

Article

ELAVL1 Promotes Proliferation and Inhibits Apoptosis of the Marek's Disease Virus (MDV)-Transformed Cell Line MSB1 via the COX-2/PGE2 Pathway

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Simple Summary

Marek's disease virus (MDV) causes lymphoproliferative tumors in chickens. Although the RNA-binding protein ELAVL1 is implicated in tumorigenesis, its role in MDV-induced oncogenesis remains unclear. This study investigated the role of ELAVL1 in the MDV-transformed MSB1 cell line, specifically in the COX-2/PGE2 pathway. Overexpression of ELAVL1 significantly enhanced cell proliferation, promoted G1-to-S/G2 phase cell cycle progression, and reduced apoptosis. These changes were accompanied by upregulated transcription and expression of COX-2 and PGE2. Conversely, siRNA-mediated knockdown of ELAVL1 inhibited cell proliferation, induced G1 phase arrest, and decreased COX-2 and PGE2 expression. The findings suggest that the RNA-binding protein ELAVL1 enhances the oncogenic properties of MDV-transformed lymphocytes by activating the COX-2/PGE2 signaling pathway, unraveling the potential mechanism through which MDV induces oncogenesis.

Abstract

Marek's disease (MD), caused by the oncogenic Marek's disease virus (MDV), is a highly contagious avian infection that induces lymphoproliferative tumors. The RNA-binding protein ELAVL1 is known to regulate tumor cell proliferation and apoptosis, but its role in MDV-induced oncogenesis remains unclear. This study investigated whether ELAVL1 modulates proliferation and apoptosis in the MDV-transformed MSB1 cell line and whether its effects involve the cyclooxygenase-2 (COX-2)/prostaglandin E2 (PGE2) pathway. MSB1 cells were transiently transfected with *ELAVL1*-overexpressing plasmids (pEGFP-C-*ELAVL1*) or *ELAVL1*-specific siRNA, with expression confirmed by real-time PCR (qRT-PCR). Cell proliferation was assessed using the CCK-8 assay, while cell cycle distribution and apoptosis rates were analyzed by flow cytometry. COX-2 and PGE2 expression levels were determined by qRT-PCR, Western blotting, and ELISA. Overexpression of *ELAVL1* significantly promoted the proliferation of MSB1 cells, decreased transition into the G1 phase, increased the proportions of S and G2 phase cells, and suppressed apoptosis. Correspondingly, both mRNA and protein levels of COX-2 and PGE2 were significantly elevated.



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Conversely, ELAVL1 knockdown significantly inhibited proliferation, induced G1 phase arrest, decreased S phase cells, and significantly decreased COX-2 and PGE2 expression. These findings indicate that ELAVL1 promotes proliferation and inhibits apoptosis in MDV-transformed MSB1 cells, potentially via the COX-2/PGE2 signaling pathway.

Keywords: ELAVL1; Marek's disease; MSB1; proliferation; apoptosis; COX-2/PGE2

1. Introduction

RNA-binding proteins (RBPs) are central regulators of post-transcriptional gene expression, RNA splicing, stability, localization, and translation [1]. By binding to specific elements in the 5' or 3' untranslated regions (UTRs) of target messenger RNAs (mRNAs), RBPs modulate mRNA fate and are critical for development, cellular homeostasis, and neoplastic disease progression [2–5]. Embryonic lethal abnormal vision-like 1 (ELAVL1), also known as human antigen R (HuR), is a key member of this family and has been strongly implicated in oncogenesis. Under normal conditions, ELAVL1 is primarily localized in the nucleus; however, it translocates to the cytoplasm in response to stimuli such as cellular stress or inflammation. In the cytoplasm, ELAVL1 binds to AU-rich elements (AREs) within the 3'UTRs of target mRNAs, enhancing their stability and translation [6,7]. Through this mechanism, ELAVL1 upregulates the expression of numerous proteins involved in cell proliferation, survival, angiogenesis, and metastasis [8]. Cyclooxygenase-2 (COX-2), an inducible enzyme that plays a significant role in inflammation and cancer progression, is a well-characterized downstream target of ELAVL1 [9,10]. Specifically, ELAVL1 increases COX-2 expression, which in turn promotes tumor cell growth and inhibits apoptosis by directly binding to and stabilizing the 3'UTR of COX-2 mRNA [11,12]. This regulatory axis also contributes to tumor-associated neovascularization [13–15], where prostaglandin E2 (PGE2), the main enzymatic product of COX-2, is a potent mediator of tumor progression [10,16–18]. Thus, the ELAVL1-mediated regulation of COX-2 is a significant axis in oncogenesis [15,19–21].

Studies indicate that several herpesviruses manipulate the post-transcriptional regulatory network of host cells to promote viral persistence and tumorigenesis by targeting ELAVL1. For example, proteins encoded by Epstein–Barr virus (EBV) regulate ELAVL1 activity in human tumors [22]. Similarly, Kaposi's sarcoma-associated herpesvirus (KSHV) exploits ELAVL1 to enhance viral gene expression. Notably, Kaposin-B stabilizes *PROX1* mRNA during KSHV-mediated lymphatic reprogramming of vascular endothelial cells [23].

The ELAVL1 protein and its mammalian orthologs are highly conserved in chickens, suggesting they may perform similar functions in avian species. Marek's disease virus (MDV; Gallid herpesvirus 2) is a highly oncogenic alpha-herpesvirus that can establish latent infections in lymphocytes, leading to transformation and the development of Marek's disease (MD), which is primarily characterized by the proliferation of lymphocytes in the infected chickens. As a major economic burden to the global poultry industry, MD underscores the need for a deeper understanding of the molecular mechanisms underlying its pathogenesis. MDV infection has been shown to activate the COX-2/PGE2 pathway, which may promote tumorigenesis by suppressing the antiviral CD4⁺ T-cell response [24]. However, the upstream molecular mechanism underlying MDV-mediated activation of the COX-2/PGE2 pathway remains poorly understood. In mammalian systems, ELAVL1 is a key post-transcriptional regulator of COX-2 expression, and both ELAVL1 and COX-2 have been extensively studied in human and other mammalian cancers [25–31]. However, their

functional interplay has not been systematically explored in avian viruses, particularly in models of MDV-induced tumorigenesis.

Accordingly, we propose the following hypothesis: ELAVL1 expression and/or activity are dysregulated in MDV-transformed T cells. This stabilizes COX-2 and other pro-survival and pro-inflammatory mRNAs, thereby driving cell proliferation, inhibiting apoptosis, and possibly promoting tumor development through PGE2-mediated immunosuppression.

In the present study, ELAVL1 expression was modulated via plasmid-mediated overexpression and siRNA-mediated knockdown in the MDV-transformed avian CD4⁺ T-cell line MSB1. The effects of ELAVL1 on MSB1 cell proliferation, cell cycle distribution, and apoptosis were investigated to determine whether ELAVL1 exerts a pro-tumorigenic effect and whether these phenotypic changes are associated with alterations in the COX-2/PGE2 pathway. These findings provide insight into the role of ELAVL1 in MDV-driven tumorigenesis and explore its potential as a therapeutic target for this economically important avian disease.

2. Materials and Methods

2.1. Cell Culture and Transfection with Plasmids and siRNA

MSB1 cells, an MDV-transformed chicken lymphoblastoid line, were maintained in complete RPMI 1640 medium (Gibco, Waltham, MA, USA) supplemented with 10% fetal bovine serum (Gibco, USA), 10% tryptose phosphate broth (Sigma, St. Louis, MO, USA), and 1% penicillin-streptomycin (HyClone, Logan, UT, USA) at 37 °C under 5% CO₂. For transfection experiments, MSB1 cells were seeded in 6-well plates at a density of 1×10^6 per well ($n = 3$ per group). Cells were transfected with a plasmid overexpressing ELAVL1 (pEGFP-C-ELAVL1) or the corresponding empty vector control (pEGFP-C) using the riboFECTTM CP kit (C10511-05, RiboBio, Guangzhou, China), according to the manufacturer's instructions. For gene silencing, MSB1 cells were transfected with ELAVL1-specific siRNA (siELAVL1: 5'-GUGUCCUGCAACUUUGUCCTT-3') or a non-targeting control siRNA (siNC: 5'-CCAUGACCAACUACGAUGA-3') (100 nM, RiboBio, China).

2.2. Quantitative RT-PCR (qRT-PCR)

The mRNA expression levels of *ELAVL1*, *COX-2*, and *PGE2* were quantified by quantitative RT-PCR (qRT-PCR), as outlined previously [32]. Briefly, total RNA was extracted from MSB1 cells in each group at 48 h post-transfection using TRIzol (15596018CN, Invitrogen, Carlsbad, CA, USA) and quantified with a NanoDrop ND-2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). cDNA was synthesized from 1 µg of total RNA using the PrimeScriptTM RT Master Mix kit (RR036A, TaKaRa Bio, Shiga, Japan). qRT-PCR was subsequently performed for *ELAVL1*, *COX-2*, and *PGE2* using the SYBR[®] Premix Ex TaqTM II (RR820A, TaKaRa Bio, Japan) on an ABI 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Each 20 µL PCR reaction contained 1 µL of cDNA, 0.4 µL (10 µM) of each specific primer, 10 µL SYBR Premix Ex Taq II, 0.4 µL ROX Reference Dye II (50×), and 7.8 µL sterile water. The cycling conditions were 95 °C for 30 s, followed by 40 cycles of 95 °C for 30 s and 60 °C for 30 s. Gene expression levels were calculated using the comparative $2^{-\Delta\Delta C_t}$ method, with β -actin as the endogenous control. Results are expressed as fold change relative to the control group (set to 1). Primer sequences are listed in Table 1.

Table 1. Sequences of primers used for qRT-PCR assays detecting the mRNA expression levels of *ELAVL1*, *COX-2*, and *PGE2* in MSB1 cells.

Name	Primer Sequences (5' → 3')	Size (bp)
gga β -actin Forward primer	TCAACACCCCAGCCATGTAT	244
gga β -actin Reverse primer	ATTCTCTCTCGGCTGTGGT	
ELAVL1 Forward primer	TACCTCCCCCAGAACATGAC	220
ELAVL1 Reverse primer	TTGGACGAGCATAGGAAACC	
COX-2 Forward primer	GAACCATCCTACCCGCTATTGT	246
COX-2 Reverse primer	CTATGGGGATTACAATGCGATG	
PGE2 Forward primer	CAACAAGTTCAGCCAGAGCGA	200
PGE2 Reverse primer	CCAGCTTTGTTTTTGCAGAGGT	

2.3. Western Blotting Analysis

The protein expression levels of ELAVL1 and COX were measured as follows: 1×10^6 MSB1 cells per well were cultured in 6-well plates ($n = 3$ per group), and lysed 48 h post-transfection using ice-cold RIPA buffer (R0010, Servicebio, Wuhan, China) supplemented with protease and phosphatase inhibitors. Protein concentrations were determined using a Bradford assay kit (G2001, Servicebio, China). Equal amounts of total protein (20 μ g per sample) were separated by 10% SDS-PAGE and transferred onto a polyvinylidene difluoride (PVDF) membrane (G6047, Servicebio, China). Membranes were blocked with 5% bovine serum albumin (BSA) (GC305006, Servicebio, China) for 30 min at room temperature and incubated overnight at 4 °C with the following primary antibodies: anti-ELAVL1 (1:1000; 11910-1-AP, Proteintech, Rosemont, IL, USA), anti-COX-2 (1:1000; 123751-1-AP, Proteintech), and anti- β -actin (1:1000; ab8226, Abcam, Waltham, MA, USA). Subsequently, membranes were incubated for two hours with the corresponding horseradish peroxidase (HRP)-conjugated secondary antibodies: anti-rabbit IgG (1:1000; A0208, Beyotime, Shanghai, China) for ELAVL1 and COX-2, and anti-mouse IgG (1:1000; SA00001-1, Proteintech) for β -actin. Protein bands were visualized using the Bio-Rad Clarity Western ECL substrate (Bio-Rad Laboratories, Inc., Hercules, CA, USA), and chemiluminescent signals were captured. Band intensities were quantified using ImageJ (version 1.53, National Institutes of Health, Bethesda, MD, USA). The gray value of ELAVL1 or COX-2 protein was first normalized to that of β -actin, then expressed as a ratio relative to the control group.

2.4. ELISA Assays

PGE2 levels in MSB1 cell lysates were measured using ELISA. Briefly, MSB1 cells (1×10^6 per well) were cultured in 6-well plates ($n = 3$ per group), and culture supernatants (2 mL per well) were collected 48 h post-transfection. Supernatants were centrifuged at 12,000 r/min for 10 min to remove cell debris, concentrated, and stored at -80 °C until analysis. PGE₂ concentrations were measured using a commercially available ELISA kit (EU2554, Wuhan Fine Biotech Co., Ltd., Wuhan, China), following the manufacturer's instructions. The PGE2 concentrations in the supernatant were extrapolated from the standard curve and ultimately normalized to total protein, with results presented as pg PGE₂/μg protein.

2.5. Cell Viability Assays

Cell proliferation was assessed using the Cell Counting Kit-8 (CCK-8; C0037, Beyotime, China), according to the manufacturer's instructions. MSB1 cells were harvested post-transfection and seeded in triplicate into 96-well plates at a density of 4000 cells per well. The CCK-8 reagent (10 μ L) was added to each well at 0, 24, 48, and 72 h of culture, followed

by a 2-h incubation at 37 °C. Optical density (OD) was measured at 450 nm using a microplate reader (Thermo Fisher Scientific, Waltham, MA, USA).

2.6. Cell Cycle Assay

The distribution of cell cycle phases was analyzed using a commercial Cell Cycle Detection Kit (KGA512, KeyGen, Changchun, China) according to the manufacturer's instructions. Briefly, cells were harvested 48 h post-transfection, washed twice with ice-cold PBS, and fixed in 70% ethanol on ice for 2 h at 4 °C. Fixed cells were washed twice with cold PBS and resuspended in a staining solution containing 50 µg/mL propidium iodide (PI) and 50 µg/mL RNase A. After incubation in the dark at 4 °C for 30 min, cell cycle distribution was analyzed by flow cytometry (CytoFLEX, Beckman Coulter, Brea, CA, USA).

2.7. AnnexinV-APC/7-AAD Staining

Cell apoptosis was evaluated by flow cytometry (CytoFLEX, Beckman Coulter, USA) using Annexin V-APC and 7-AAD staining. At 48 h post-transfection, MSB1 cells were harvested, washed twice with ice-cold PBS, and processed using an Annexin V-APC/7-AAD Apoptosis Detection Kit (A213, Vazyme, Nanjing, China) following the manufacturer's instructions. Cells were resuspended in 500 µL of 1× binding buffer and stained with 5 µL of Annexin V-APC and 7-AAD for 15 min at room temperature in the dark. Flow cytometric analysis was then performed, and the proportions of early apoptotic (Annexin V⁺/7-AAD⁻) and late apoptotic (Annexin V⁺/7-AAD⁺) cells were quantified.

2.8. Statistical Analysis

All experiments were performed with three independent biological replicates ($n = 3$). For RT-qPCR, CCK-8, and Western blotting, each biological replicate was assayed in technical triplicate. Data are presented as the mean ± SD. Statistical analyses were performed using SPSS 20.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 9.0 (GraphPad Software, Boston, MA, USA). Differences between the two groups were analyzed using a two-tailed Student's *t*-test or a Welch's *t*-test when variances were unequal. Comparisons among multiple groups were performed using a one-way ANOVA followed by Dunnett's post hoc test. A *p* value less than 0.05 was considered statistically significant (* $p < 0.05$, ** $p < 0.01$), with ns denoting no significant difference.

3. Results

3.1. ELAVL1 Overexpression and Knockdown in MSB1 Cells

To investigate the functional role of ELAVL1 in MSB1 cells, overexpression and knock-down models were established by transfecting MSB1 cells with pEGFP-C1-ELAVL1 or siELAVL1, respectively. qRT-PCR analysis confirmed that *ELAVL1* mRNA levels were markedly upregulated following pEGFP-C1-ELAVL1 transfection and significantly down-regulated upon siELAVL1 treatment (Figure 1). This differential expression system provided a reliable experimental platform for subsequent functional studies.

3.2. ELAVL1 Expression Plays a Key Role in the Cell Viability and Cycle Progression of MSB1 Cells

CCK-8 assays were performed to examine whether ELAVL1 regulates MSB1 cell proliferation. Overexpression of ELAVL1 significantly increased the cell viability, whereas its knockdown suppressed it (Figure 2). Flow cytometric analysis revealed that ELAVL1 overexpression accelerated the G1/S transition, as evidenced by a decreased proportion of cells in G1 and increased proportions in S and G2 phases. Conversely, ELAVL1 knockdown induced G1 arrest, with accumulation of cells in the G1 phase and a concomitant reduction

in the S phase. No significant change in the G2 phase distribution was observed upon ELAVL1 knockdown (Figure 3). These results demonstrate that ELAVL1 promotes MSB1 cell proliferation, at least in part, by facilitating the G1/S phase progression.

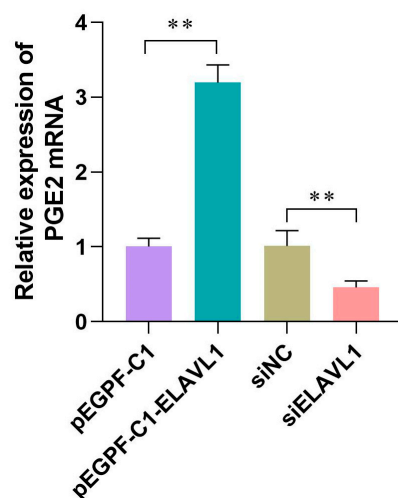


Figure 1. Expression of *ELAVL1* in MSB1 cells following transfection with pEGFP-C1-*ELAVL1*, pEGFP-C1, si*ELAVL1*, or siNC. β -*Actin* was used as the control. Gene expression was normalized to the Control group. Each biological replicate was assayed in technical triplicate. Data are presented as mean $\pm$ SD from three independent biological replicates ($n = 3$). Comparisons between two groups were performed using a two-tailed Student's *t*-test or Welch's *t*-test when variances were unequal. ** $p < 0.01$ indicates a significant difference compared with the corresponding control group.

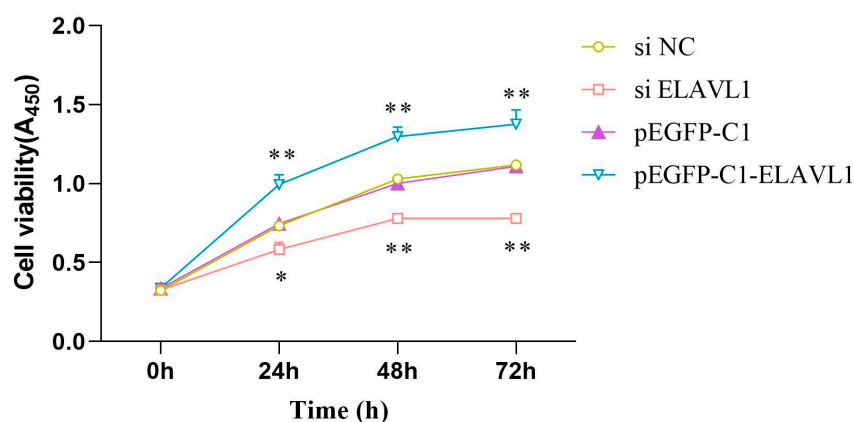


Figure 2. Cell viability of MSB1 cells after transfection with pEGFP-C1, pEGFP-C1-*ELAVL1*, siNC, or si*ELAVL1*. Each biological replicate was assayed in technical triplicate. Data are presented as mean $\pm$ SD from three independent biological replicates ($n = 3$). Comparisons among multiple groups were performed using one-way ANOVA followed by Dunnett's post hoc test to compare each treatment group with its corresponding control. (* $p < 0.05$, ** $p < 0.01$).

3.3. Overexpression of *ELAVL1* Inhibits the Apoptosis of MSB1 Cells

Next, we examined whether *ELAVL1* influences apoptosis in MSB1 cells. Overexpression of *ELAVL1* significantly reduced the apoptotic rate, whereas *ELAVL1* knockdown markedly increased apoptosis (Figure 4). These results indicate that *ELAVL1* protects MSB1 cells from apoptotic cell death, consistent with its pro-survival role suggested by proliferation and cell cycle analyses.

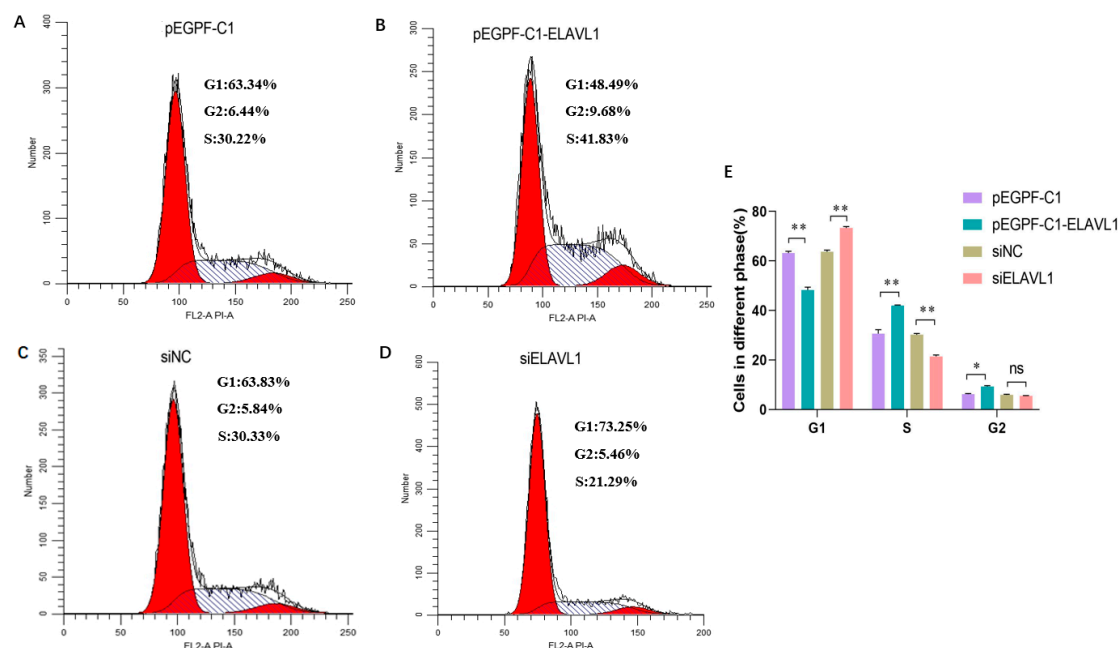


Figure 3. Flow cytometry analysis of the cell cycle in MSB1 cells following transfection with (A) pEGFP-C1; (B) pEGFP-C1-ELAVL1; (C) siNC; or (D) pEGFP-C1-siELAVL1; (E) Cell cycle distribution across experimental groups. G1: pre-DNA synthesis phase; S: DNA synthesis phase; G2: post-DNA synthesis phase. Data are presented as mean $\pm$ SD from three independent biological replicates ($n = 3$). Comparisons among multiple groups were performed using one-way ANOVA followed by Dunnett's post hoc test to compare each treatment group with its corresponding control. (* $p < 0.05$, ** $p < 0.01$, ns, not significant).

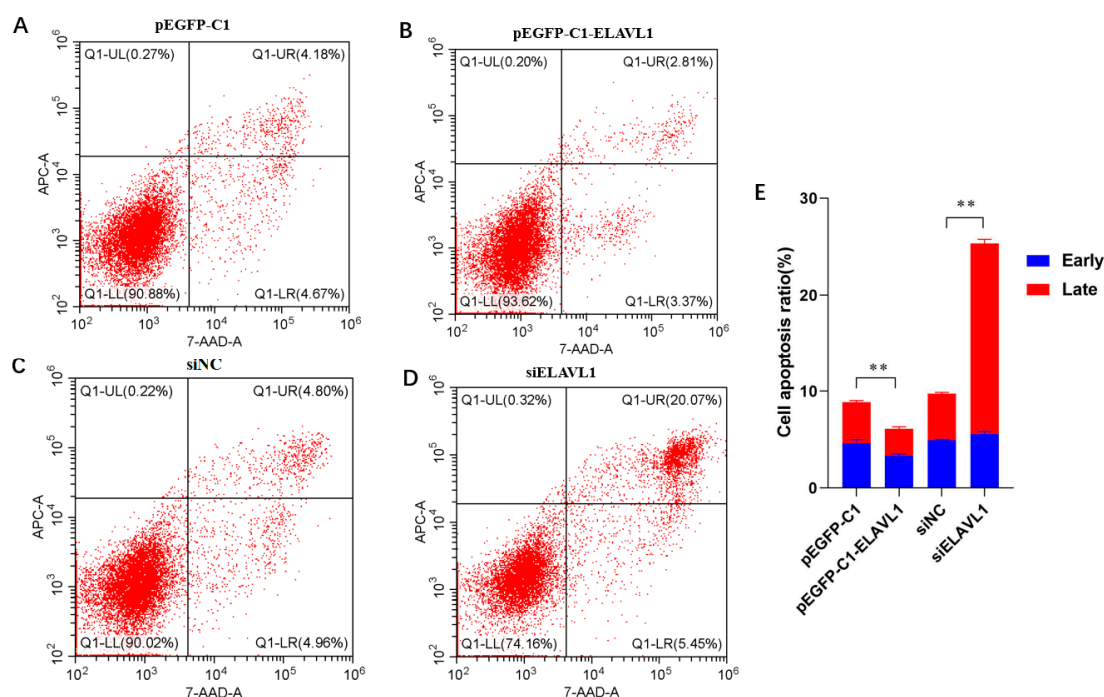


Figure 4. Flow cytometric results for the apoptosis rate of MSB1 cells following modulation of ELAVL1 expression. Representative plots are shown for (A) pEGFP-C1 group, (B) pEGFP-C1-ELAVL1 group, (C) siNC group, and (D) pEGFP-C1-siELAVL1 group; (E) The proportion of apoptotic cells in the different experimental groups. Data are presented as mean $\pm$ SD from three independent biological replicates ($n = 3$). Comparisons between two groups were performed using a two-tailed Welch's t -test. (** $p < 0.01$).

3.4. ELAVL1 Regulates the Expression of COX-2/PGE2 in MSB1 Cells

To further investigate the effect of ELAVL1 on the COX-2/PGE2 pathway, we analyzed the mRNA transcription and protein expression of ELAVL1, COX-2, and PGE2. Overexpression of ELAVL1 significantly upregulated both the mRNA and protein levels of COX-2, whereas ELAVL1 knockdown markedly suppressed COX-2 expression (Figure 5A,B). Similarly, PGE2, a key downstream effector of COX-2, was elevated upon ELAVL1 overexpression and reduced following ELAVL1 knockdown (Figure 5D,E). These results demonstrate that ELAVL1 positively regulates the COX-2/PGE₂ pathway at both the transcriptional and translational levels.

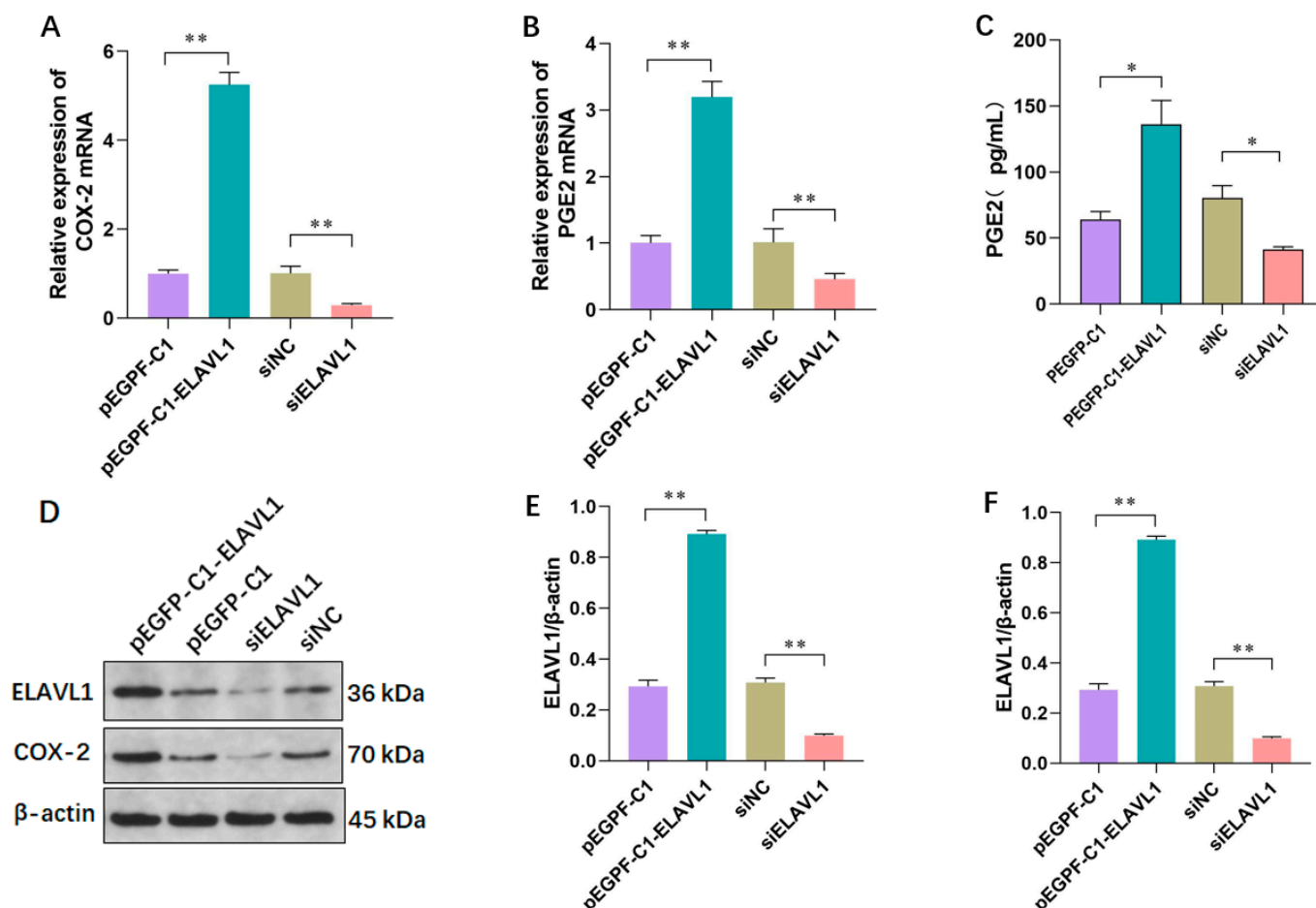


Figure 5. The effect of ELAVL1 on the expression of COX-2 and PGE2. (A,B) Relative mRNA levels of COX-2 and PGE2 in MSB1 cells transfected with pEGFP-C1-ELAVL1, pEGFP-C1-siELAVL1, or siNC, with β -actin as the loading control; (C) PGE2 concentration in cell culture supernatant, quantified using ELISA; (D) Representative Western blot images showing ELAVL1 and COX-2 protein levels; (E,F) Densitometric quantification of ELAVL1 and COX-2 protein levels normalized to β -actin. Each biological replicate was assayed in technical triplicate. Data are presented as mean $\pm$ SD from three independent biological replicates ($n = 3$). Comparisons between two groups were performed using a two-tailed Student's *t*-test or Welch's *t*-test when variances were unequal. (* $p < 0.05$, ** $p < 0.01$).

4. Discussion

The biological functions of ELAVL1 and its role in the pathogenesis of neoplastic diseases have attracted considerable scientific interest [33,34]. Studies have shown that ELAVL1 expression is significantly upregulated in the cytoplasm of various tumor tissues [35,36], where it regulates tumor cell proliferation, invasion, lymphangiogenesis, and angiogenesis across multiple cancer types [37–41]. Consequently, ELAVL1 has been pro-

posed as a potential therapeutic target for chemotherapy and for slowing down tumor progression [42–45]. However, most research on ELAVL1 has focused on human cancers, and its functions and underlying mechanisms in avian neoplastic diseases remain largely unexplored.

Uncontrolled cell division, dysregulated cell cycle progression, and abnormal proliferation are hallmarks of cancer. Activation of cyclins, which in turn activate cyclin-dependent kinases (CDKs), is a key driver of cell cycle progression [46]. ELAVL1 can bind to the 3'UTR of cyclin D1 and cyclin E1 mRNAs, enhancing their stability and promoting their expression. The resulting activation of cyclin D1 and E1 stimulates CDK4, CDK6, and CDK2, thereby facilitating the transition from the G1 to the S phase while promoting cell proliferation [43,47]. This study investigated the effects of ELAVL1 on proliferation, cell cycle distribution, and apoptosis in MSB1 cells using overexpression and knockdown approaches. The results revealed a consistent trend: ELAVL1 overexpression significantly enhanced cell viability, accelerated cell cycle progression, and inhibited apoptosis in MSB1 cells, whereas ELAVL1 knockdown exerted the opposite effect, including inducing G1 phase arrest, delaying progression to S phase, reducing cell viability, and enhancing apoptosis. These findings indicate that ELAVL1 promotes proliferation and inhibits apoptosis in MSB1 tumor cells, supporting previous reports in other tumors [38,48–50].

Studies have shown that COX-2 and its enzymatic product PGE2 play key roles in tumor cell proliferation and apoptosis [51,52]. In normal cells, COX-2 expression is tightly regulated by ARE-binding proteins. However, during tumorigenesis, this regulatory capacity is often compromised, leading to sustained COX-2 overexpression [53]. Elevated COX-2 expression in cancer cells has been linked to ELAVL1, which interacts with COX-2 mRNA, and *ELAVL1* expression levels positively correlate with COX-2 levels. Thus, silencing ELAVL1 reduces COX-2 protein expression [12,15,53]. Inhibition of the COX-2/PGE2 signaling pathway has been shown to suppress the proliferation, migration, and invasion of cancer cells while inducing apoptosis, confirming its role in tumor growth and survival [54,55]. In the present study, modulation of ELAVL1 expression in MSB1 cells affected cell cycle progression and positively correlated with COX-2 and PGE2 levels. Conversely, ELAVL1 knockdown suppressed the expression of these two proteins, accompanied by cell cycle arrest and reduced proliferation. These findings suggest that ELAVL1 regulates proliferation and apoptosis in MSB1 cells, possibly through the COX-2/PGE2 pathway.

Future studies should include additional MDV-induced tumor cell lines or tumor samples to validate the broader relevance of these findings. Although this study examined the correlation between ELAVL1 and COX-2/PGE2 levels in vitro, the specific regulatory relationship remains to be further investigated. Techniques such as RNA immunoprecipitation (RIP) or 3'UTR luciferase reporter assays could clarify whether avian ELAVL1 directly interacts with COX-2 mRNA. Inhibition of COX-2 and PGE2, followed by assessment of proliferation and apoptosis in MDV-induced tumor cells, would further clarify the mechanisms by which ELAVL1 and COX-2/PGE2 regulate the proliferation and apoptosis of MDV-induced tumor cells. Moreover, in vivo studies of MDV infection are warranted to elucidate the mechanistic role of ELAVL1 in viral oncogenesis and to provide a more comprehensive assessment of the COX/PGE2 pathway as a viable therapeutic target.

5. Conclusions

In this study, we investigated the function of ELAVL1 in MSB1 cells, an MDV-induced model of avian T-cell lymphoma. The results demonstrate that ELAVL1 promotes proliferation, accelerates cell cycle progression, and inhibits apoptosis. Notably, ELAVL1 overexpression was associated with increased COX-2 expression and PG2 production, suggesting a functional link between ELAVL1 and the COX-2/PGE2 axis. These findings

identify ELAVL1 as a candidate therapeutic target for this economically important avian disease and highlight the need for further investigation into its downstream mechanisms.

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Institutional Review Board Statement: Ethical review and approval were waived for this study, as this study was conducted exclusively using established, commercially available cell lines, and it is not classified as human or animal subject research.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data supporting the findings of this study are included within the article. Further inquiries can be directed to the corresponding authors.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

MD	Marek's disease
MDV	Marek's disease virus
ELAVL1	Embryonic lethal abnormal vision-like 1
HuR	Human antigen R
RBP	RNA-binding proteins
DMSO	Dimethyl sulfoxide
FBS	Fetal bovine serum
TPB	Tryptose phosphate broth
qRT-PCR	Quantitative Real-time Polymerase Chain Reaction
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
UTR	Untranslated region
COX-2	Cyclooxygenase-2
PGE2	Prostaglandin E2
CDK	Cyclin-dependent kinases
OD	Optical density

References

1. Fang, Y.; Liu, X.; Liu, Y.; Xu, N. Insights into the Mode and Mechanism of Interactions Between RNA and RNA-Binding Proteins. *Int. J. Mol. Sci.* **2024**, *25*, 11337. [[CrossRef](#)] [[PubMed](#)]
2. Pereira, B.; Billaud, M.; Almeida, R. RNA-Binding Proteins in Cancer: Old Players and New Actors. *Trends Cancer* **2017**, *3*, 506–528. [[CrossRef](#)] [[PubMed](#)]
3. Li, W.; Deng, X.; Chen, J. RNA-binding proteins in regulating mRNA stability and translation: Roles and mechanisms in cancer. *Semin. Cancer Biol.* **2022**, *86*, 664–677. [[CrossRef](#)]
4. Jungfleisch, J.; Gebauer, F. RNA-binding proteins as therapeutic targets in cancer. *RNA Biol.* **2025**, *22*, 1–8. [[CrossRef](#)]
5. Smirnov, A. Research Progress in RNA-Binding Proteins. *Int. J. Mol. Sci.* **2022**, *24*, 58. [[CrossRef](#)]
6. Abdelmohsen, K.; Lal, A.; Kim, H.H.; Gorospe, M. Posttranscriptional orchestration of an anti-apoptotic program by HuR. *Cell Cycle* **2007**, *6*, 1288–1292. [[CrossRef](#)]
7. Brennan, C.M.; Steitz, J.A. HuR and mRNA stability. *Cell. Mol. Life Sci.* **2001**, *58*, 266–277. [[CrossRef](#)]

8. Grammatikakis, I.; Abdelmohsen, K.; Gorospe, M. Posttranslational control of HuR function. *Wiley Interdiscip. Rev. RNA* **2017**, *8*. [\[CrossRef\]](#)
9. Kassab, A.E. Recent advances in targeting COX-2 for cancer therapy: A review. *RSC Med. Chem.* **2025**. [\[CrossRef\]](#)
10. Zheng, X.; Wang, J.; OuYang, Y.; Yao, K.; Zheng, J.; Zeng, L.; Wang, J.; Chen, H.; Du, H.; Fu, D.; et al. Breaking immune evasion in breast cancer by targeting COX-2/PGE2 pathway. *Mol. Cell. Endocrinol.* **2025**, *608*, 112617. [\[CrossRef\]](#) [\[PubMed\]](#)
11. Janakiraman, H.; House, R.P.; Talwar, S.; Courtney, S.M.; Hazard, E.S.; Hardiman, G.; Mehrotra, S.; Howe, P.H.; Gangaraju, V.; Palanisamy, V. Repression of caspase-3 and RNA-binding protein HuR cleavage by cyclooxygenase-2 promotes drug resistance in oral squamous cell carcinoma. *Oncogene* **2017**, *36*, 3137–3148. [\[CrossRef\]](#) [\[PubMed\]](#)
12. Mitsunari, K.; Miyata, Y.; Asai, A.; Matsuo, T.; Shida, Y.; Hakariya, T.; Sakai, H. Human antigen R is positively associated with malignant aggressiveness via upregulation of cell proliferation, migration, and vascular endothelial growth factors and cyclooxygenase-2 in prostate cancer. *Transl. Res.* **2016**, *175*, 116–128. [\[CrossRef\]](#) [\[PubMed\]](#)
13. Giaginis, C.; Alexandrou, P.; Delladetsima, I.; Karavokyros, I.; Danas, E.; Giagini, A.; Patsouris, E.; Theocharis, S. Clinical Significance of Hu-Antigen Receptor (HuR) and Cyclooxygenase-2 (COX-2) Expression in Human Malignant and Benign Thyroid Lesions. *Pathol. Oncol. Res.* **2016**, *22*, 189–196. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Aguado, A.; Rodríguez, C.; Martínez-Revelles, S.; Avendaño, M.S.; Zhenyukh, O.; Orriols, M.; Martínez-González, J.; Alonso, M.J.; Briones, A.M.; Dixon, D.A.; et al. HuR mediates the synergistic effects of angiotensin II and IL-1 β on vascular COX-2 expression and cell migration. *Br. J. Pharmacol.* **2015**, *172*, 3028–3042. [\[CrossRef\]](#)
15. Kurosu, T.; Ohga, N.; Hida, Y.; Maishi, N.; Akiyama, K.; Kakuguchi, W.; Kuroshima, T.; Kondo, M.; Akino, T.; Totsuka, Y.; et al. HuR keeps an angiogenic switch on by stabilising mRNA of VEGF and COX-2 in tumour endothelium. *Br. J. Cancer* **2011**, *104*, 819–829. [\[CrossRef\]](#)
16. Li, X.; Zhu, X.; Diba, P.; Shi, X.; Vrieling, F.; Jansen, F.A.C.; Balvers, M.G.J.; de Bus, I.; Levasseur, P.R.; Sattler, A.; et al. Tumor-derived cyclooxygenase-2 fuels hypothalamic inflammation. *Brain Behav. Immun.* **2025**, *123*, 886–902. [\[CrossRef\]](#)
17. Li, X.; Zhu, Y.; Zhao, T.; Zhang, X.; Qian, H.; Wang, J.; Miao, X.; Zhou, L.; Li, N.; Ye, L. Role of COX-2/PGE2 signaling pathway in the apoptosis of rat ovarian granulosa cells induced by MEHP. *Ecotoxicol. Environ. Saf.* **2023**, *254*, 114717. [\[CrossRef\]](#)
18. Parvathareddy, S.K.; Siraj, A.K.; Annaiyappanaidu, P.; Al-Sobhi, S.S.; Al-Dayel, F.; Al-Kuraya, K.S. Prognostic Significance of COX-2 Overexpression in BRAF-Mutated Middle Eastern Papillary Thyroid Carcinoma. *Int. J. Mol. Sci.* **2020**, *21*, 9498. [\[CrossRef\]](#)
19. Husain, M.A.; Smith, R.; Sorge, R.E.; Kaimari, A.; Si, Y.; Hassan, A.Z.; Guha, A.; Smith, K.A.; Cardozo, C.P.; DeBerry, J.J.; et al. Inhibition of the RNA Regulator HuR Mitigates Spinal Cord Injury by Potently Suppressing Post-Injury Neuroinflammation. *FASEB J.* **2025**, *39*, e70588. [\[CrossRef\]](#)
20. Matsuo, T.; Miyata, Y.; Asai, A.; Sagara, Y.; Furusato, B.; Fukuoka, J.; Sakai, H. Green Tea Polyphenol Induces Changes in Cancer-Related Factors in an Animal Model of Bladder Cancer. *PLoS ONE* **2017**, *12*, e0171091. [\[CrossRef\]](#)
21. Ohnishi, M.; Yukawa, R.; Akagi, M.; Ohsugi, Y.; Inoue, A. Bradykinin and interleukin-1 β synergistically increase the expression of cyclooxygenase-2 through the RNA-binding protein HuR in rat dorsal root ganglion cells. *Neurosci. Lett.* **2019**, *694*, 215–219. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Tompkins, V.S.; Valverde, D.P.; Moss, W.N. Human regulatory proteins associate with non-coding RNAs from the EBV IR1 region. *Bmc Res. Notes* **2018**, *11*, 139. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Yoo, J.; Kang, J.; Lee, H.N.; Aguilar, B.; Kafka, D.; Lee, S.; Choi, I.; Lee, J.; Ramu, S.; Haas, J.; et al. Kaposin-B enhances the PROX1 mRNA stability during lymphatic reprogramming of vascular endothelial cells by Kaposi's sarcoma herpes virus. *PLoS Pathog.* **2010**, *6*, e1001046. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Kamble, N.; Gurung, A.; Kaufer, B.B.; Pathan, A.A.; Behboudi, S. Marek's Disease Virus Modulates T Cell Proliferation via Activation of Cyclooxygenase 2-Dependent Prostaglandin E2. *Front. Immunol.* **2021**, *12*, 801781. [\[CrossRef\]](#)
25. Lee, S.Y.; Choi, H.K.; Lee, K.J.; Jung, J.Y.; Hur, G.Y.; Jung, K.H.; Kim, J.H.; Shin, C.; Shim, J.J.; In, K.H.; et al. The immune tolerance of cancer is mediated by IDO that is inhibited by COX-2 inhibitors through regulatory T cells. *J. Immunother.* **2009**, *32*, 22–28. [\[CrossRef\]](#)
26. Cha, J.D.; Li, S.; Cha, I.H. Association between expression of embryonic lethal abnormal vision-like protein HuR and cyclooxygenase-2 in oral squamous cell carcinoma. *Head Neck* **2011**, *33*, 627–637. [\[CrossRef\]](#)
27. Lim, S.J.; Lee, S.H.; Joo, S.H.; Song, J.Y.; Choi, S.I. Cytoplasmic expression of HuR is related to cyclooxygenase-2 expression in colon cancer. *Cancer Res. Treat.* **2009**, *41*, 87–92. [\[CrossRef\]](#)
28. Lim, S.J.; Kim, H.J.; Kim, J.Y.; Park, K.; Lee, C.M. Expression of HuR is associated with increased cyclooxygenase-2 expression in uterine cervical carcinoma. *Int. J. Gynecol. Pathol.* **2007**, *26*, 229–234. [\[CrossRef\]](#)
29. Sengupta, S.; Jang, B.C.; Wu, M.T.; Paik, J.H.; Furneaux, H.; Hla, T. The RNA-binding protein HuR regulates the expression of cyclooxygenase-2. *J. Biol. Chem.* **2003**, *278*, 25227–25233. [\[CrossRef\]](#)

30. Johann, A.M.; Weigert, A.; Eberhardt, W.; Kuhn, A.M.; Barra, V.; von Knethen, A.; Pfeilschifter, J.M.; Brüne, B. Apoptotic cell-derived sphingosine-1-phosphate promotes HuR-dependent cyclooxygenase-2 mRNA stabilization and protein expression. *J. Immunol.* **2008**, *180*, 1239–1248. [\[CrossRef\]](#)
31. Hsiao, Y.W.; Li, C.F.; Chi, J.Y.; Tseng, J.T.; Chang, Y.; Hsu, L.J.; Lee, C.H.; Chang, T.H.; Wang, S.M.; Wang, D.D.; et al. CCAAT/enhancer binding protein δ in macrophages contributes to immunosuppression and inhibits phagocytosis in nasopharyngeal carcinoma. *Sci Signal.* **2013**, *6*, ra59. [\[CrossRef\]](#) [\[PubMed\]](#)
32. Ding, K.; Yu, Z.H.; Yu, C.; Jia, Y.Y.; He, L.; Liao, C.S.; Li, J.; Zhang, C.J.; Li, Y.J.; Wu, T.C.; et al. Effect of gga-miR-155 on cell proliferation, apoptosis and invasion of Marek's disease virus (MDV) transformed cell line MSB1 by targeting RORA. *BMC Vet Res.* **2020**, *16*, 23. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Finan, J.M.; Sutton, T.L.; Dixon, D.A.; Brody, J.R. Targeting the RNA-Binding Protein HuR in Cancer. *Cancer Res.* **2023**, *83*, 3507–3516. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Schultz, C.W.; Preet, R.; Dhir, T.; Dixon, D.A.; Brody, J.R. Understanding and targeting the disease-related RNA binding protein human antigen R (HuR). *Wiley Interdiscip. Rev. RNA* **2020**, *11*, e1581. [\[CrossRef\]](#)
35. Majumder, M.; Chakraborty, P.; Mohan, S.; Mehrotra, S.; Palanisamy, V. HuR as a molecular target for cancer therapeutics and immune-related disorders. *Adv. Drug Deliv. Rev.* **2022**, *188*, 114442. [\[CrossRef\]](#)
36. Wu, M.; Tong, C.W.S.; Yan, W.; To, K.K.W.; Cho, W.C.S. The RNA Binding Protein HuR: A Promising Drug Target for Anticancer Therapy. *Curr. Cancer Drug Targets* **2019**, *19*, 382–399. [\[CrossRef\]](#)
37. Xiao, H.; Ye, X.; Vishwakarma, V.; Preet, R. Dixon DACRC-derived exosomes containing the RNA-binding protein HuR promote lung cell proliferation by stabilizing c-Myc, m.R.N.A. *Cancer Biol. Ther.* **2022**, *23*, 139–149. [\[CrossRef\]](#)
38. Ma, Q.; Xu, C.; Han, X.; Wang, X.; Zhang, W.; Liu, Z.; Wu, R.; Wu, F.; Liu, X.; Zhang, T.; et al. The effects of modified RNA-binding proteins HuR on the biological behavior of the bladder cancer T24 cell line. *Transl. Androl. Urol.* **2022**, *11*, 348–357. [\[CrossRef\]](#)
39. Majumder, M.; Janakiraman, H.; Chakraborty, P.; Vijayakumar, A.; Mayhue, S.; Yu, H.; Dincman, T.; Martin, R.; O'Quinn, E.; Mehrotra, S.; et al. RNA-binding protein HuR reprograms immune T cells and promotes oral squamous cell carcinoma. *Oral Oncol. Rep.* **2024**, *10*, 100296. [\[CrossRef\]](#)
40. Ye, X.; Fu, Q.; Xiao, H. The Role of RNA-Binding Protein HuR in Lung Cancer by RNA Sequencing Analysis. *Front. Genet.* **2022**, *13*, 813268. [\[CrossRef\]](#)
41. Weiße, J.; Rosemann, J.; Krauspe, V.; Kappler, M.; Eckert, A.W.; Haemmerle, M.; Gutschner, T. RNA-Binding Proteins as Regulators of Migration, Invasion and Metastasis in Oral Squamous Cell Carcinoma. *Int. J. Mol. Sci.* **2020**, *21*, 6835. [\[CrossRef\]](#)
42. Raguraman, R.; Shanmugarama, S.; Mehta, M.; Elle Peterson, J.; Zhao, Y.D.; Munshi, A.; Ramesh, R. Drug delivery approaches for HuR-targeted therapy for lung cancer. *Adv. Drug Deliv. Rev.* **2022**, *180*, 114068. [\[CrossRef\]](#) [\[PubMed\]](#)
43. Goutas, D.; Pergaris, A.; Giaginis, C.; Theocharis, S. HuR as Therapeutic Target in Cancer: What the Future Holds. *Curr. Med. Chem.* **2022**, *29*, 56–65. [\[CrossRef\]](#) [\[PubMed\]](#)
44. Peng, J.; Quan, J.; Wang, X. Integrated pan-cancer analysis of RNA binding protein HuR investigates its biomarker potential in prognosis, immunotherapy, and drug sensitivity. *PLoS Comput. Biol.* **2025**, *21*, e1013374. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Wei, L.; Kim, S.H.; Armaly, A.M.; Aubé, J.; Xu, L.; Wu, X. RNA-binding protein HuR inhibition induces multiple programmed cell death in breast and prostate cancer. *Cell Commun. Signal.* **2024**, *22*, 580. [\[CrossRef\]](#)
46. Almalki, S.G. The pathophysiology of the cell cycle in cancer and treatment strategies using various cell cycle checkpoint inhibitors. *Pathol. Res. Pract.* **2023**, *251*, 154854. [\[CrossRef\]](#)
47. Ghosh, U.; Adhya, S. Posttranscriptional regulation of cyclin D1 by ARE-binding proteins AUF1 and HuR in cycling myoblasts. *J. Biosci.* **2018**, *43*, 685–691. [\[CrossRef\]](#)
48. Jia, M.Y.; Wu, C.; Fu, Z.; Xu, W.B.; Liu, J.; Wu, C.Y.; Zeng, X.Y.; Wu, Y.L.; Yan, H. Targeting the HuR/E2F7 axis synergizes with bortezomib against multiple myeloma. *Acta Pharmacol. Sin.* **2025**, *46*, 2296–2309. [\[CrossRef\]](#)
49. Zhang, Z.; Huang, A.; Zhang, A.; Zhou, C. HuR promotes breast cancer cell proliferation and survival via binding to CDK3 mRNA. *Biomed. Pharmacother.* **2017**, *91*, 788–795. [\[CrossRef\]](#)
50. Huang, Z.; Luo, Y.; Chen, C.; Zhou, C.; Su, Z.; Cai, C.; Li, X.; Wu, W. miR-325-3p Reduces Proliferation Promotes Apoptosis of Gastric Cancer Cells by Inhibiting Human Antigen. *R. Can. J. Gastroenterol. Hepatol.* **2023**, *2023*, 6882851. [\[CrossRef\]](#)
51. Jin, K.; Qian, C.; Lin, J.; Liu, B. Cyclooxygenase-2-Prostaglandin E2 pathway: A key player in tumor-associated immune cells. *Front. Oncol.* **2023**, *13*, 1099811. [\[CrossRef\]](#)
52. Denkert, C.; Koch, I.; von Keyserlingk, N.; Noske, A.; Niesporek, S.; Dietel, M.; Weichert, W. Expression of the ELAV-like protein HuR in human colon cancer: Association with tumor stage and cyclooxygenase-2. *Mod. Pathol.* **2006**, *19*, 1261–1269. [\[CrossRef\]](#)
53. Young, L.E.; Moore, A.E.; Sokol, L.; Meisner-Kober, N.; Dixon, D.A. The mRNA stability factor HuR inhibits microRNA-16 targeting of COX-2. *Mol. Cancer Res.* **2012**, *10*, 167–180. [\[CrossRef\]](#)

54. Karpisheh, V.; Nikkhoo, A.; Hojjat-Farsangi, M.; Namdar, A.; Azizi, G.; Ghalamfarsa, G.; Sabz, G.; Yousefi, M.; Yousefi, B.; Jadidi-Niaragh, F. Prostaglandin E2 as a potent therapeutic target for treatment of colon cancer. *Prostaglandins Other Lipid Mediat.* **2019**, *144*, 106338. [[CrossRef](#)]
55. Cai, S.; Gao, Z. Atorvastatin inhibits proliferation and promotes apoptosis of colon cancer cells via COX-2/PGE2/ β -Catenin Pathway. *J. Buon* **2021**, *26*, 1219–1225.

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