells were randomly assigned to three areas for shooting and counting analyses.

2.6 Western Blot

RIPA lysate (CAS # P0013B, Beyotime, Shanghai, China) containing the protease inhibitor mixture (CAS # 04693116001, Roche, Mannheim, Germany) was used to extract total proteins. The bicinchoninic acid test (CAS # P0010S, Beyotime, Shanghai, China) was employed to



Fig. 1. DEXD/H box helicase 60 (DDX60) expression was upregulated in HNSCC tissues and cells. (A) Analysis results of the data from the GEPIA database show that DDX60 was upregulated in DLBC, ESCA, GBM, HNSCC, LAML, LGG, PAAD, and THYM. (B) Analysis results of the data from the ENCORI database confirmed that DDX60 was upregulated in HNSCC. (C,D) Survival curves of patients with HNSCC predicted by the GEPIA database (C) and ENCORI database (D) based on the mRNA levels of DDX60. (E) The mRNA levels of DDX60 in 30 pairs of HNSCC and its paraneoplastic tissues were detected by SQ RT-PCR. (F) The DDX60 expression levels in 4 pairs of HNSCC and its paraneoplastic tissues were detected by western blot. (G,H) The DDX60 mRNA and protein levels in human normal oral epithelial keratinocytes (HOK) and four HNSCC cells (CAL27, SAS, CAL33, and SCC25) were detected by SQ RT-PCR (G) and western blot (H). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. DLBC, Lymphoid neoplasm diffuse large B-cell lymphoma; ESCA, Esophageal carcinoma; GBM, Glioblastoma; HNSCC, Head and neck squamous cell carcinoma; LAML, Acute myeloid leukemia; LGG, Lower-grade glioma; PAAD, Pancreatic adenocarcinoma; THYM, Thymoma; mRNA, messenger RNA; SQ RT-PCR, Semi-Quantitative Real-Time PCR; GEPIA, Gene Expression Profiling Interactive Analysis.

determine the total protein concentration. Thirty micrograms of protein lysates were separated with 6% to 15% SDS-PAGE according to the molecular weight. Subsequently, they were transferred onto the PVDF membrane (CAS # 0000187216, 0.45 μ m, Millipore Corp., Burlington, MA, USA). The PVDF membrane was first blocked by 5% BSA (CAS # ST2249-100g, Beyotime, Shanghai, China) and then subjected to incubation in the primary antibody throughout the night at 4 °C. Then, the goat antirabbit secondary antibody, labeled with HRP, was introduced to incubate the membrane (1:5000, CAS #pr30011, Proteintech, Wuhan, Hubei, China) for 2 h at room temperature (RT). Subsequently, the Tanon-5200 automatic chemiluminescence imaging analysis system (Tanon, Shanghai, China) was applied to identify the bands. ImageJ (version 1.60; National Institutes of Health, Bethesda, MA, USA) was used to quantify the data obtained using western blotting. Primary antibodies included DDX60 polyclonal antibody (Rabbit, 1:500, CAS #ab139807, Abcam, Boston, MA, USA), N-cadherin polyclonal antibody (Rabbit, 1:3000, CAS # 22018-1-AP, Proteintech, Wuhan, China), E-cadherin polyclonal antibody (Rabbit, 1:5000, CAS # 20874-1-AP, Proteintech, Wuhan, China), Vimentin polyclonal antibody (Rabbit, 1:5000, CAS # 10366-1-AP,



Fig. 2. DDX60 enhanced the migratory and invasive capacities of HNSCC cells. (A,B) Effect of DDX60 overexpression and knockdown on migration in HNSCC cells. (C,D) Effect of DDX60 overexpression and knockdown on invasion in HNSCC cells. Bars = 100 μ m. **p < 0.01, ***p < 0.001, ****p < 0.0001. NC, negative control.

Proteintech, Wuhan, China), p65 recombinant antibody (Rabbit, 1:5000, CAS # 80979-1-RR, Proteintech, Wuhan, China), p-p65 monoclonal antibody (Rabbit, 1:1000, CAS # ab76302, Abcam, Cambridge, MA, USA), IFI27 polyclonal antibody (Rabbit, 1:500, CAS # ab171919, Abcam, Cambridge, MA, USA), and β -Tubulin polyclonal antibody (Rabbit, 1:8000, CAS # 10094-1-AP, Proteintech, Wuhan, China).

2.7 Statistical Analysis

The experiments were repeated three times. The format of mean \pm standard error of the mean (SEM) was used to present the collected data. The data were statistically analyzed, and GraphPad Prism 8.0 (GraphPad Software, Inc., San Diego, CA, USA) was applied for plotting. Student's *t*-tests were used to understand the differences between the two subgroups. Multiple subgroups were compared via a one-way analysis of variance and Tukey post-hoc test. Based on the distribution patterns of indicators, Pearson or Spearman correlation tests were used to estimate the association between variables. Using the survival package, univariate and multivariate Cox regression models were used to assess the survival significance of variables (p < 0.05; statistically significant).

3. Results

3.1 The Expression Level of DDX60 was Upregulated in HCC and was Correlated with a Dismal Prognosis

We explored the expression level of DDX60 and verified its regulatory mechanism in HNSCC to investigate the role of DDX60. First, the Gene Expression Profiling Interactive Analysis (GEPIA; http://gepia.cancer-pku.cn/) database was searched to predict the DDX60 mRNA level in pan-cancers. Through analysis, DDX60 was upregulated in several cancers as well as HNSCC (Fig. 1A). In addition, the Encyclopedia of RNA Interactomes (ENCORI; https://starbase.sysu.edu.cn/) database was searched to further verify the mRNA level of DDX60 in HNSCC (Fig. 1B). The survival curve was analyzed via GEPIA and ENCORI, and patients who were suffering from HNSCC and were characterized by high mRNA levels of DDX60 exhibited a lower 10-year overall survival rate than patients with HN-SCC who were characterized by a lower expression level of DDX60 (Fig. 1C,D). Thirty pairs of tissues (HNSCC tumor and adjoining normal tissues) were used to study the DDX60 mRNA levels. The results revealed the importance of upregulated DDX60 in HNSCC tissues. The DDX60 mRNA level was increased in HNSCC tissues as opposed to the adjoining non-tumor tissues (Fig. 1E). Moreover, HNSCC tissues also exhibited DDX60 upregulation as op-





Fig. 3. HNSCC cells' epithelial-mesenchymal transition (EMT) was enhanced by DDX60. (A–D) The mRNA levels of DDX60 (A), N-cadherin (B), Vimentin (C), and E-cadherin (D) in CAL27 and SAS of HNSCC cells were detected by SQ RT-PCR. (E) Western blotting was implemented to determine the expression levels of DDX60, N-cadherin, Vimentin, and E-cadherin expressed by CAL27 and SAS HNSCC cells. (F–I) Quantification of protein levels of DDX60 (F), N-cadherin (G), Vimentin (H), and E-cadherin (I) in (E). *p < 0.05; **p < 0.01; ***p < 0.001; ***p < 0.001.

posed to normal tissues (Fig. 1F). To investigate the role of DDX60 in HNSCC, four cell lines originated from tongue were selected: CAL27, SAS, CAL33, and SCC25. We also studied the mRNA and protein levels of DDX60 in human normal oral epithelial keratinocytes (HOK) and these four HNSCC cells. Results showed that both DDX60 mRNA and protein levels were found to be elevated in the four HN-SCC cell lines relative to the normal cell line (Fig. 1G,H).

3.2 DDX60 Enhanced the Migratory and Invasive Capacities of HNSCC Cells

Tumor initiation, invasion, and metastasis all require the involvement of epithelial-mesenchymal transition (EMT), one of the hallmarks of malignant tumors [21–23]. Therefore, we initially investigated whether DDX60 affected EMT in HNSCC cells. Transwell migration and invasion assay results revealed that DDX60 overexpression could enhance cell migration and invasion abilities of HN- SCC cells, whereas DDX60 expression was silenced, and these processes were inhibited (Fig. 2A–D). Thus, we hypothesize that DDX60 promoted EMT in HNSCC cells.

3.3 DDX60 Regulated the EMT Markers in HNSCC Cells

EMTs are characterized by the absence of epithelial cell markers (e.g., E-cadherin) and upregulation of mesenchymal cell markers (like vimentin and N-cadherin) [24]. We investigated how DDX60 affected EMT markers, such as Vimentin, E-cadherin, and N-cadherin. SQ RT-PCR results showed that forced overexpression of DDX60 remarkably elevated the mRNA levels of N-cadherin and Vimentin while reducing those of E-cadherin (Fig. 3A–D). Conversely, DDX60 knockdown could reduce the mRNA levels of N-cadherin and Vimentin while increasing those of E-cadherin (Fig. 3A–D). In addition, increased protein levels of N-cadherin and Vimentin but reduced those of



Fig. 4. DDX60 was positively corrected with interferon alpha inducible protein 27 (IFI27) in HNSCC cells. (A) The relationship between DDX60 and the mRNA level of IFI27 was established by the GEPIA database. (B) IFI27 mRNA levels in HNSCC tissues and control tissues were predicted utilizing data from the GEPIA database. (C) A correlation between IFI27 mRNA levels and HNSCC patient survival was predicted using the GEPIA database. (D) IFI27 mRNA level was measured by SQ RT-PCR in 30 matched pairs of HNSCC and its paraneoplastic tissues. (E) Analysis of the correlation between DDX60 and IFI27 according to their mRNA levels in 30 pairs of HNSCC and its paraneoplastic tissues detected by SQ RT-PCR. (F) The mRNA levels of DDX60 and IFI27 in CAL27 and SAS of HNSCC cells were detected by SQ RT-PCR. (G) The expression levels of DDX60 and IFI27 in CAL27 and SAS of HNSCC cells were detected by SQ RT-PCR. (G) The expression levels of DDX60 and IFI27 in CAL27 and SAS of HNSCC cells were detected by SQ RT-PCR. (G) The expression levels of DDX60 and IFI27 in CAL27 and SAS of HNSCC cells were blot. (H,I) Quantification of DDX60 (H) and IFI27 (I) protein levels in (G). *p < 0.05; **p < 0.01; ***p < 0.001; ***p < 0.001. TPM, transcript per million.

E-cadherin were observed after DDX60 overexpression, whereas the opposite results occurred by downregulation of DDX60 in HNSCC cells (Fig. 3E–I).

3.4 DDX60 Positively Regulated the IF127 Expression in HNSCC Cells

To further explore the potential mechanism of DDX60 in HNSCC, we performed the correlation analysis using GEPIA. According to the findings, IFI27 (interferon alpha inducible protein 27) mRNA level was considerably elevated in HNSCC tissues in comparison to the normal tissues, and IFI27 was positively corrected with DDX60 (Fig. 4A,B). Patients with HNSCC who had high levels of IFI27 mRNA had a lower 10-year overall survival rate in comparison to those with lower levels (Fig. 4C). Furthermore, the above prediction results were verified by SQ RT-PCR (Fig. 4D,E). Moreover, overexpression or knockdown of DDX60 promoted or inhibited IFI27 at the protein and mRNA levels (Fig. 4F–I).



Fig. 5. DDX60 regulated the EMT via the IFI27-mediated mechanism in HNSCC cells. (A–E) After co-transfecting HNSCC cells with NC + si-NC, DDX60 + si-NC, and DDX60 + si-IFI27, the mRNA levels of DDX60 (A), IFI27 (B), N-cadherin (C), Vimentin (D), and E-cadherin (E) were determined via SQ RT-PCR. (F) Western blotting was used to determine DDX60, IFI27, E-cadherin, Vimentin, and N-cadherin expression levels. (G–K) Quantification of protein expression levels of DDX60 (G), IFI27 (H), N-cadherin (I), Vimentin (J), and E-cadherin (K) in (F). (L) After co-transfecting HNSCC cells with si-NC + NC, si-DDX60 + NC, si-DDX60 + IFI27, the migratory and invasive capacities of HNSCC cells were measured using transwell assays. (M) Transwell migration and (N) transwell invasion abilities were quantified from (F). Bars = 100 μ m. *p < 0.05; **p < 0.01; ***p < 0.001; ***p < 0.001; s. no significance.



Fig. 6. DDX60 regulated IFI27 by activating the NF-*κ***B pathway.** (A) GSEA results indicated that DDX60 was significantly and positively correlated with NF-*κ*B in the analysis of the TCGA datasets. (B) After overexpression or knockdown of DDX60, the p65 and p-p65 protein levels were detected by western blot. (C,D) Quantification of p65 (C) and p-p65 (D) protein levels in B. (E) After transfection NC+si-NC, DDX60+si-NC, and DDX60+si-p65, western blotting was used to determine the protein levels of DDX60, p65, p-p65, and IFI27. (F–I) Quantification of protein expression levels DDX60 (F), p65 (G), p-p65 (H) and IFI27 (I) in E. (J) Schematic representation of the proposed DDX60 regulation of the IFI27 expression via the NF-*κ*B–mediated mechanism. *p < 0.05; **p < 0.01; ***p < 0.001; ns, no significance. GSEA, gene set enrichment analysis; TCGA, The Cancer Genome Atlas.

3.5 DDX60 Regulated the EMT via Upregulation of IF127 in HNSCC Cells

Based on the above results, we ask whether DDX60 regulated the EMT through the IFI27-mediated mechanism. A rescue experiment was implemented to validate the hypothesis, and the results illustrated that silencing IFI27 in HNSCC cells attenuated the DDX60-induced upregulation of N-cadherin and Vimentin mRNA levels and the E-cadherin inhibition (Fig. 5A–E). In addition, a similar phenomenon has been observed at the protein level (Fig. 5F–K). Then, further research was conducted to determine whether DDX60 influenced the migratory and invasive ca-

pacities of HNSCC cells via modulation of IFI27. The analysis showed that HNSCC cells' migratory and invasive capacities were remarkably reduced upon DDX60 knockdown, whereas co-transfection with IFI27 overexpressing plasmid removed this inhibition (Fig. 5L–N). Thus, we hypothesize that these processes can be promoted by DDX60 via the upregulation of the IFI27 in HNSCC cells.

3.6 DDX60 Upregulated IFI27 via NF-*k*B-Mediated Mechanism in HNSCC Cells

We delved further into the molecular mechanism via which DDX60 upregulates IFI27 in HNSCC cells. When comparing patients with high and low DDX60 expression, GSEA revealed that the NF- κ B pathway was significantly enriched in the former group (Fig. 6A), indicating that the NF- κ B pathway might be activated by DDX60.

To further our understanding of how DDX60 works in HNSCC cells, we examined how changed DDX60 expression affected the protein levels of p65 and p-protein levels, two key molecular components in the NF- κ B pathway. We discovered that the protein level of p65 did not change significantly regardless of DDX60 levels (Fig. 6B-D). However, while p-p65 protein levels rose remarkably during DDX60 overexpression, they dropped remarkably after DDX60 knockdown (Fig. 6B-D). The direct transcription of IFI27 is induced by the NF- κ B pathway activation, and previous research has shown that the promoter region of IFI27 contains NF- κ B-p65 binding sites [25]. Therefore, we investigated whether DDX60 could upregulate IFI27 via activating the NF- κ B pathway. We discovered that DDX60 overexpression could significantly increase the p-p65 protein level. A significant reduction in the p65 and p-p65 protein levels was also observed, while p65 expression levels were also inhibited in DDX60 overexpressed cells (Fig. 6E-H). In addition, reducing p65 protein levels could attenuate the promoting effect of DDX60 overexpression on IFI27 expression (Fig. 6E-I). The results indicated that IFI27 expression levels in HNSCC cells may be upregulated by DDX60 via the NF- κ B pathway (Fig. 6J).

4. Discussion

DDX60, an important regulatory molecule, was initially proven to be involved in RNA-mediated processes via hydrolysis [26]. It is also associated with antiviral activities, radiosensitivity of cancer cells, and immunotherapies [26–28]. However, the role of DDX60 in cancers such as HNSCC, is still much unknown. Herein, we studied how DDX60 affected HNSCC progression. The results revealed that the upregulation of DDX60 levels in primary tissues and cell lines was associated with HNSCC. The findings matched those from a search of online databases. We showed that HNSCC cells' ability to migrate and invade was enhanced by *DDX60*, an oncogene.

The associated mechanism was studied, and the results revealed that DDX60 could upregulate IFI27 in HNSCC cells. IFI27, which is stably induced by interferon, is a vital member of the FAM14 family and is implicated in the biological processes of numerous malignancies [28–30]. Prognosis in pancreatic cancer patients is strongly correlated with IFI27 expression level, and its upregulation is linked to a dismal prognosis [31]. Existing literature has shown that EMT may occur in ovarian cancer cells when IFI27 is upregulated [32]. Downregulation of IFI27 by activating transcription factor 3 (ATF3) could inhibit the growth and migration of tongue squamous cell carcinoma cells [33]. Li *et al.* [25] demonstrated that a disintegrin-like metalloproteinase with thrombospondin motifs-16 (ADAMTS16) could upregulate IFI27 expression by activating the NF- κ B pathway in gastric carcinogenesis. We showed that DDX60 was another regulator of IFI27 in HNSCC cells.

The NF- κ B pathway is a classic signaling pathway. Notably, the onset and advancement of HNSCC are affected when the NF- κ B pathway is abnormally activated. This pathway can be activated by several protein molecules, such as DNA-damage-inducible transcript 4 (DDIT4), mucosaassociated lymphoid tissue lymphoma translocation protein 1 (MALT1), and ADAMTS16 [25,34,35]. Activation of NF- κ B pathway could trigger NF- κ B-dependent gene expression and play an essential role in cancers [36]. Herein, we observed that the upregulation of DDX60 can significantly increase the p-p65 protein level in HNSCC cells. However, the level of p-p65 reduced significantly following the knockdown of DDX60. Furthermore, we discovered that DDX60 regulated the IFI27 expression by activating the NF- κ B pathway in HNSCC cells. These results indicated that DDX60 was another activator of the NF- κB pathway, which could lead to IFI27 expression in HNSCC cells.

Our study found that DDX60 has better sensitivity in HNSCC. It is concluded that DDX60 has good diagnostic efficacy in patients with HNSCC, and the results suggest that DDX60 protein can be used as a potential biomarker and therapeutic target for HNSCC. However, due to research limitations, such as sample size or potential biases, our research still has some shortcomings, and we hope that subsequent studies can further determine the above results by expanding the sample size and classification research. However, our study provides a basis for further research on the function and mechanism of DDX60 in HNSCC.

5. Conclusion

We found that DDX60, as an oncogene in HNSCC cells, can activate the NF- κ B pathway, thereby increasing the expression of IFI27. Our results provided an experimental basis for understanding the tumorigenic mechanism of DDX60 associated with HNSCC. The results can potentially help identify new HNSCC biomarkers and targets for targeted therapy.

Availability of Data and Materials

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

Author Contributions

Conceptualization, YH; Data curation, YH, JG; Formal analysis, YH, JG; Investigation, YH, JG, LL; Methodology, YH, JG, LL; Supervision, YH, JG, LL; Writing — original draft, YH; Writing — review & editing, YH, JG, LL. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

The study was approved by the Ethics Committee of The Third Central Hospital of Tianjin (approval no.: TCHT20230411). Patient-informed consent was provided under the existing ethics approval procedures.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10. 31083/j.fbl2901014.

References

- Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, *et al.* Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: a Cancer Journal for Clinicians. 2021; 71: 209–249.
- [2] Ferlay J, Colombet M, Soerjomataram I, Parkin DM, Piñeros M, Znaor A, et al. Cancer statistics for the year 2020: An overview. International Journal of Cancer. 2021. (online ahead of print)
- [3] Johnson DE, Burtness B, Leemans CR, Lui VWY, Bauman JE, Grandis JR. Head and neck squamous cell carcinoma. Nature Reviews. Disease Primers. 2020; 6: 92.
- [4] Solomon B, Young RJ, Rischin D. Head and neck squamous cell carcinoma: Genomics and emerging biomarkers for immunomodulatory cancer treatments. Seminars in Cancer Biology. 2018; 52: 228–240.
- [5] Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. CA: a Cancer Journal for Clinicians. 2022; 72: 7–33.
- [6] Chen B, Leung LL, Qu X, Chan JYK. Extracellular Vesicles as Biomarkers in Head and Neck Squamous Cell Carcinoma: From Diagnosis to Disease-Free Survival. Cancers. 2023; 15: 1826.
- [7] McDermott JD, Bowles DW. Epidemiology of Head and Neck Squamous Cell Carcinomas: Impact on Staging and Prevention Strategies. Current Treatment Options in Oncology. 2019; 20: 43.
- [8] Ruffin AT, Li H, Vujanovic L, Zandberg DP, Ferris RL, Bruno TC. Improving head and neck cancer therapies by immunomodulation of the tumour microenvironment. Nature Reviews. Cancer. 2023; 23: 173–188.
- [9] Glazar DJ, Johnson M, Farinhas J, Steuer CE, Saba NF, Bonomi M, *et al*. Early response dynamics predict treatment failure in patients with recurrent and/or metastatic head and neck squamous cell carcinoma treated with cetuximab and nivolumab. Oral Oncology. 2022; 127: 105787.

- [10] Liu JC, Kaplon A, Blackman E, Miyamoto C, Savior D, Ragin C. The impact of the multidisciplinary tumor board on head and neck cancer outcomes. The Laryngoscope. 2020; 130: 946–950.
- [11] Banroques J, Cordin O, Doère M, Linder P, Tanner NK. Analyses of the functional regions of DEAD-box RNA "helicases" with deletion and chimera constructs tested in vivo and in vitro. Journal of Molecular Biology. 2011; 413: 451–472.
- [12] Balko JM, Arteaga CL. Dead-box or black-box: is DDX1 a potential biomarker in breast cancer? Breast Cancer Research and Treatment. 2011; 127: 65–67.
- [13] Fuller-Pace FV. DEAD box RNA helicase functions in cancer. RNA Biology. 2013; 10: 121–132.
- [14] Germain DR, Graham K, Glubrecht DD, Hugh JC, Mackey JR, Godbout R. DEAD box 1: a novel and independent prognostic marker for early recurrence in breast cancer. Breast Cancer Research and Treatment. 2011; 127: 53–63.
- [15] Huang CC, Ou CY, Lee WT, Hsiao JR, Tsai ST, Wang JD. Life expectancy and expected years of life lost to oral cancer in Taiwan: a nation-wide analysis of 22,024 cases followed for 10 years. Oral Oncology. 2015; 51: 349–354.
- [16] Jankowsky E. RNA helicases at work: binding and rearranging. Trends in Biochemical Sciences. 2011; 36: 19–29.
- [17] Linder P, Jankowsky E. From unwinding to clamping the DEAD box RNA helicase family. Nature Reviews. Molecular Cell Biology. 2011; 12: 505–516.
- [18] Miyashita M, Oshiumi H, Matsumoto M, Seya T. DDX60, a DEXD/H box helicase, is a novel antiviral factor promoting RIG-I-like receptor-mediated signaling. Molecular and Cellular Biology. 2011; 31: 3802–3819.
- [19] Fu TY, Wu CN, Sie HC, Cheng JT, Lin YS, Liou HH, et al. Subsite-specific association of DEAD box RNA helicase DDX60 with the development and prognosis of oral squamous cell carcinoma. Oncotarget. 2016; 7: 85097–85108.
- [20] Zeng Y, Xu Q, Xu N. Long non-coding RNA LOC107985656 represses the proliferation of hepatocellular carcinoma cells through activation of the tumor-suppressive Hippo pathway. Bioengineered. 2021; 12: 7964–7974.
- [21] Pastushenko I, Blanpain C. EMT Transition States during Tumor Progression and Metastasis. Trends in Cell Biology. 2019; 29: 212–226.
- [22] Mittal V. Epithelial Mesenchymal Transition in Tumor Metastasis. Annual Review of Pathology. 2018; 13: 395–412.
- [23] Dongre A, Weinberg RA. New insights into the mechanisms of epithelial-mesenchymal transition and implications for cancer. Nature Reviews. Molecular Cell Biology. 2019; 20: 69–84.
- [24] Prieto-García E, Díaz-García CV, García-Ruiz I, Agulló-Ortuño MT. Epithelial-to-mesenchymal transition in tumor progression. Medical Oncology (Northwood, London, England). 2017; 34: 122.
- [25] Li T, Zhou J, Jiang Y, Zhao Y, Huang J, Li W, *et al*. The Novel Protein ADAMTS16 Promotes Gastric Carcinogenesis by Targeting IFI27 through the NF-κb Signaling Pathway. International Journal of Molecular Sciences. 2022; 23: 11022.
- [26] Oshiumi H, Miyashita M, Okamoto M, Morioka Y, Okabe M, Matsumoto M, et al. DDX60 Is Involved in RIG-I-Dependent and Independent Antiviral Responses, and Its Function Is Attenuated by Virus-Induced EGFR Activation. Cell Reports. 2015; 11: 1193–1207.
- [27] Xin D, Liu J, Gu J, Ji Y, Jin J, Sun L, *et al.* Low Expression of DDX60 Gene Might Associate with the Radiosensitivity for Patients with Breast Cancer. Journal of Oncology. 2020; 2020: 8309492.
- [28] Geng N, Hu T, He C. Identification of DDX60 as a Regulator of MHC-I Class Molecules in Colorectal Cancer. Biomedicines. 2022; 10: 3092.
- [29] Cervantes-Badillo MG, Paredes-Villa A, Gómez-Romero V, Cervantes-Roldán R, Arias-Romero LE, Villamar-Cruz O, *et al.*

IFI27/ISG12 Downregulates Estrogen Receptor α Transactivation by Facilitating Its Interaction With CRM1/XPO1 in Breast Cancer Cells. Frontiers in Endocrinology. 2020; 11: 568375.

- [30] Suomela S, Cao L, Bowcock A, Saarialho-Kere U. Interferon alpha-inducible protein 27 (IFI27) is upregulated in psoriatic skin and certain epithelial cancers. The Journal of Investigative Dermatology. 2004; 122: 717–721.
- [31] Huang S, Zhao J, Song J, Li Y, Zuo R, Sa Y, *et al.* Interferon alpha-inducible protein 27 (IFI27) is a prognostic marker for pancreatic cancer based on comprehensive bioinformatics analysis. Bioengineered. 2021; 12: 8515–8528.
- [32] Li S, Xie Y, Zhang W, Gao J, Wang M, Zheng G, et al. Interferon alpha-inducible protein 27 promotes epithelial-mesenchymal transition and induces ovarian tumorigenicity and stemness. The Journal of Surgical Research. 2015; 193: 255–264.
- [33] Xu L, Zu T, Li T, Li M, Mi J, Bai F, et al. ATF3 downmodulates

its new targets IFI6 and IFI27 to suppress the growth and migration of tongue squamous cell carcinoma cells. PLoS Genetics. 2021; 17: e1009283.

- [34] Lin X, Yoshikawa N, Liu W, Matsukawa T, Nakamura K, Yoshihara M, et al. DDIT4 Facilitates Lymph Node Metastasis via the Activation of NF-κB Pathway and Epithelial-Mesenchymal Transition. Reproductive Sciences (Thousand Oaks, Calif.). 2023; 30: 2829–2841.
- [35] Minderman M, Lantermans HC, Grüneberg LJ, Cillessen SAGM, Bende RJ, van Noesel CJM, *et al.* MALT1-dependent cleavage of CYLD promotes NF-κB signaling and growth of aggressive B-cell receptor-dependent lymphomas. Blood Cancer Journal. 2023; 13: 37.
- [36] Hoesel B, Schmid JA. The complexity of NF-κB signaling in inflammation and cancer. Molecular Cancer. 2013; 12: 86.