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The Hu antigen R/interferon-β axis modulates the sensitivity of esophageal squamous cancer cells to cisplatin

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The incidence rate of esophageal squamous cell carcinoma (ESCC) has risen significantly in recent years. RNA binding protein (RBP) has been attracting increased attention in the treatment of ESCC. Therefore, the primary aim of this study was to explore the roles of the RBP Hu antigen R (HuR) in ESCC. The mRNA levels were detected via reverse transcription-quantitative PCR, while the expression levels of protein were evaluated using western blotting. Cell proliferation was estimated by cell counting kit-8 assay and colony formation assay. Flow cytometry was applied to measure cell apoptosis. Luciferase assay and RIP assay were applied to verify whether interferon-β (IFN-β) was targeted by HuR. The results unambiguously demonstrated that HuR was upregulated in ESCC. Overexpression of HuR alleviated chemosensitivity to cisplatin in ESCC cells, as evidenced by increased cell proliferation and decreased apoptosis. Moreover, IFN-β was found to be a target of HuR and downregulated in ESCC cells. And overexpression of IFN-β abrogated the effects of HuR on cisplatin-sensitivity of ESCC cells. Taken together, these findings suggested that HuR may alleviate the chemosensitivity of ESCC cells to cisplatin via binding to IFN-β. Therefore, the HuR/IFN-β axis may be a novel biomarker for improving the chemosensitivity of ESCC.

Key words: Hu antigen R; interferon-beta; cisplatin sensitivity; esophageal squamous cell carcinoma

Esophageal cancer (EC) is characterized by high morbidity and mortality rates. Among all EC cases, esophageal squamous cell carcinoma (ESCC) takes up >90% [1]. Over recent years, there has been an increasing incidence of EC worldwide [2]. As the 'epicenter' of ESCC, China contributes to 1/2 of new cases worldwide [3]. Although significant breakthroughs have been achieved in clinical treatment, the 5-year survival rate of ESCC patients is still unsatisfactory [4], which may be due to emerging chemoresistance and distant metastasis [5, 6]. Thus, investigating potential molecular mechanisms that promote the chemosensitivity of ESCC is important.

RNA-binding proteins (RBPs) are a family of heterogeneous proteins, which are key factors in post-transcriptional regulation and modulation of RNA stability [7]. Accumulating evidence shows that aberrant RBP expression is involved in the occurrence and development of various diseases, including cancer [8, 9]. Hu antigen R (HuR), also referred to as ELAV-like protein 1 (ELAVL1), is a ubiquitous member of cancer-related RBPs [10]. Activated HuR contributes to its translocalization from the nucleus to the cytoplasm, inducing the upregulation of cancer-related gene expression [11]. Furthermore, HuR interacts with its target genes to promote cancer cells proliferation, migration, invasion, and epithelial-mesenchymal metastasis [12–14]. Therefore, HuR may serve as an oncogene in cancer. For instance, HuR-induced transcriptional upregulation of isocitrate dehydrogenase 1 promotes pancreatic cancer cells' survival and restores chemoresistance under low nutrient conditions [15]. HuR also binds to CDK3 to enhance the proliferation of breast cancer cells and inhibit their apoptosis [16]. Moreover, the LINC00324/HuR/FAM83B axis promotes the proliferation of gastric cancer cells [17]. In ESCC, high HuR expression levels are associated with poor prognosis, lymph node metastasis, tumor invasion depth, and advanced stage [18, 19]. However, HuR knockdown induces the upregulation of

IL-18 and suppresses the ESCC cells' proliferation and metastasis [20]. Thus, HuR may play the role of an oncogene in ESCC. However, the potential molecular mechanisms have not been fully elucidated.

The present study aimed to investigate the effects and the underlying mechanisms of HuR on the chemosensitivity of ESCC cells to cisplatin. By elucidating whether overexpression of HuR can inhibit the ESCC cells' chemosensitivity via blocking the IFN-β interaction, in order to determine whether targeting the HuR/IFN-β axis may serve as a novel strategy for ESCC treatment.

Materials and methods

Cell culture. Four human ESCC cell lines TE-8, Eca109, TE-1, and KYSE30, and the normal HEEC cell line were obtained directly from the American Type Culture Collection. All cells were grown in the DMEM medium supplemented with 10% FBS and 1% penicillin/streptomycin at 37 °C with a 5% CO_2 atmosphere.

Cell transfection. All the overexpression lentiviral vectors and siRNAs were purchased from GenePharma (Shanghai, China). HuR or IFN-β overexpressing lentivirus was used to infect ESCC cells. TE-8 and KYSE30 cells (approximately 5×10^3) in the logarithmic growth phase were seeded into six-well plates, respectively. When the cells grow to 50% confluence, lentiviruses of different groups were added to infect cells. After 72 h, the fluorescent protein expression was observed under an inverted fluorescence microscope. Then, 1 μg/ml puromycin was added to each well and continued to culture for 2 weeks to obtain stable transfected cells. si-HuR was used to construct HuR knockdown cells and si-NC was used as a control. According to the manufacturer's protocol, siRNA at a final concentration of 5 nM was used for transient transfection using Lipofectamine 2000 (Invitrogen).

The information for used plasmids and siRNA is as follows: si-HuR: GAACGAAUUUGAUCGUCAACUdTdT; si-IFN-β: GCUCUCCUGUGUGCUUCUCCACUAdTdT.

For the overexpression of HuR and IFN-β, the full length of the CDS region of HuR and IFN-β were synthesized and cloned into a plvx vector, respectively. BamHI and KpnI were used to build the overexpressing constructs of IFN-β. NotI and KpnI were used to build the overexpressing constructs of HuR.

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was collected from the cells. RNA was reversed transcription to cDNA using a First-Strand cDNA Synthesis Kit (Invitrogen; Thermo Fisher Scientific, Inc.). qPCR experiments were performed using SYBR Premix Ex Taq II (Qiagen, Inc.) on a Step One Plus Real-Time PCR system (Applied Biosystems) under the following thermal cycling conditions: initial denaturation for 2 min at 50 °C, 10 min at 95 °C, 40 cycles at 95 °C for 15 s, and 60 °C for 1 min. GAPDH was utilized as an internal control. The mRNA expression level was calculated with the $2^{-\Delta\Delta Ct}$ method. Each experiment was performed at least in triplicate. The sequences of the primers used in this study are as follows: HuR: 5'-TCCCAACCGGGGTAATACCTG-3' (sense), 5'-AATCCCGCTTACTGCACACTG-3' (antisense); IFN-β: 5'-CAGAAGGAGGACGCCGCATTGAC-3' (sense), 5'-CCAGGCACAGTGACTGTACTCC-3' (antisense); and GAPDH: 5'-TGTGGGCATCAATGGATTTGG-3' (sense), 5'-ACACCATGTATTCCGGGTCAAT-3' (antisense).

RNA immunoprecipitation (RIP) assay. The interaction between HuR and IFN-β was determined with a RIP kit (EMD Millipore). Briefly, the cells (approximately 4×10^6) were lysed in NP-40 lysis buffer (10 mM Tris-HCl, pH 7.4; 10 mM NaCl; 0.5% NP40; 1 mM DTT; 200 U/ml RNase OUT, EDTA-free protease inhibitor cocktail). Then, the lysis mixture was centrifuged and the supernatant was incubated with specific antibody (5 μl)-magnetic beads in RIP buffer (150 mM NaCl; 10 mM Tris-HCl, pH 7.4; 1 mM EDTA; 1 mM EGTA, pH 8.0; 1% Triton X-100; 0.5% NP40, 1 mM DTT, 200 U/ml RNase OUT, EDTA-free protease inhibitor cocktail) at 4 °C overnight. 10% lysis supernatant was used as Input. On the following day, the mixture was centrifuged to obtain the protein-RNA complex and further digested with proteinase K to extract RNA. Finally, the abundance of mRNA expression level was measured by RT-qPCR. The input was used for normalization. The antibodies used in this experiment were as follows: anti-HuR (ab242410, Abcam), anti-IFN-β (ab275880, Abcam), anti-GAPDH (ab128915, Abcam), anti-IgG (ab6715, Abcam), HRP-linked secondary antibody (6401-05, Biovision).

Western blot analysis. Total protein was harvested from cells. Protein concentrations were calculated with the BCA kits (Beyotime Institute of Biotechnology). Next, an equal amount of protein was loaded onto 12% SDS-polyacrylamide gels and run at 120 V. Then, the separated protein was transferred onto PVDF membranes and then blocked with 5% skimmed milk. After PVDF membranes washing thrice with PBS, the membranes were incubated with primary antibodies (anti-HuR, ab242410, Abcam; anti-IFN-β, ab275880, Abcam; anti-GAPDH, ab128915, Abcam) overnight at 4 °C in the dark. On the following day, the membranes were incubated with an anti-rabbit antibody (6401-05, Biovision) at room temperature for 2 h. Subsequently, the bands were visualized using an ECL kit, following the analysis with Quantity One software (Bio-Rad).

Colony formation assay. After transfection for 48 h, cells were digested and then washed with PBS. Then cells suspension was prepared and seeded into a six-well plate at the density of 1000 cells/well. Next, cell plates were cultured in the incubator with a humidified 5% CO_2 atmosphere at 37 °C for 14 days. When the visible clones of cells appeared, the cells were fixed with 4% ethanol. Subsequently, the cells were stained with 1% crystal violet (Beyotime) and then dehydrated for 15 min. Finally, the colonies were imaged with a microscope and the colonies containing at least 15 cells were counted manually.

Cell Counting Kit-8 (CCK-8) assay. The cell viability was determined by a CCK-8 kit (Sigma-Aldrich). Briefly, cells (3×10^4/well) were plated in a 96-well plate. Then, cells were incubated with 10 µl of CCK-8 regents for 2 h at 37 °C. The viability of cells was calculated at 0, 12, 24, 48, and 72 h based on the optical density values at 450 nm. Each experiment was conducted thrice.

Flow cytometry. The apoptosis detection kit (Dojindo Molecular Technologies) was employed to measure cell apoptosis. After 48 h of transfection, cells (approximately 1×10^5) were collected and stained with Annexin-V-FITC and PI in the dark for 15 min. Flow cytometer (BD Biosciences) was applied to measure the apoptosis of stained cells and FlowJo software (FlowJo, LLC) was used to analyze the apoptotic rate.

Dual-luciferase reporter assay. A wide-type IFN-β 3' untranslated region (3'UTR) containing the HuR binding sequence was introduced into a psiCHECK-2 luciferase vector (Promega). Mutated IFN-β 3'UTR without HuR binding sequence was also introduced into the psiCHECK-2 luciferase vector. Lipofectamine® 2000 (Invitrogen) was used to co-transfect cells with HuR or NC overexpression plasmids and IFN-β 3'UTR WT or IFN-β 3'UTR MUT. Luciferase activity was measured using a luciferase reporter kit (Promega). *Renilla* luciferase was used as an internal reference. Each experiment was performed three times.

Statistical analysis. The data in this study were analyzed using GraphPad 5.0 (GraphPad Software). All values were presented as mean ± SD. The difference between the two groups was evaluated with a Student's t-test. Used one-way ANOVA to compare the multiple group differences. A p-value <0.05 was indicated statistically significant.

Results

Cisplatin downregulates HuR in ESCC cells. To study the effects of HuR in ESCC, we first examined the HuR expression level in ESCC cells by RT-qPCR. We found that HuR was significantly upregulated in ESCC cells (Figure 1A). Similarly, the protein levels of HuR were also obviously reduced in ESCC cells (Figure 1B). And TE-8 and KYSE30 cell lines with the highest and lowest upregulation were selected for the following experiments. Next, in order to investigate whether HuR was related to the chemotherapy resistance of ESCC, we treated the cells with cisplatin. As expected, the data demonstrated that the mRNA (Figure 1C) and protein (Figure 1D) expression levels of HuR were markedly decreased by cisplatin in a dose-dependent manner. These results illustrated

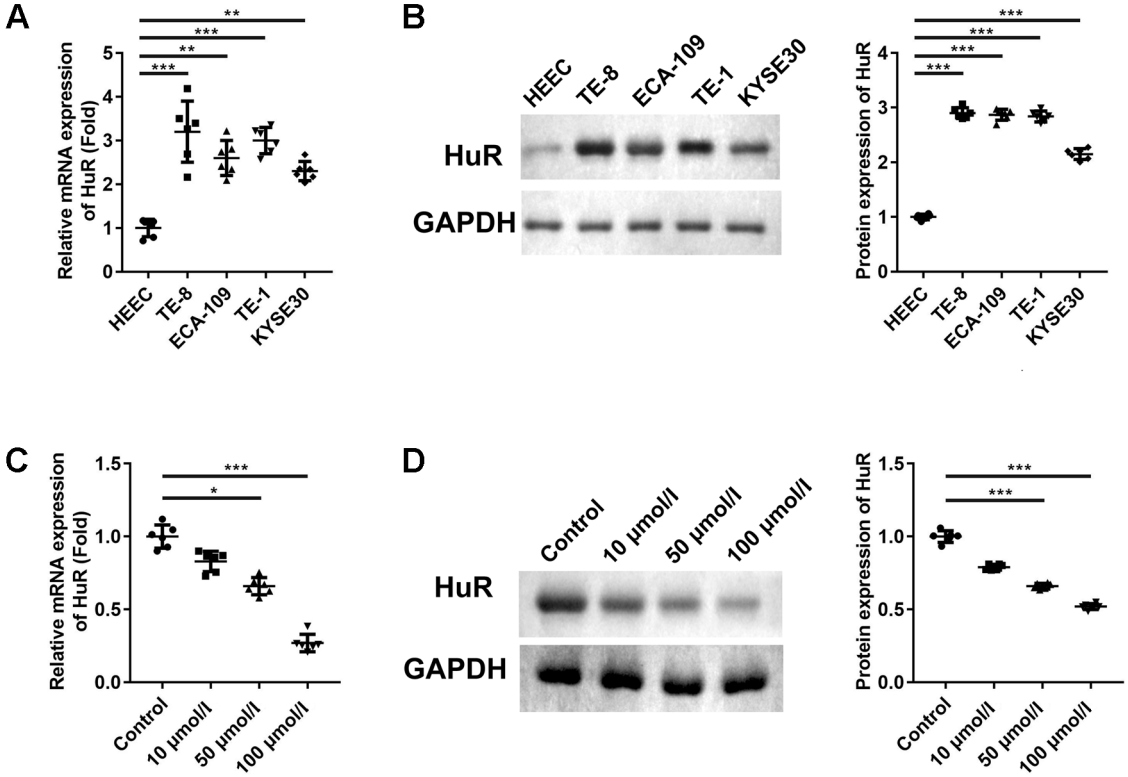

Figure 1. Cisplatin downregulates HuR in ESCC cells. RT-qPCR and western blot were performed to calculate the HuR mRNA (A) and protein (B) expression level in ESCC (TE-8, Eca109, TE-1, and KYSE30) and normal (HEEC) cell lines. HuR mRNA (C) and protein (D) expression levels in different concentrations of cisplatin-treated cells. Abbreviations: ESCC-esophageal squamous cell carcinoma; HuR-Hu antigen R; RT-qPCR-reverse transcription-quantitative PCR. *p<0.05, **p<0.01, ***p<0.001

that HuR may act an important role in ESCC chemotherapy resistance.

Upregulation of HuR alleviates the ESCC cells' chemosensitivity. To further investigate the biological functions of HuR in ESCC, we performed cell transfection to construct HuR overexpressing cells after cisplatin treatment. RT-qPCR and western blot results suggested that HuR mRNA (Figure 2A) and protein (Figure 2B) expression were obviously increased in cells with HuR overexpression plasmids transfection, which indicates that cells have been

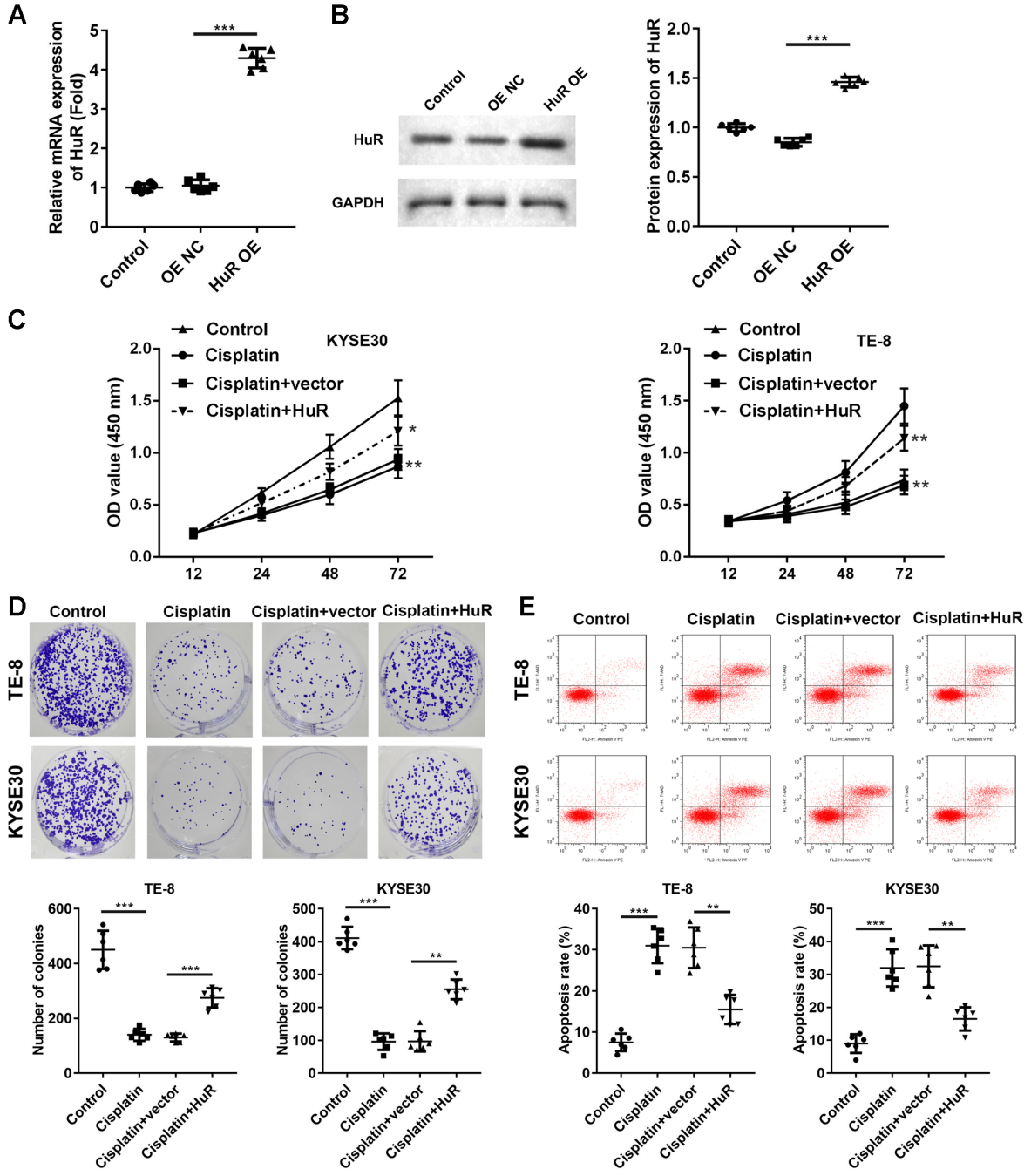

Figure 2. Upregulation of HuR alleviates the chemosensitivity of ESCC cells. Transfected TE-8 and KYSE30 cells with HuR OE plasmid and NC OE plasmid. A) RT-qPCR was done to determine the HuR mRNA expression level in transfected cells. B) HuR protein expression level in transfected cells. C) CCK-8 assay was applied to investigate the viability of transfected TE-8 and KYSE30 cells. D) Colony formation assay was performed to measure the proliferation of transfected TE-8 and KYSE30 cells. E) Flow cytometry was conducted to calculate the apoptotic rates in transfected TE-8 and KYSE30 cells. Abbreviations: OE-overexpression; NC-negative control; ESCC-esophageal squamous cell carcinoma; HuR-Hu antigen R. *p<0.05, **p<0.01, ***p<0.001

successfully transfected. Next, we conducted the CCK-8 assay to detect cell proliferation. The results indicated that overexpression of HuR antagonized the cisplatin-induced decrease of ESCC cell viability in TE-8 and KYSE30 cells (Figure 2C). Consistent with this, the results of the colony formation assay showed that overexpression of HuR reversed the decrease of colony formation ability induced by cisplatin (Figure 2D). Furthermore, HuR overexpression also attenuated the increase of the apoptosis rate in TE-8 and KYSE30 cells caused by cisplatin. (Figure 2E). These results suggested that HuR overexpression reduced the chemosensitivity of ESCC cells to cisplatin.

IFN-β is a direct target of HuR. According to reports, HuR is widely involved in the post-transcriptional regulation of genes as an mRNA binding protein. There are three RNA recognition motifs on the HuR protein, which can bind to specific sites of the ARE element on the mRNA. Surprisingly, we found that there are typical ARE elements on the 3'UTR of IFN-β using bioinformatics software, which may mean that HuR can bind to IFN-β. Therefore, RIP and luciferase assays were conducted to verify the relationship between IFN-β and HuR. We observed that the luciferase activity was obviously reduced in cells co-transfected with IFN-β 3'UTR WT and HuR overexpression plasmid. However, there was no significant difference in the IFN-β 3'UTR MUT group (Figure 3A). The RIP assay further verified the enrichment of HuR with IFN-β (Figure 3B). Then, we clarified whether HuR could regulate the expression of IFN-β. Specifically, the changes of IFN-β levels were assessed in cells after HuR knockdown or overexpression. The data revealed that the levels of IFN-β mRNA (Figure 3C) and protein (Figure 3D) expression were repressed with overexpression of HuR, and increased with knockdown of HuR. Finally, we also verified the expression of IFN-β in ESCC cells by RT-qPCR. It was found that the expression of IFN-β was significantly decreased in TE-8 cells (Figure 3E). These results suggested that HuR modulated the IFN-β expression in ESCC cells.

Upregulation of IFN-β reverses the effects of HuR on the chemosensitivity of ESCC. To further confirm the mechanistic roles of HuR and IFN-β in ESCC, rescue experiments were performed. Firstly, RT-qPCR was conducted to verify the transfection efficiency of IFN-β overexpression in cells. The results illustrated that IFN-β mRNA (Figure 4A) and protein (Figure 4B) expressions were significantly increased indicating that cells have been successfully transfected. Next, the protein levels were checked in four different groups. Consistent with the previous results, HuR was decreased in cells treated with cisplatin, while IFN-β showed the opposite results (Figure 4C). Then, cell prolif-

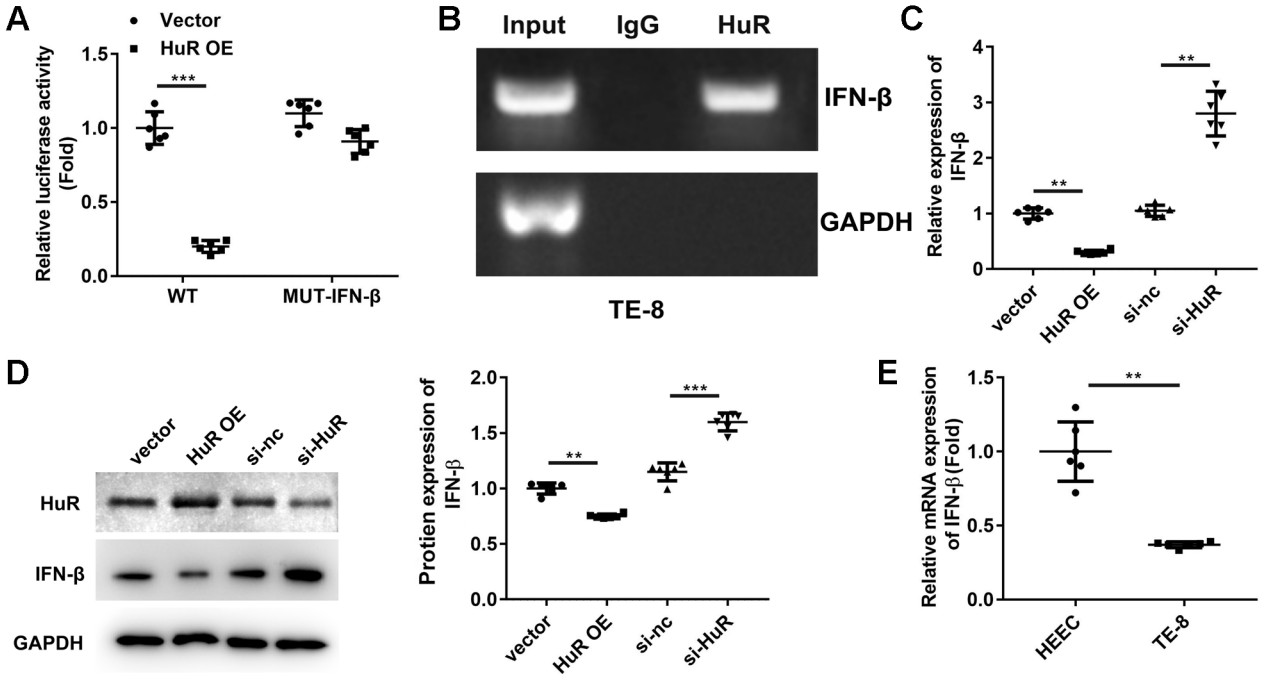

Figure 3. IFN-β is a target of HuR. A) A complementary binding between HuR and the IFN-β 3'UTR was verified with a luciferase assay. B) HuR and IFN-β interaction was verified using RNA immunoprecipitation assay. GAPDH was used as an internal negative control. C) The IFN-β mRNA expression level was measured via RT-qPCR in cells transfected with HuR OE plasmid, si-HuR, or si-NC. D) Western blot analysis was performed to determine the HuR and IFN-β protein expression level in cells transfected with HuR OE plasmid, si-HuR, or si-NC. E) RT-qPCR was performed to determine the IFN-β mRNA expression level in ESCC (TE-8) and normal (HEEC) cell lines. Abbreviations: OE-overexpression; NC-negative control; ESCC-esophageal squamous cell carcinoma; HuR-Hu antigen R; RT-qPCR-reverse transcription-quantitative PCR; siRNA-small interfering RNA; UTR-untranslated region. *p<0.05, **p<0.01, ***p<0.001

eration was examined by CCK-8 assay and colony formation assay. We found that overexpression of HuR abated the decline in cell proliferation (Figure 4D) and colony formation ability (Figure 4E) induced by cisplatin, which was reversed by the overexpression of IFN-β. Finally, cell apoptosis was also measured by flow cytometry. Overexpression of IFN-β antagonized the effects of HuR on cisplatin-induced apoptosis of TE-8 and KYSE30 cells (Figure 4F). These results confirmed that HuR regulated cell proliferation, apoptosis, and chemosensitivity to cisplatin by IFN-β.

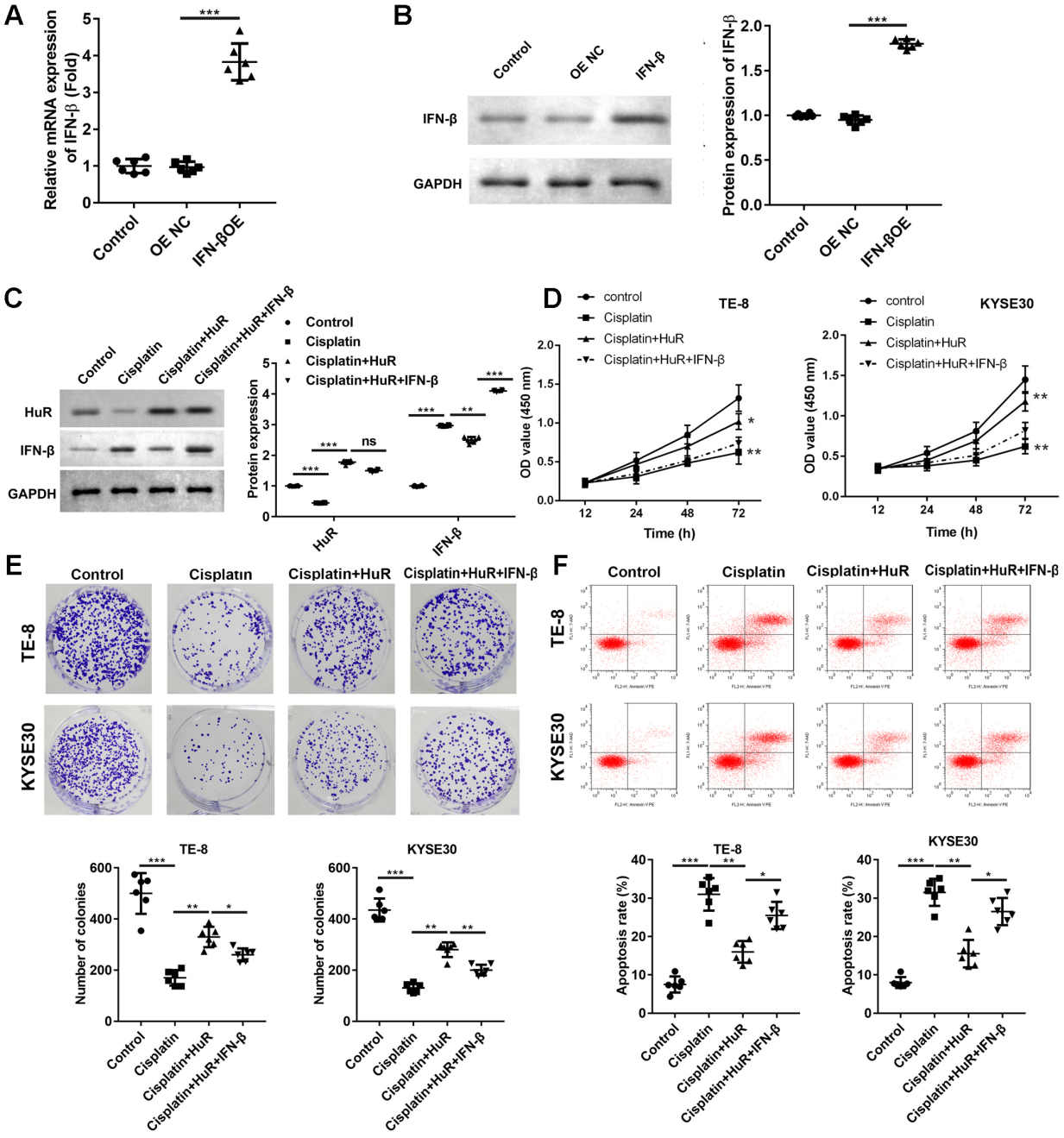

Figure 4. Upregulation of IFN-β alleviates the effects of HuR on the progression of ESCC. TE-8 and KYSE30 cells were transfected with HuR OE plasmid, NC OE plasmid, HuR OE plasmid+IFN-β OE plasmid. A) RT-qPCR was carried out to determine the IFN-β mRNA expression level. B) IFN-β protein expression level. C) HuR and IFN-β protein levels by western blot. D) Cell viability was revealed using CCK-8 assay in TE-8 and KYSE30 cells. E) Cell proliferation was detected with colony formation assay in TE-8 and KYSE30 cells. F) Apoptotic rates were calculated by flow cytometry in transfected TE-8 and KYSE30 cells. Abbreviations: OE-overexpression; NC-negative control; ESCC-esophageal squamous cell carcinoma; HuR-Hu antigen R. *p<0.05, **p<0.01, ***p<0.001

Discussion

Accumulating evidence has revealed that RBP participates in the initiation and growth of tumors [8–11]. Dysregulated RBP functions as an oncogene or antineoplastic gene to modulate cancer chemosensitivity and development, including ESCC [13–17]. However, the potential roles of HuR in the chemosensitivity of ESCC remain unknown. In the current study, HuR was upregulated in ESCC cells. However, cisplatin exposure decreased the expression of HuR. Thus, it was suggested that HuR may fulfill the oncogene role in ESCC, and HuR overexpression alleviated the chemosensitivity of ESCC cells to cisplatin, which was manifested by increased proliferation and decreased apoptotic rates of ESCC cells. Therefore, overexpression of HuR may abate the effects of chemotherapeutics on ESCC treatment. However, potential mechanisms have not yet been elucidated.

HuR participates in the progression and drug resistance of multiple types of cancer [12–15, 21, 22]. For example, the aberrant expression of HuR contributes to treatment resistance and tumor growth by increasing the expression of Bcl-2 in glioma [23]. Therefore, the possible roles of HuR in the chemosensitivity of cancer have attracted increasing attention. According to the published literature, the downregulation of HuR and YAP1 promotes the osteosarcoma cells' chemosensitivity [24]. Moreover, suppressing the interaction between HuR and MDR1 alleviates adriamycin resistance in chronic myelogenous leukemia [25]. Overexpression of HuR contributes to the development and chemosensitivity of ESCC [18–20]. However, the potential underlying mechanisms have not been fully elucidated.

Previous studies have reported that RBP modulates the progression of cancer by upregulating or degrading its target via binding to its target genes through a 3'UTR sequence [26]. HuR-induced inhibition of caspase-2 enhances the chemoresistance of colon cancer [27]. HuR also interacts with ELAVL1 to promote tumor metastasis of malignant peripheral nerve sheath tumors [28]. In addition, the HuR/MDR1 axis may serve as a biomarker for alleviating the chemoresistance of chronic myelogenous leukemia cells to adriamycin [25]. Thus, blocking the interaction between HuR and its target gene may be a promising strategy to improve chemotherapy.

Our results found that HuR could directly target IFN-β in ESCC cells. IFN-β, a member of type I IFNs, is essential for the innate and adaptive antiviral host defense and the pathogenesis of autoimmunity. Additionally, recent studies revealed that IFN-β possesses anti-tumor properties, including anti-angiogenic, immunomodulatory, antiproliferative, pro-apoptotic, and promotes the chemosensitivity of tumor cells. IFN-β combined with chemotherapy, such as anti-PD-1/PD-L1, has beneficial clinical results [29]. In ESCC, IFN-β-mediated upregulation of IRF1-antisense inhibits carcinogenesis by inducing an IFN response. Therefore, it was suggested that IFN-β may enhance the chemosensitivity of ESCC cells.

In this study, IFN-β was downregulated in ESCC cells and HuR negatively regulated the expression of IFN-β. It is contrary to the regulation of IFN-β by HuR in cell resistance to viral infections. Previous studies have shown that HuR can stabilize the expression level of IFN-β in HeLaS3 cells. When HuR expression is decreased by siRNA, the expression of IFN-β mRNA in response to poly I:C stimulation is severely impaired in HeLaS3 cells, which severely hindered the type I IFN response [30, 31]. These opposite effects may be due to the differences in the regulatory mechanisms of various types of tumor cells in response to different stimuli. The mechanism of cancer occurrence and drug resistance is complicated. This dual effect is very common in cancer, which depends on the type of tumor and the characteristics of treatment. For example, IL-27 had a direct inhibitory effect on proliferation in human pediatric acute myeloid leukemia (AML) cells expressing the WSX1/gp130 receptor. But contrary to the findings of pediatric AML, IL-27 promoted the proliferation of adult AML cell lines co-expressing WSX1 and gp130 [32-34]. Moreover, our results demonstrated that IFN-β promoted the chemosensitivity of ESCC cells, inhibited ESCC cell proliferation, and increased cell apoptosis. Based on these results, the HuR/IFN-β axis may be a promising target for ESCC. The regulatory effect and mechanism of HuR/IFN-β on drug resistance in tumors still need more research to clarify in the future.

In summary, our study demonstrated that HuR was upregulated significantly in ESCC. HuR overexpression alleviated the chemosensitivity of ESCC cells to cisplatin by targeting IFN-β. These findings suggest a novel strategy for sensitizing ESCC cells to chemotherapeutics.

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References

[1] TORRE LA, BRAY F, SIEGEL RL, FERLAY J, LORTET-TIEULENT J et al. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87–108. https://doi.org/10.3322/caac.21262

[2] SIEGEL RL, MILLER KD, JEMAL A. Cancer statistics, 2019. CA Cancer J Clin 2019; 69: 7–34. https://doi.org/10.3322/caac.21551

[3] JIAO W, ZHANG J, WEI Y, FENG J, MA M et al. MiR-139-5p regulates VEGFR and downstream signaling pathways to inhibit the development of esophageal cancer. Dig Liver Dis 2019; 51: 149–156. https://doi.org/10.1016/j.dld.2018.07.017

[4] CHEN W, ZHENG R, BAADE PD, ZHANG S, ZENG H et al. Cancer statistics in China, 2015. CA Cancer J Clin 2016; 66: 115–132. https://doi.org/10.3322/caac.21338

[5] SONG J, ZHANG P, LIU M, XIE M, GAO Z et al. Novel-miR-4885 promotes migration and invasion of esophageal cancer cells through targeting CTNNA2. Dna Cell Biol 2019; 38: 151–161. https://doi.org/10.1089/dna.2018.4377

[6] SONG S, HONJO S, JIN J, CHANG SS, SCOTT AW et al. The hippo coactivator YAP1 mediates EGFR overexpression and confers chemoresistance in esophageal cancer. Clin Cancer Res 2015; 21: 2580–2590. https://doi.org/10.1158/1078-0432.CCR-14-2191

[7] KIM MY, HUR J, JEONG S. Emerging roles of RNA and RNA-binding protein network in cancer cells. Bmb Rep 2009; 42: 125–130. https://doi.org/10.5483/bmbrep.2009.42.3.125

[8] ZHOU XJ, WU J, SHI L, LI XX, ZHU L et al. PTEN expression is upregulated by a RNA-binding protein RBM38 via enhancing its mRNA stability in breast cancer. J Exp Clin Cancer Res 2017; 36: 149. https://doi.org/10.1186/s13046-017-0620-3

[9] FAKHRALDEEN SA, CLARK RJ, ROOPRA A, CHIN EN, HUANG W et al. Two isoforms of the RNA binding protein, coding region determinant-binding protein (CRD-BP/IGF2BP1), are expressed in breast epithelium and support clonogenic growth of breast tumor cells. J Biol Chem 2015; 290: 13386–13400. https://doi.org/10.1074/jbc.M115.655175

[10] ZHANG Y, YANG L, LING C, HENG W. HuR facilitates cancer stemness of lung cancer cells via regulating miR-873/CDK3 and miR-125a-3p/CDK3 axis. Biotechnol Lett 2018; 40: 623–631. https://doi.org/10.1007/s10529-018-2512-9

[11] CAI J, WANG H, JIAO X, HUANG R, QIN Q et al. The RNA-Binding protein HuR confers oxaliplatin resistance of colorectal cancer by upregulating CDC6. Mol Cancer Ther 2019; 18: 1243–1254. https://doi.org/10.1158/1535-7163.MCT-18-0945

[12] LIU Y, CHEN X, CHENG R, YANG F, YU M et al. The Jun/miR-22/HuR regulatory axis contributes to tumourigenesis in colorectal cancer. Mol Cancer 2018; 17: 11. https://doi.org/10.1186/s12943-017-0751-3

[13] XU X, SONG C, CHEN Z, YU C, WANG Y et al. Downregulation of HuR inhibits the progression of esophageal cancer through interleukin-18. Cancer Res Treat 2018; 50: 71–87. https://doi.org/10.4143/crt.2017.013

[14] PU J, ZHANG X, LUO H, XU L, LU X et al. Adrenaline promotes epithelial-to-mesenchymal transition via HuR-TGFβ regulatory axis in pancreatic cancer cells and the implication in cancer prognosis. Biochem Biophys Res Commun 2017; 493: 1273–1279. https://doi.org/10.1016/j.bbrc.2017.09.146

[15] ZAREI M, LAL S, PARKER SJ, NEVLER A, VAZIRI-GOHAR A et al. Posttranscriptional upregulation of IDH1 by HuR establishes a powerful survival phenotype in pancreatic cancer cells. Cancer Res 2017; 77: 4460–4471. https://doi.org/10.1158/0008-5472.CAN-17-0015

[16] ZHANG Y, YANG L, LING C, HENG W. HuR facilitates cancer stemness of lung cancer cells via regulating miR-873/CDK3 and miR-125a-3p/CDK3 axis. Biotechnol Lett 2018; 40: 623–631. https://doi.org/10.1007/s10529-018-2512-9

[17] ZOU Z, MA T, HE X, ZHOU J, MA H et al. Long intergenic non-coding RNA 00324 promotes gastric cancer cell proliferation via binding with HuR and stabilizing FAM83B expression. Cell Death Dis 2018; 9: 717. https://doi.org/10.1038/s41419-018-0758-8

[18] WANG Y, ZHAO H, ZHI W. SEMA4D under the posttranscriptional regulation of HuR and miR-4319 boosts cancer progression in esophageal squamous cell carcinoma. Cancer Biol Ther 2020; 21: 122–129. https://doi.org/10.1080/15384047.2019.1669996.

[19] ZHANG C, XUE G, BI J, GENG M, CHU H et al. Cytoplasmic expression of the ELAV-like protein HuR as a potential prognostic marker in esophageal squamous cell carcinoma. Tumour Biol 2014; 35: 73–80. https://doi.org/10.1007/s13277-013-1008-4

[20] XU X, SONG C, CHEN Z, YU C, WANG Y et al. Downregulation of HuR inhibits the progression of esophageal cancer through interleukin-18. Cancer Res Treat 2018; 50: 71–87. https://doi.org/10.4143/crt.2017.013

[21] DOU Q, XU Y, ZHU Y, HU Y, YAN Y et al. LncRNA FAM83H-AS1 contributes to the radioresistance, proliferation, and metastasis in ovarian cancer through stabilizing HuR protein. Eur J Pharmacol 2019; 852: 134–141. https://doi.org/10.1016/j.ejphar.2019.03.002

[22] BURKHART RA, PINEDA DM, CHAND SN, ROMEO C, LONDIN ER et al. HuR is a post-transcriptional regulator of core metabolic enzymes in pancreatic cancer. Rna Biol 2013; 10: 1312–1323. https://doi.org/10.4161/rna.25274

[23] WANG J, GUO Y, CHU H, GUAN Y, BI J et al. Multiple functions of the RNA-binding protein HuR in cancer progression, treatment responses and prognosis. Int J Mol Sci 2013; 14: 10015–10041. https://doi.org/10.3390/ijms140510015

[24] XU W, CHEN C, XU R, LI Y, HU R et al. Knockdown of HuR represses osteosarcoma cells migration, invasion and stemness through inhibition of YAP activation and increases susceptibility to chemotherapeutic agents. Biomed Pharmacother 2018; 102: 587–593. https://doi.org/10.1016/j.biopha.2018.03.098

[25] ZHANG F, NI H, LI X, LIU H, XI T et al. LncRNA FENDRR attenuates adriamycin resistance via suppressing MDR1 expression through sponging HuR and miR-184 in chronic myelogenous leukaemia cells. Febs Lett 2019; 593: 1993–2007. https://doi.org/10.1002/1873-3468.13480

[26] LIANG Y, CHEN X, WU Y, LI J, ZHANG S et al. LncRNA CASC9 promotes esophageal squamous cell carcinoma metastasis through upregulating LAMC2 expression by interacting with the CREB-binding protein. Cell Death Differ 2018; 25: 1980–1995. https://doi.org/10.1038/s41418-018-0084-9

[27] EBERHARDT W, NASRULLAH U, HAEUSSLER K. Inhibition of caspase-2 translation by the mRNA binding protein HuR: A novel path of therapy resistance in colon carcinoma cells? Cells-Basel 2019; 8. https://doi.org/10.3390/cells8080797

[28] PALOMO-IRIGOYEN M, PÉREZ-ANDRÉS E, IRUARRIZAGA-LEJARRETA M, BARREIRA-MANRIQUE A, TAMAYO-CARO M et al. HuR/ELAVL1 drives malignant peripheral nerve sheath tumor growth and metastasis. J Clin Invest 2020; 130: 3848–3864. https://doi.org/10.1172/JCI130379

[29] LAI Q, WANG H, LI A, XU Y, TANG L et al. Decitibine improve the efficiency of anti-PD-1 therapy via activating the response to IFN/PD-L1 signal of lung cancer cells. Oncogene 2018; 37: 2302–2312. https://doi.org/10.1038/s41388-018-0125-3

[30] HERDY B, KARONITSCH T, VLADIMER GI, TAN CS, STUKALOV A et al. The RNA-binding protein HuR/ELAVL1 regulates IFN-β mRNA abundance and the type I IFN response. Eur J Immunol 2015; 45: 1500–1511. https://doi.org/10.1002/eji.201444979

[31] TAKEUCHI O. HuR keeps interferon-β mRNA stable. Eur J Immunol 2015; 45: 1296–1299. https://doi.org/10.1002/eji.201545616.

[32] FABBI M, CARBOTTI G, FERRINI S. Dual roles of IL-27 in cancer biology and immunotherapy. Mediators Inflamm 2017; 2017: 3958069. https://doi.org/10.1155/2017/3958069

[33] ZORZOLI A, DI CARLO E, COCCO C, OGNIO E, RIBATTI D et al. Interleukin-27 inhibits the growth of pediatric acute myeloid leukemia in NOD/SCID/Il2rg-/- mice. Clin Cancer Res 2012; 18: 1630–1640. https://doi.org/10.1158/1078-0432.CCR-11-2432

[34] JIA H, DILGER P, BIRD C, WADHWA M. IL-27 promotes proliferation of human leukemic cell lines through the MAPK/ERK signaling pathway and suppresses sensitivity to chemotherapeutic drugs. J Interferon Cytokine Res 2016; 36: 302–316. https://doi.org/10.1089/jir.2015.0091