High-mobility group A1 (*HMGA1*) gene expressions in various colorectal cancer cell lines and correlation with prognosis

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Background: The high-mobility group A1 gene (HMGA1) plays a major role in the development of malignant cancers. However, the mechanisms underlying the correlation between HMGA1 expression level and patients' overall survival rate in various malignant cancers is unclear.

Methods: We used The Cancer Genome Atlas (TCGA) database (https://genome-cancer.ucsc.edu/) to search for mRNA expression levels of *HMGA1* in tumor patients and grouped them by receiver operating characteristic (ROC) curve. This divided patients into a high expression cohort and low expression cohort, and Kaplan-Meier analysis revealed the overall survival of the cancer patients. We also used real-time quantitative PCR (qPCR) to detect the expression of *HMGA1*, *CBX*7, *E-cadherin*, and *β-catenin* gene was detected by normalized to the expression of β-actin in colorectal cancer cell lines.

Results: High expression group correlated with worse survival prognosis statistically significant ($P<0.05$), and scatter plots showed *HMGA1* high expression in the different cancers (lung cancers; lung adenocarcinoma and lung squamous cell carcinoma; stomach and colorectal cancers; liver and pancreatic cancer; kidney papillary cell carcinoma; kidney clear cell carcinoma, brain lower grade glioma; adrenocortical cancer; acute myeloid leukemia; and sarcoma; head and neck squamous cell carcinoma, cholangio and bladder urothelial cancers). Further, we also found that the mRNA expressions of *HMGA1*, *CBX*7, *E-cadherin*, and *β-catenin* genes significantly in colorectal cancer cell lines (P value: 0.0005), consistent with the results of HMGA1 in TCGA database.

Conclusions: *HMGA1* is highly expressed in various cancers than normal tissues, and high expression levels of HMGA1 correlated with a worse prognosis. The gene expressions and the TCGA data clearly supports that targeting *HMGA1* in the management of cancers increases the survival rate of cancer patients.

Keywords: HMGA1; The Cancer Genome Atlas (TCGA); receiver operating characteristic (ROC); cancer; prognosis

Introduction

The high-mobility group A protein family is characterized by small nuclear proteins with elevated mobility. The HMGA family has four members, including three HMGA1 proteins from alternative splicing, which are HMGA1a, HMGA1b, and HMGA1c, with the fourth protein member being HMGA2. HMGA1 isoforms are located on chromosome 6p21, whereas HMGA2 is transcribed by another gene on chromosome 12q15 (1,2). HMGA1 lacks self-transcriptional activity; thus, it mainly regulates chromatin structures and promotes the interaction between the transcriptional regulatory proteins and downstream DNA, which contains three AT richer domains, known as "AT-hook," and is also known as an architectural transcription factor.

Previous studies proved that HMGA1 not only promotes tumorigenesis but also enhances the malignant progression of different types of cancer (lung cancer, stomach adenocarcinoma, colorectal cancer, hepatocellular carcinoma, pancreatic adenocarcinoma, kidney carcinoma, bladder urothelial carcinoma, head, and neck squamous cell carcinoma). It was reported that HMGA1 has an important role in promoting thyroid cancer through inhibition of p53 and the induction of TGF-β1 (3), moreover, TGF-β1 induces HMGA1 expression (4). HMGA1 also promotes colorectal cancer development, primarily through transcriptional regulation via the Wnt signaling pathway (5,6), miR-137, and miR-214 (7,8). The rise of metabolomics in recent years has also shown that HMGA1 can increase glucose uptake, promote aerobic glycolysis (9), and promote the development of colorectal cancer (10,11). IL-24 modulates the high mobility group (HMG) A1/miR222/AKT signaling in lung cancer cells (12).

In this study, we aimed to investigate the oncogenic role of HMGA1 in various cancers by analyzing the clinical overall ten-year survival rate and expression level of HMGA1 in the following cancers: head and neck squamous cell carcinoma, lung cancer, stomach and colorectal cancer, liver and pancreatic cancer, cholangiocarcinoma, kidney cancer, and bladder urothelial carcinoma. Our results suggest that HMGA1 has a role in various cancers, and thus targeting this protein family will have beneficial impacts on the survival rate of the cancer patients.

Methods

Cell culture

We obtained DLD1, HCT116, HCT8, LOVO, HT29,

SW480, SW620, and RKO cell lines from the American Type Culture Collection, and these were maintained using Dulbecco's Modified Eagle Medium (DMEM + 10% DMEM medium + 10% fetal bovine serum + 1% penicillin and streptomycin) (GIBCO) at 37 ℃ in a 50 mL/L CO_2 atmosphere.

Public dataset analysis

The cancer expression levels of profiling studies that included matched clinical information were acquired from The Cancer Genome Atlas database. The datasets were classified into two groups: expression datasets in tumor tissues and normal matched adjacent tissues. Using receiver operating characteristic (ROC) curve analysis, combined with sensitivity and specificity chosen as a cutoff point, the expression of *HMGA1* in tumor patients was used to create two groups: the lower expression group and the higher expression group. Based on the overall survival time of patients derived from the clinical dataset, we analyzed the difference between the lower and higher *HMGA1* expression groups and compared the 10-year overall survival rate by Kaplan-Meier analysis.

mRNA extraction and q-PCR

We used real-time quantitative PCR (QPCR) to analyze the expression of the *HMGA1*, *CBX7*, *E-cadherin*, and *β-catenin* genes (normalized to the expression of β-actin). Total RNAs were extracted using TRIzol (TIANGEN) according to the manufacturer's instruction. Total RNA (2 µg) was reverse transcribed using Prime Script RT reagent kit (TaKaRa). The reverse transcription step was 37 ℃ for 15 min and 95 ℃ for 5 min. QPCR was performed using SYBR green (Bimake) and the ABI Step One Plus real-time PCR system (Applied Biosystems). We mixed the SYBR Green PCR Master Mix 10 µL with primers 200 nM, cDNA template 1 µL, and deionized water with up to 20 µL of volume. The steps of PCR were 95 ℃ for 3 min for denaturation, 95 ℃ for 15 s for annealing, and 60 ℃ for 30 s for the extension, for 40 cycles. The primer sets used to amplify the gene expression in the study are shown in *Table 1*.

Statistical analysis

We used the Statistical Package for the Social Sciences (SPSS version 20.0; IBM New York, NY, USA) for statistical

Table 1 Forward and reverse primer sets of *β-actin*, *HMGA1*, *E-cadherin*, *CBX7*, and *β-catenin* respectively used in the study

Gene	Primers
β-actin	F: CACAGAGCCTCGCCTTTGCC
	R: ACCCATGCCCACCATCACG
HMGA1	F: GCTGGTAGGGAGTCAGAAGGA
	R: CGAAAGGCCTTCAACTGCAAAT
E-cadherin	F: CGAAAGGCCTTCAACTGCAAAT
	R: ACTGGTACTTCTTGACATCTG
CBX7	F: GCGTGCGGAAGGGTAAAGT
	R: GCTTGGGTTTCGGACCTCTC
β-catenin	F: AAAGCGGCTGTTAGTCACTGG
	R: CGAGTCATTGCATACTGTCCAT

analysis, and P value <0.05 was considered statistically significant.

Results

HMGA1 expression level up-regulated in lung cancer (Figure 1)

In the TCGA database, we observed two subtypes of lung cancers: lung adenocarcinoma and lung squamous cell carcinoma. *HMGA1* was more highly expressed in cancers than in normal adjacent tissues (*Figure 1B,D*), and its higher expression level predicted worse clinical prognosis (*Figure 1A,C*), with statistical significance (P<0.05). Surprisingly, we found *HMGA1* to be one of the biomarkers in lung cancer, not only because of its elevated expression
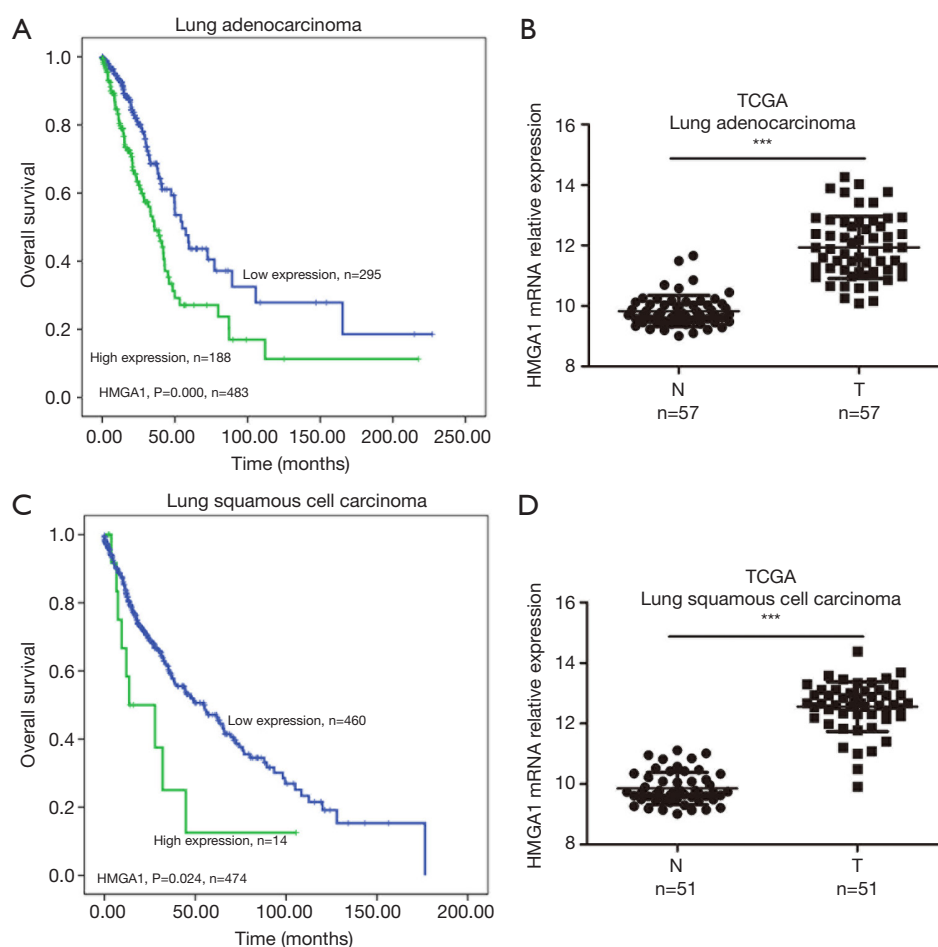

Figure 1 High expression of *HMGA1* in lung cancer correlated with worse overall survival. (A) Kaplan-Meier analysis of *HMGA1* expression level in lung adenocarcinoma patients; (B) Scatter plot of *HMGA1* in matched normal and lung adenocarcinoma tissues; (C) Kaplan-Meier analysis of *HMGA1* expression level in lung squamous cell carcinoma patients; (D) Scatter plot of *HMGA1* in matched normal and lung squamous cell carcinoma tissues. ***, P<0.001.
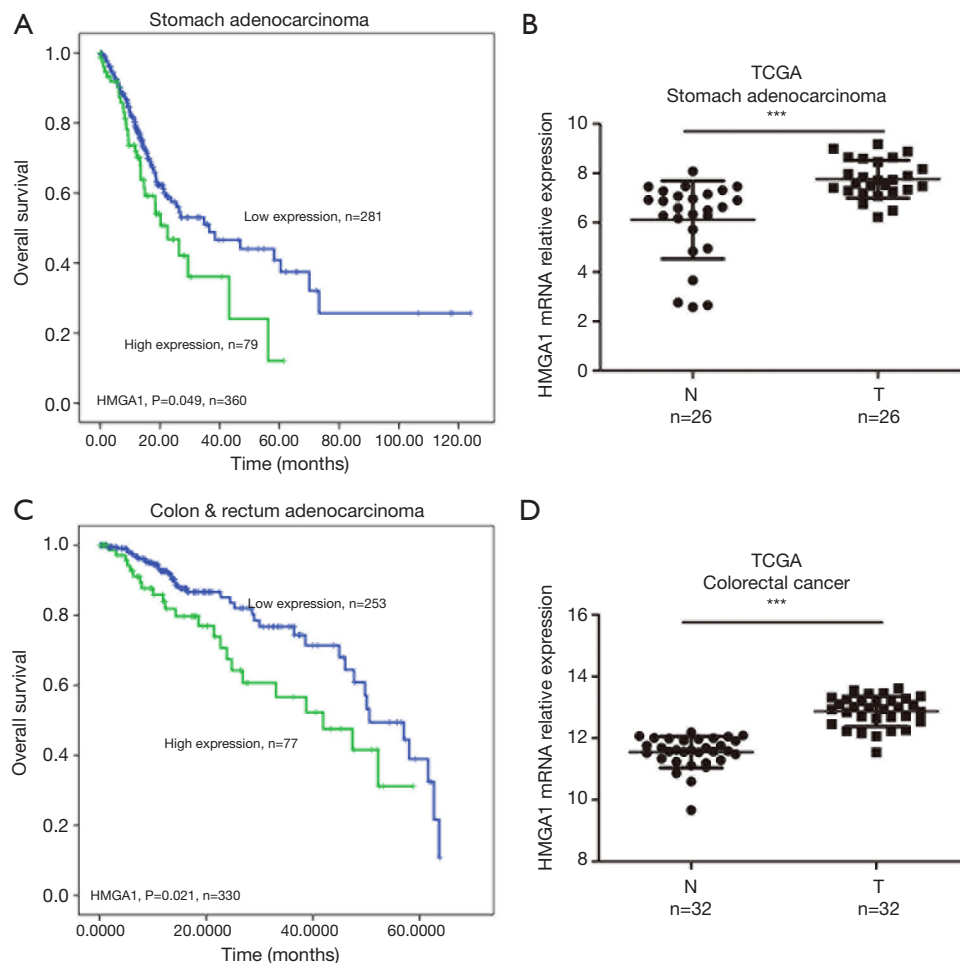
Figure 2 High expression of *HMGA1* in stomach and colorectal cancer correlated with worse overall survival. (A) Kaplan-Meier analysis of HMGA1 expression level in stomach adenocarcinoma patients; (B) Scatter plot of *HMGA1* in matched normal and stomach adenocarcinoma tissues; (C) Kaplan-Meier analysis of *HMGA1* expression level in colon and rectum adenocarcinoma patients; (D) Scatter plot of HMGA1 in matched normal and colorectal cancer tissues. ***, P<0.001.

but also because it was an indicator of the worse overall clinical survival.

HMGA1 expression level up-regulated in the stomach and colorectal cancer (Figure 2)

TCGA database showed similar results for the stomach and colorectal cancers. We found higher expression levels of *HMGA1* in tumor tissues compared to adjacent normal tissues (*Figure 2B,D*), and the higher expression group had a significantly worse prognosis for 10-year survival compared to the lower expression group (*Figure 2A,C*) (P<0.05). A high expression level of *HMGA1* was significant and correlated with worse prognosis in the stomach and

colorectal cancers.

High expression levels of HMGA1 in the liver and pancreatic cancer (Figure 3)

We found the liver and pancreatic cancer patients with elevated levels (prognostic values by Kaplan-Meier analysis) of *HMGA1* had a worse prognosis for survival compared to the lower *HMGA1* level patients with the same cancers (*Figure 3A,C*). We also analyzed the *HMGA1* expressions in normal control tissues and compared them with liver and pancreatic cancer tissues (*Figure 3B,D*). The clinical data showed a significant (P<0.05) correlation with *HMGA1* expression in cancer (liver and pancreatic) development and prognosis.
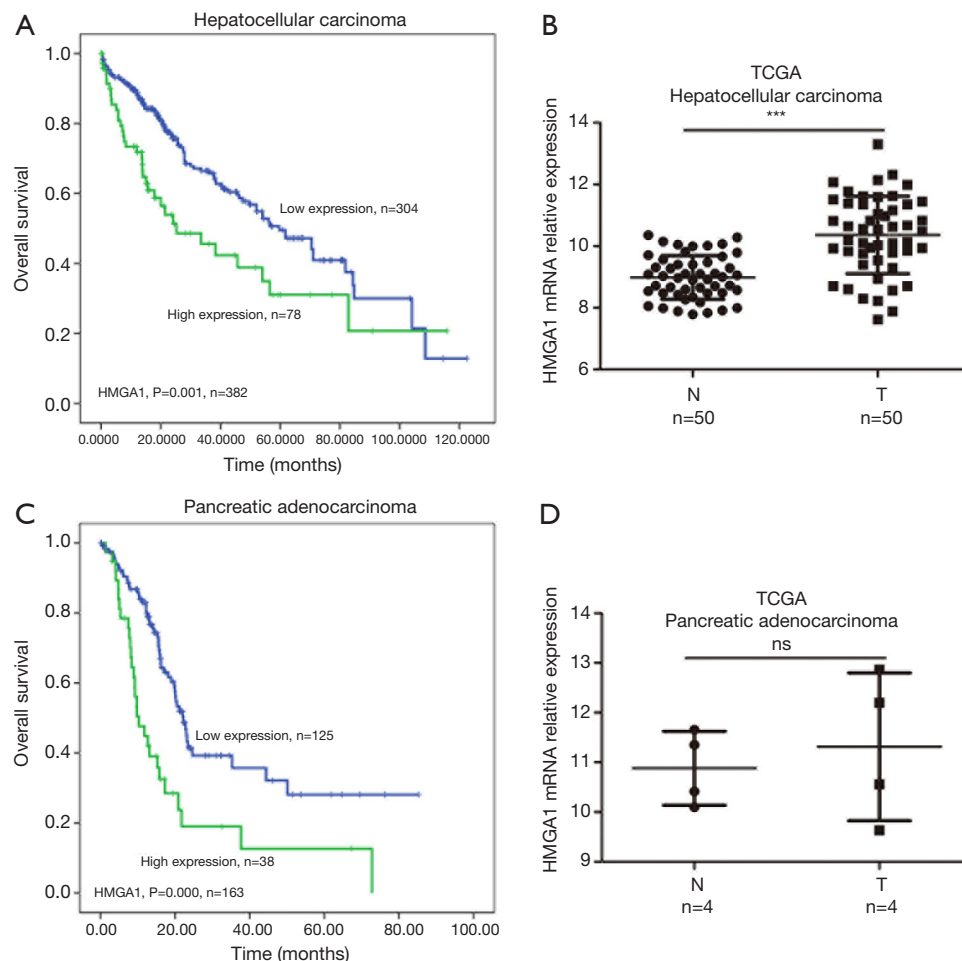
Figure 3 High expression of *HMGA1* in liver and pancreatic cancer correlated with worse overall survival. (A) Kaplan-Meier analysis of *HMGA1* expression level in hepatocellular carcinoma patients; (B) Scatter plot of *HMGA1* in matched normal and hepatocellular carcinoma tissues; (C) Kaplan-Meier analysis of HMGA1 expression level in pancreatic adenocarcinoma patients; (D) Scatter plot of *HMGA1* in matched normal and pancreatic adenocarcinoma tissues. ***, P<0.001.

High expression levels of HMGA1 in kidney cancer (Figure 4)

We analyzed the *HMGA1* expression level in kidney papillary cell carcinoma and kidney clear cell carcinoma. We found that *HMGA1* expression level correlated with the clinical overall survival time of patients. The data showed that the lower expression *HMGA1* group had a significantly higher (P<0.05) ten-year survival rate than the high expression group (*Figure 4A,C*). We also analyzed the expression level of *HMGA1* in kidney tumor tissues and matched normal tissues; the results revealed that *HMGA1* had a higher expression (significantly, P<0.05) in tumor tissue than in normal tissues (*Figure 4B,D*). These findings

indicate that *HMGA1* also serves as a biomarker and can predict clinical overall survival time in kidney cancers.

High expression levels of HMGA1 in head and neck squamous cells, cholangio, and bladder urothelial cancers were correlated with worse prognosis

The mRNA values of *HMGA1* expression levels of head and neck squamous cell carcinoma, cholangiocarcinoma, and urothelial bladder carcinoma showed similar results as the above. In head and neck squamous cell carcinoma, we found a higher expression level of *HMGA1* in tumor tissues compared to normal adjacent tissues. The overall survival time indicated that the higher expression *HMGA1*
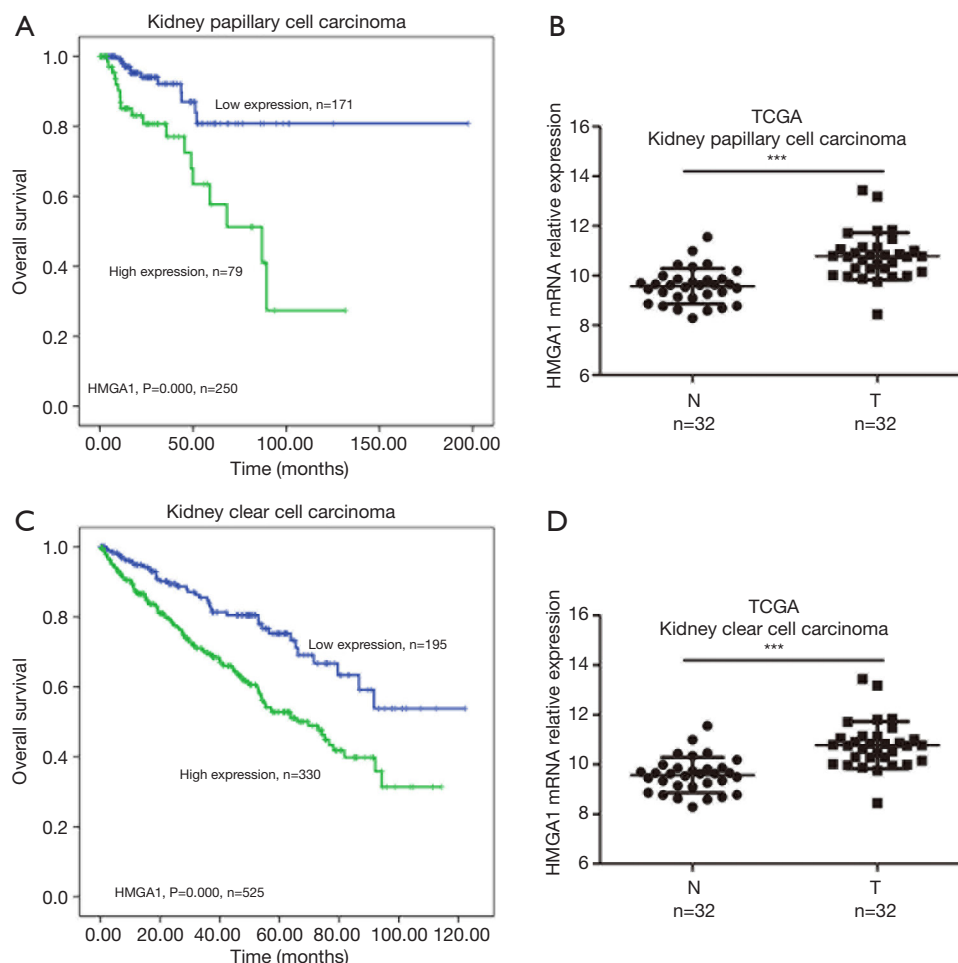
Figure 4 High expression of *HMGA1* in kidney correlated with worse overall survival. (A) Kaplan-Meier analysis of *HMGA1* expression level in kidney papillary cell carcinoma patients; (B) Scatter plot of *HMGA1* in matched normal and kidney papillary cell carcinoma tissues; (C) Kaplan-Meier analysis of *HMGA1* expression level in kidney clear cell carcinoma patients; (D) Scatter plot of *HMGA1* in matched normal and kidney clear cell carcinoma tissues. ***, P<0.001.

group correlated with shorter survival time (*Figure 5A,B*). In cholangiocarcinoma, we observed the similar to the above expression level in normal tissues and tumor tissues. The higher expression group of *HMGA1* showed worse clinical survival time (*Figure 5C,D*). In urothelial bladder carcinoma, we found similar results as the above: there was a higher expression level in tumors, which indicated a worse overall clinical survival time (P<0.05) (*Figure 5E,F*).

The HMGA1 expression level was correlated with worse overall survival in the following cancers

We found TCGA clinical data related to *HMGA1* in different cancers: lower-grade glioma of the brain (*Figure 6A*),

adrenocortical cancer (*Figure 6B*), acute myeloid leukemia (*Figure 6C*), and sarcoma (*Figure 6D*). The analysis of these results differentiated *HMGA1* expression level of tumor patients into high and low groups, and the overall survival of the high expression group was significantly lower (P<0.05) compared to the low expression group.

mRNA expression of HMGA1 in different clinical cancer cell lines

The *HMGA1* gene is overexpressed in cancer, with higher levels indicating poor prognosis across various tumor types. To further verify that *HMGA1* is a tumor marker, we selected eight strains of colorectal cancer cell lines (DLD1,
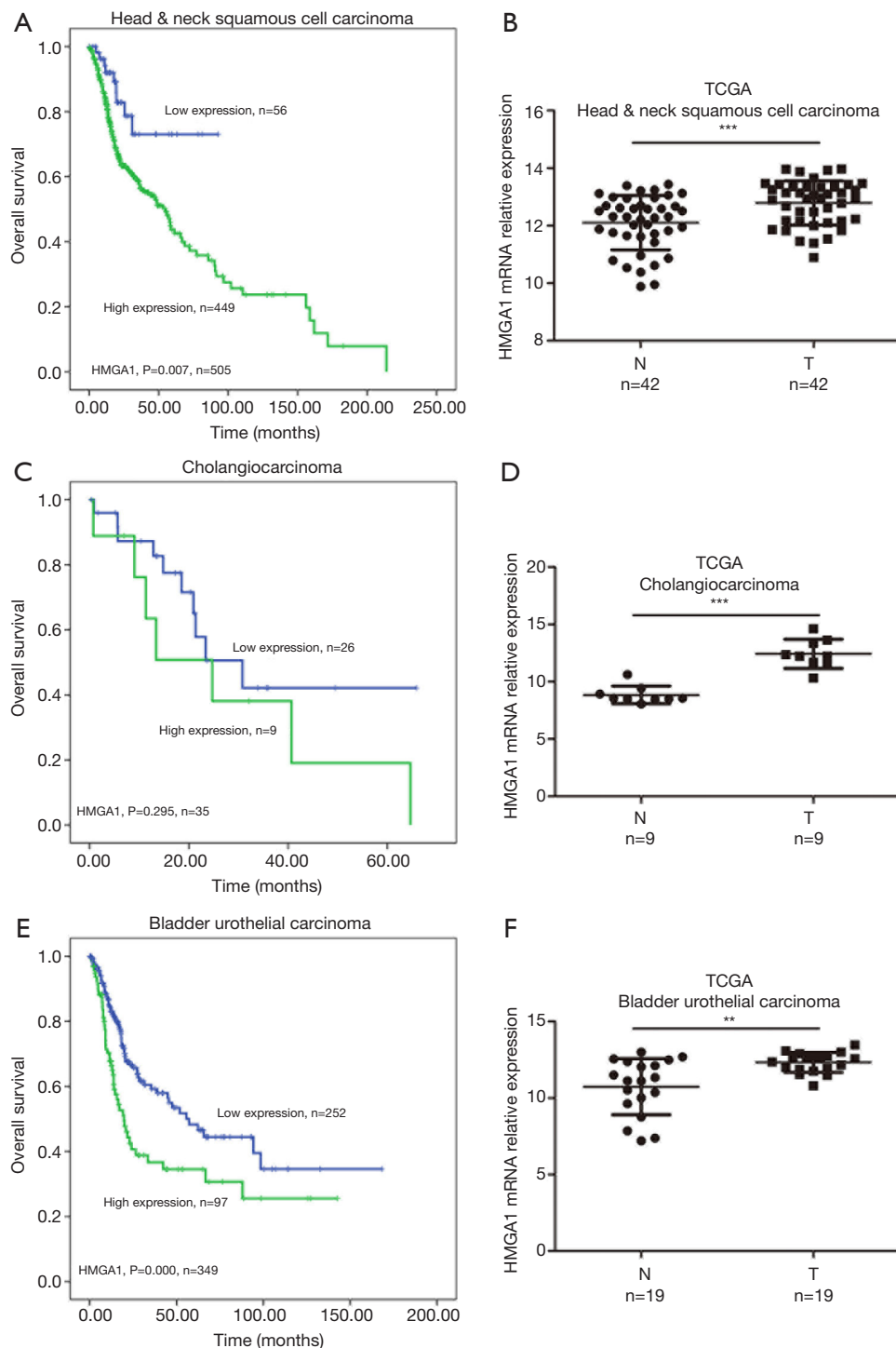
Figure 5 High expression of *HMGA1* in head and neck squamous cell carcinoma, cholangiocarcinoma, and bladder urothelial carcinoma correlated with worse overall survival. (A) Kaplan-Meier analysis of *HMGA1* expression level in head and neck squamous cell carcinoma patients; (B) Scatter plot of *HMGA1* in matched normal and head and neck squamous cell carcinoma tissues; (C) Kaplan-Meier analysis of *HMGA1* expression level in cholangiocarcinoma patients; (D) Scatter plot of *HMGA1* in matched normal and cholangiocarcinoma tissues; (E) Kaplan-Meier analysis of *HMGA1* expression level in bladder urothelial carcinoma patients; (F) Scatter plot of *HMGA1* in matched normal and bladder urothelial carcinoma tissues. **, $P<0.01$; ***, $P<0.001$.
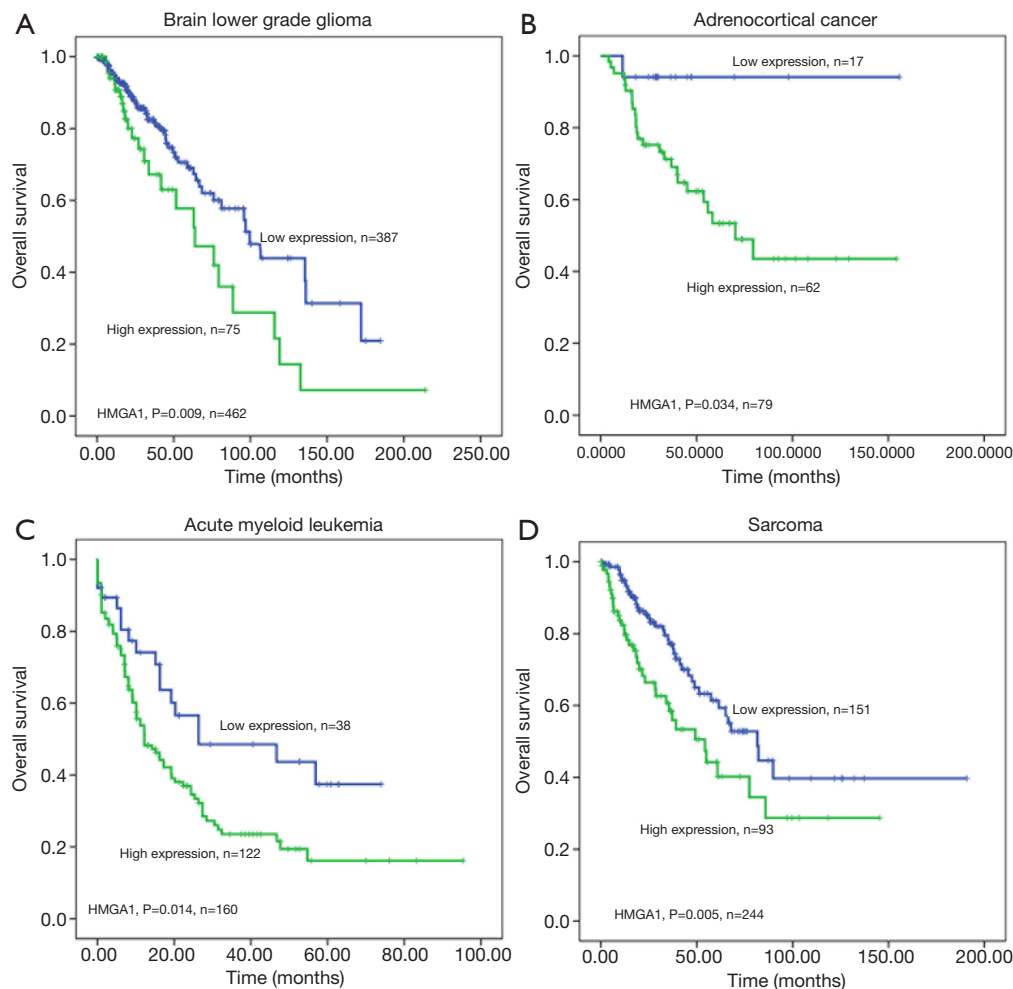
Figure 6 HMGA1 expression level correlated with worse prognosis in other cancer types. (A) Kaplan-Meier analysis of *HMGA1* expression level in lower grade glioma of the brain; (B) Kaplan-Meier analysis of *HMGA1* expression level in adrenocortical cancer; (C) Kaplan-Meier analysis of *HMGA1* expression level in acute myeloid leukemia; (D) Kaplan-Meier analysis of *HMGA1* expression level in sarcoma patients.

HCT116, HCT8, LOVO, HT29, SW480, SW620, and RKO), a normal human colon mucosal epithelial cell line NCM460 and mRNA expression levels of four genes (*HMGA1*, *E-cadherin*, *CBX*7, and *β-catenin*) using q-PCR. A large number of studies have reported a low expression of *CBX*7 and *E-cadherin* in tumor cells, while in colorectal cancer, *β-catenin* is highly expressed. We found that in the eight strains of colorectal cancer tumor cell lines, the mRNA level of *HMAG1* was significantly higher than that of *CBX*7 and *E-cadherin*, and the expression level was similar to that of *β-catenin*. Although the mRNA level of *HMGA1* and *β-catenin* were higher than that of *CBX*7 and *E-cadherin* in NCM460, the relative mRNA level was much lower than that of eight strains of colorectal cancer cell lines. These results show strongly suggest that the level of HMGA1 expression in colorectal cancer is elevated, and HMGA1 is an effective tumor marker (*Figure 7*).

Discussion

HMGA1 is an oncoprotein that is involved in tumorigenesis and malignant tumor progression. As an oncogene, HMGA1 is upregulated in many different cancers. Huang *et al.* [2015] revealed that overexpression of HMGA1 correlates with the malignant status and prognosis of breast cancer (13). HMGA1 promotes gastric cancer oncogenic and glycolytic phenotypes by regulating c-myc expression (14). Cheng *et al.* [2019] demonstrated
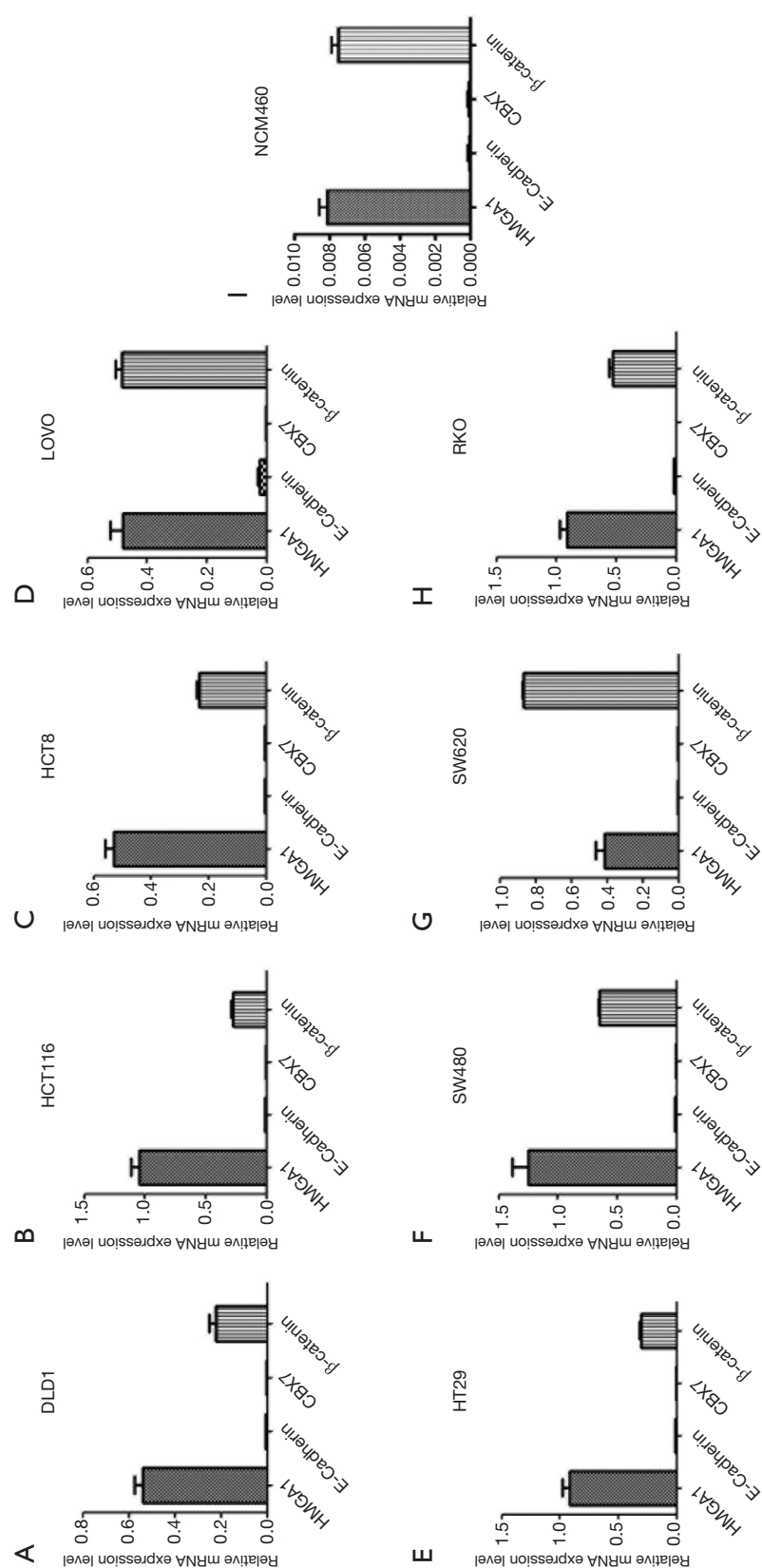
Figure 7 HMGA1 mRNA expression in different cancer cell lines. We used qPCR to analyze the gene expression and found higher expression levels of *HMGA1* and *β-catenin* compared to *E-cadherin* and *CBX7* respectively. (A,B,C,D,E,F,G,H,I) DLD1, HCT116, HCT8, LOVO, HT29, SW480, SW620, RKO and NCM460 cell mRNA was extracted using Trizol and used to analyze the gene expressions of *HMGA1*, *E-cadherin*, *CBX7* and *β-catenin*.

that HMGA1 exacerbates tumor progression by activating miR-222 through PI3K/Akt/MMP-9 signaling pathway in uveal melanoma (15). In the present study, by analyzing data from the TCGA database, we revealed that HMGA1 is an oncogene in malignant cancers and is highly expressed in malignant cancer patients. The patients with a high expression of HMGA1 showed a shorter overall survival rate compared to lower expression patients in different types of cancers. Recent studies have shown that HMGA1 knockdown or mutation will increase the efficacy of gefitinib in lung cancer cells (16). D'Angelo *et al.* [2014] reported that HMGA1 proteins lead to chemo-resistance against cetuximab and 5-fluorouracil in colon and thyroid cancer (17). These findings and the present study's results suggest that the targeting of the HMGA1 protein and a deeper understanding of the underlying mechanisms in tumorigenesis and advanced progression will help in the treatment and management of different types of malignant cancers. Further studies are needed to characterize HMGA1 coordination with downstream functional pathways and correlation with functional proteins.

Conclusions

HMGA1 is an oncofetal gene involved in tumorigenesis and malignant progression. In our study, we revealed that *HMGA1* was highly expressed in different cancers, and the high expression level of *HMGA1* correlates with worse clinical prognosis. Furthermore, we found a high expression of *HMGA1* in lung cancers. With molecular precision treatment becoming the direction of future clinical cancer treatment, our results and the TCGA data suggest that HMGA1-targeted precision therapy can reduce the occurrence of malignant progression of tumors and benefit the survival rate of the patients.

Acknowledgments

Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2019.12.10). The authors have no conflicts

of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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