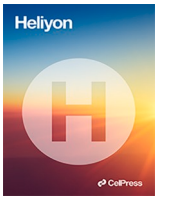
Heliyon 10 (2024) e24458

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Role and molecular mechanism of APOBEC3B in the development and progression of gastric cancer

Nana Su [a,1], Erle Zhou [a,1], Min Cui [b], Hong Li [a], Shuhua Wu [a], Qian Zhang [a,**], Zhang Cao [a,*]

[a] Department of Pathology, Binzhou Medical University Hospital, Binzhou, 256603, China
[b] Department of Pediatrics, Binzhou City People's Hospital, Binzhou, 256600, China

ARTICLE INFO

Keywords:
APOBEC3B
Cyclin D1
Gastric cancer
MCM2
P53

ABSTRACT

Gastric cancer is a common malignant tumor with a high mortality rate. Abnormal APOBEC3B (apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like 3B) expression increases tumor susceptibility. However, the exact molecular mechanism of APOBEC3B expression in the development of gastric cancer is still unknown. We investigated the effect of APOBEC3B on the malignant biological behavior of gastric cancer cells and discussed the role of APOBEC3B in the development and progression of gastric cancer. APOBEC3B protein levels were measured in 161 gastric cancer samples using western blotting and immunohistochemistry. Both *in vitro* and *in vivo* assays were performed, and molecules were analyzed using bioinformatics analysis and western blotting. APOBEC3B was overexpressed in gastric cancer. Moreover, APOBEC3B significantly enhanced cell proliferation *in vitro* and tumorigenicity *in vivo*. Regarding the underlying mechanism, APOBEC3B promoted the proliferation of gastric cancer cells by upregulating P53, MCM2 (minichromosome maintenance protein 2), and cyclin D1. Our results suggest that APOBEC3B is involved in cancer progression, providing a new theoretical basis for the prevention and treatment of gastric cancer.

1. Introduction

Gastric cancer (GC) is a common malignant tumor with a high mortality rate. Moreover, GC has the fifth-highest incidence rate among malignant solid tumors worldwide. Although early detection and prevention have reduced the incidence and mortality of GC, it remains among the major obstacles limiting human life expectancy in the 21st century [1,2]. Therefore, it is important to elucidate the mechanisms underlying tumor progression to identify novel biomarkers and effective therapeutic targets for patients with GC.

The apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like (APOBEC) family is a group of proteins that alter DNA through deamination, affect genomic stability, and increase tumor susceptibility [3]. The APOBEC protein family comprises the following 11 members: A1, Activation Induced Deaminase (AID), APOBEC2, APOBEC3A, APOBEC3B, APOBEC3C, APOBEC3D, APOBEC3E, APOBEC3F, APOBEC3G, APOBEC3H, and APOBEC4 [4,5]. Among them, APOBEC3B is actively expressed in breast cancer, glioma, melanoma, and other cancer cell lines and is the main candidate for solid tumor mutagenesis [6–8]. Mao et al. [6] reported that

* Corresponding author.
** Corresponding author.
E-mail addresses: zhangqianbl@163.com (Q. Zhang), caoning1997@163.com (Z. Cao).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.heliyon.2024.e24458
Received 13 October 2023; Received in revised form 9 January 2024; Accepted 9 January 2024
Available online 17 January 2024

APOBEC3B expression is associated with an adverse prognosis in breast cancer. In hepatocellular carcinoma cells, APOBEC3B expression can directly stimulate multiple binding sites in the NF-κB signaling pathway and promote cell proliferation [9]. Fan et al. [10] reported that APOBEC3B knockdown significantly inhibited the migration and invasion of cervical cancer cells. Further, APOBEC3B may be involved in the development and progression of various cancers [11–13].

P53 is a crucial tumor suppressor gene that regulates cell biological behavior under stressful conditions by inducing cell apoptosis and DNA metabolism and repair [14,15]. Studies [16,17] have revealed that >50 % of cancers are associated with P53 mutations, highlighting the significant role these mutations play in tumor development. Burns et al. [18] demonstrated an upregulation of APOBEC3B expression in breast cancer, leading to an increased occurrence of P53 mutations. Similarly, Wang et al. [19] observed a positive correlation between APOBEC3B expression and P53 mutations in non-small cell lung cancer.

The minichromosome maintenance protein (MCM) family represents a highly conserved group of DNA-unwinding proteins [20,21]. Precise regulation of DNA replication is crucial for preserving genomic stability and cell cycle progression [22]. These investigations propose that MCM2 serves as a potential marker for cell proliferation, with increased levels indicating malignant cell growth. Cyclin D1, an important regulator of the cell cycle, plays a crucial role in the pathogenesis of cancer by determining uncontrolled cell proliferation [23]. Research has confirmed that cyclin D1 is an oncogene that drives the development of solid tumors and hemopathies. Studies have shown that upregulation of MCM7, a member of the MCM protein family, can promote the occurrence and development of liver cancer. Researchers [24] have found a positive correlation between MCM7 and cyclin D1 expression in mouse models and human tumor tissues, indicating that patients with liver cancer and high levels of MCM7 have a poor prognosis.

Abnormal APOBEC3B, P53, MCM2, and cyclin D1 expression increases tumor susceptibility. However, the mechanisms underlying their actions remain unclear. Therefore, we investigated the effect of these factors on the malignant biological behavior of GC cells and discussed their role in the development and progression of GC. Our findings provide a novel therapeutic target for GC.

2. Materials and methods

2.1. Patients and tissue samples

A total of 161 GC specimens with complete clinicopathological data were collected from the Department of Pathology at Binzhou Medical University Hospital between April 2018 and December 2019. Among them, we obtained fresh cancer and adjacent tissues from 10 patients with GC. Of the 161 specimens, 59 were obtained from women and 102 from men (median age: 53 [range, 27–78] years). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. The study design was approved by the Ethics Committee of Binzhou Medical University Hospital (approval number: 2016–26). None of the patients had undergone preoperative radiotherapy, chemotherapy, targeted therapy, or other anticancer therapies. All patients provided informed consent.

2.2. Cell culture

Human GC cell lines (SGC7901 and AGS), as well as the human normal gastric mucosa cell line (GES-1), were preserved by the Central Laboratory of Binzhou Medical University Hospital. GES-1 cells were cultured in high glucose DMEM supplemented with 10 % FBS. AGS and SGC7901 cells were maintained in RPMI 1640 medium supplemented with 10 % FBS. The cells were cultured and passed 10 times in a cell incubator at 37 °C and 5 % CO_2, and subsequent experiments were carried out.

2.3. Bioinformatics analysis

2.3.1. Correlation analysis between APOBEC3B expression and P53 mutations

The cBioPortal (http://cbioportal.org/) [25] is an open platform for exploring multidimensional cancer genomics data. From the cBioPortal database, we selected the "stomach adenocarcinoma (TCGA, PanCancer Atlas)" dataset. Subsequently, after entering "P53," we analyzed the *P53* mutation status in GC tissues in the OncoPrint interface. Using the UALCAN database (http://ualcan.path.uab.edu/index.html) [26], "TCGA gene analysis," we input "APOBEC3B″ and selected the function interface TCGA dataset, "stomach." APOBEC3B expression according to *P53* mutation status was analyzed in the correlation analysis interface of expression status.

2.3.2. Genetic screening

The GEPIA database [27] can be used for cancer and normal gene expression profiling and interaction analysis. Using the correlation analysis function of the GEPIA database, related genes were input into "Gene A″ and "Gene B," respectively. "STAD tumor," "STAD normal," and "stomach" were added to the expression dataset for data acquisition and analysis.

2.4. Transfection

This experiment was divided into four groups—Blank, blank; plasmid containing the APOBEC3B gene, APOBEC3B; empty plasmids, vector; and APOBEC3B knockdown, si-APOBEC3B. Based on the *APOBEC3B* gene sequence in GenBank (NM004900; https://ncbi.nlm.nih.gov/nuccore), the plasmid containing the APOBEC3B gene and empty vector plasmids were constructed by Shanghai Jikai Co., Ltd. Plasmids were transfected into GC cell lines using Lipofectamine 2000 reagent (Thermo Fisher Scientific Inc.), following

the manufacturer's instructions, for 2 days at 37 °C. After transfection for 48 h, the infected cells were cultured with neomycin for 30 days at 37 °C to yield stably infected cells for mouse xenograft studies. For APOBEC3B knockdown, GC cells were transfected with APOBEC3B siRNA (Ruibo Biotechnology) using Lipofectamine 2000 reagent (Thermo Fisher Scientific Inc.), following the manufacturer's instructions, for 2 days at 37 °C. The following sequences were used: genOFFTM st-h-APOBEC3B-001, AGACCTACTTGTGCTATGA; genOFFTM st-h-APOBEC3B-002, CAGTACCACGCAGAAATGT; and genOFFTM st-h-APOBEC3B-003, TGGGCTTTCTATGCAACGA. Transfected cells were collected for subsequent experiments.

2.5. MTT assay

Cells with good growth after transfection were seeded into 96-well plates (3000 cells/well, 6 wells/group) and incubated for 24 h at 37 °C. Subsequently, MTT reagent was added to the culture medium at a final concentration of 0.1 mg/mL. Next, 150 μL dimethyl sulfoxide was added to the cells to dissolve the formazan crystals. Optical density was measured at 490 nm using a SpectraMax M2 microplate reader (Molecular Devices, Shanghai, China).

2.6. Colony formation assay

After transfection, the cells were seeded in 100-mm Petri dishes (2000 cells/dish). The medium was replenished every 3 days, and the cells were allowed to grow for 9 days to form colonies. The colonies were fixed in 4 % paraformaldehyde (Biosharp) and stained with 0.1 % crystal violet (Solarbio). The number of colonies with >50 cells was counted.

2.7. Wound-healing assay

Three equally spaced horizontal lines were drawn on the back of a 6-well plate, using a sterilized marker, followed by the inoculation of cells from each group. When the degree of cell fusion reached 90 %, the culture medium was removed and washed with PBS. A 10-μL spearhead was used to scratch the cells perpendicularly to the horizontal line. The migration rate was calculated as follows: Migration rate = (1 − average number of migrating cells in the experimental group/average number of migrating cells in the control group) × 100 %.

2.8. Transwell assay

Matrix glue was added to the upper Transwell® chamber. The corresponding cell suspension was added after hydration and solidification. Culture medium was added to the lower Transwell® chamber, and the cells were cultured for 24 h. Next, 4 % paraformaldehyde was added, and the cells were fixed for 1 h. Subsequently, the cells were washed with PBS and stained with crystal violet for 20 min.

2.9. Western blot assay

Total proteins were extracted from cells and GC tissues using radioimmunoprecipitation assay lysis buffer containing protease inhibitors. Approximately 20 μg total protein from each group was resolved via 12 % sodium dodecyl sulfate–polyacrylamide gel electrophoresis (80 V). The duration of electrophoresis was determined based on molecular weight. Subsequently, the resolved protein bands were transferred onto a polyvinylidene difluoride membrane (Millipore, Bedford, MA, USA), which was blocked with 5 % skimmed milk powder for 2 h at 37 °C and then incubated with primary antibodies against APOBEC3B (1:1,000, Abcam); P53 (1:1,000, Abcam); MCM2 (1:1,000, Abcam); cyclin D1 (1:1,000, Beyotime); and GAPDH (1:1,000, Goodhere) overnight at 4 °C. This was followed by incubation with horseradish peroxidase-conjugated secondary antibodies (1:2,000, ZSGB-BIO) at 37 °C for 2 h. GAPDH was used as an internal control for determining relative protein expression. Protein bands were quantified using ImageJ software (NIH).

2.10. Immunohistochemistry

Immunohistochemical staining was performed using the Max Vision method, DAB color kit, and Max Vision TM kit (Solarbio). APOBEC3B and P53 antibodies were used at dilutions of 1:250 and 1:50, respectively. After conventional dewaxing and hydration, subsequent steps were performed, according to the manufacturer's instructions, for the test agent box, DAB color rendering, hematoxylin redyeing, and dehydrated medium gum sealing.

2.11. Scoring

Two pathologists interpreted 10 high-power microscopic fields, each containing >100 cells. GC cells expressed APOBEC3B in the nucleus and cytoplasm. The staining results were reported as the staining ratio and intensity. The staining ratio was scored as follows: 0 points, 0–10 %; 1 point, 10–25 %; 2 points, 26–50 %; 3 points, 51–75 %; and 4 points, >75 %. The staining intensity was scored as follows: 0 points, no staining; 1 point, weak staining; 2 points, moderate staining; 3 points, strong staining. The sum of the staining ratio and intensity was classified as follows: 0–3 points, negative; 4–7 points, positive [28]. P53 expression in GC cells was localized in the nucleus; when >10 % of nuclear-stained GC cells were included in the section, the tumor was considered P53 positive [29]. The

percentage and intensity of cell staining were assessed in a double-blinded manner.

2.12. H&E staining

Paraffin-embedded blocks of GC tissues were cut into 3-μm-thick sections. Dehydrated medium gum sealing was performed, following the H&E staining instructions.

2.13. Mouse xenografts of GC cells

Thirty-six male BALB/c nude mice (weight, 5–12 g; age, 4–6 weeks) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. All animal experiments were approved by the Ethics Committee of Binzhou Medical University Hospital and in accordance with the NIH's Guide for the Care and Use of Laboratory Animals. All mice were fed with common feed and sterile water *ad libitum* and were housed at 25 °C, with 55 % humidity and a 12-h light/dark cycle. The experiment was performed after 1 week of observation.

Each group of cells (2×10^6) was subcutaneously injected into the flanks of nude mice to establish the xenograft model. The length and width of the tumors were measured at 5-day intervals using Vernier calipers. Approximately 20 days after inoculation, the mice were euthanized via cervical dislocation. The mouse weight, tumor weight, and tumor size were recorded. The tumor was divided into two parts: one part was fixed in formalin; the other was stored at −80 °C for subsequent experiments.

2.14. Statistical analysis

Variables are presented as mean ± SD. Between- and among-group comparisons were performed using Student's *t*-test and one-way ANOVA, respectively. Spearman's rank correlation analysis was performed to determine correlations among groups. Statistical significance was set at $P < 0.05$. All experiments were performed in triplicate.

3. Results

3.1. APOBEC3B expression in GC tissues and cells

We observed high APOBEC3B expression in GC tissues and cells (see Fig. 1a and b).

3.2. Correlation between APOBEC3B and P53 activity

The positive rate of APOBEC3B expression was 78.3 % (126/161) among the 161 patients with GC (see Fig. 2a and b and Table 1). There was a significant positive correlation between APOBEC3B and P53 expression (see Fig. 2c and d and Table 1). To confirm the relationship between APOBEC3B and P53 in GC cells, mutation data were obtained from cBioPortal, which revealed that the P53 mutation rate in GC cells was 49 % (see Fig. 2e). Further, the P53 mutation group showed significantly higher APOBEC3B expression than the P53 non-mutation group (see Fig. 2f).

3.3. APOBEC3B promotes GC cell proliferation

MTT and cell cloning assays were used to determine the effect of APOBEC3B expression on GC cell proliferation. Both assays revealed that, compared with the control group, APOBEC3B and si-APOBEC3B transfection significantly promoted and inhibited cell proliferation, respectively (see Fig. 3a–c).

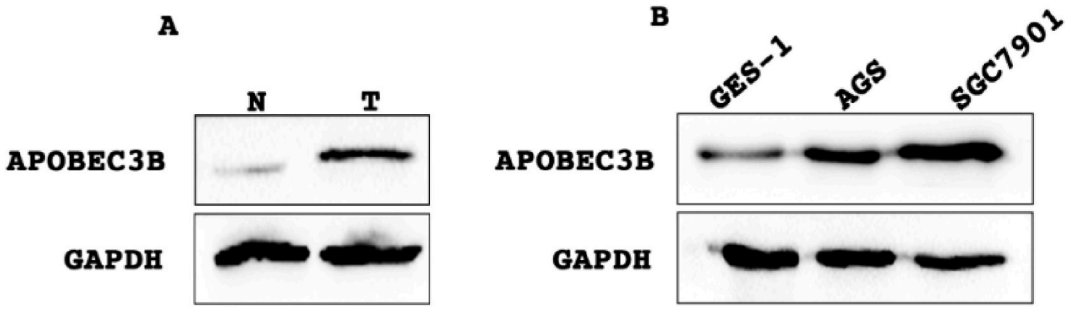


Fig. 1. APOBEC3B expression in gastric cancer (GC) tissues and cells. (**a**) APOBEC3B expression in GC and adjacent tissues. (**b**) APOBEC3B expression in GC cell lines.

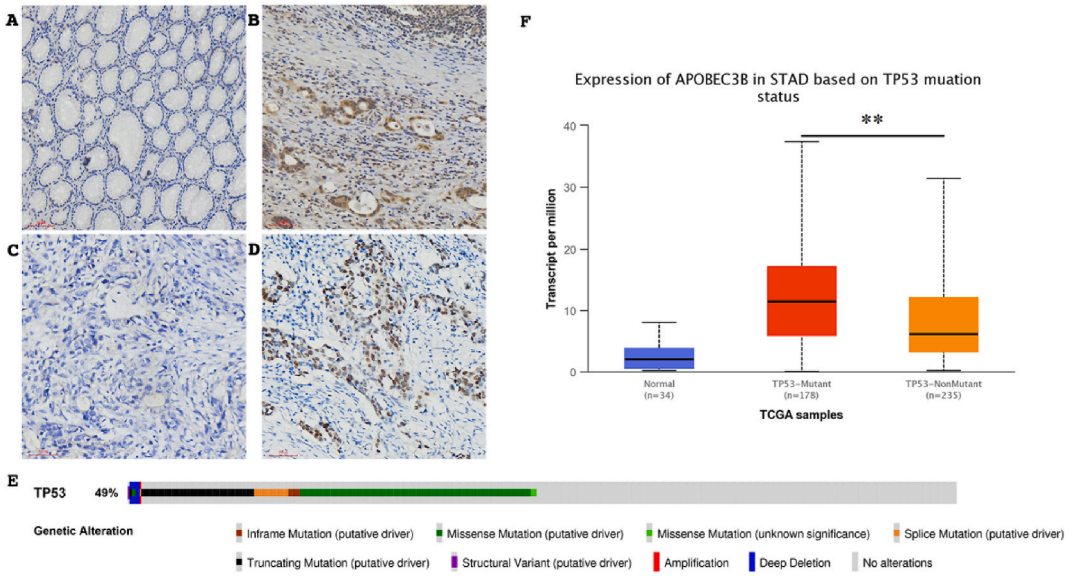


Fig. 2. Correlation between APOBEC3B expression and P53 activity. (**a**) Negative APOBEC3B expression in normal gastric tissue. (**b**) Positive APOBEC3B expression in gastric cancer (GC) tissue (× 200). (**c**) Negative P53 expression in GC tissue. (**d**) Positive P53 expression in GC tissue. (**e**) P53 mutation rate in GC cells. (**f**) Correlation between *APOBEC3B* and *P53* mutations. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

Table 1
Correlation between APOBEC3B and P53 expression in gastric cancer tissues.

APOBEC3B	n	P53		*r*	*P*
		+	−		
+	126	64	62	0.16	0.42
−	35	11	24		

3.4. APOBEC3B promotes GC cell migration and invasion

Wound healing and Transwell assays were performed to determine the effect of APOBEC3B expression on the migration and invasion of GC cells. Compared with the scrambled control, APOBEC3B overexpression and knockdown promoted and inhibited GC cell motility, respectively (see Fig. 4a–d).

3.5. APOBEC3B expression promotes GC tumorigenicity In vivo

To determine the effect of APOBEC3B expression on tumor growth in xenograft mice, nude mice were subcutaneously implanted with GES-1 and AGS cells transfected with an empty vector or APOBEC3B. Tumor growth was measured four times, with measurements taken every 5 days. For both cell lines, APOBEC3B exhibited significantly increased tumor volume and weight compared with the empty vector and blank groups (see Fig. 5a–c). For H&E staining of APOBEC3B in tumor tissues, see Fig. 5d and e (× 100).

3.6. APOBEC3B promotes GC development by regulating P53, MCM2, and cyclin D1

The GEPIA database was used to obtain differentially expressed P53 and MCM2 genes in GC cells after regulating APOBEC3B expression (see Fig. 6a). Using the correlation analysis function of the GEPIA database, cyclin D1 (a classic cell proliferation-related protein) was positively correlated with APOBEC3B expression in GC tissues (see Fig. 6a). Western blotting showed that mice with APOBEC3B overexpression had markedly increased cell proliferation compared with the control group. Additionally, APOBEC3B enhanced P53, MCM2, and cyclin D1 expression (see Fig. 6b). Similarly, in GES-1 and AGS cells, compared with the control group, APOBEC3B overexpression and knockdown significantly increased and decreased P53, MCM2, and cyclin D1 expression, respectively (see Fig. 6c). These results suggested that APOBEC3B may influence GC progression by regulating P53, MCM2, and cyclin D1 expression.

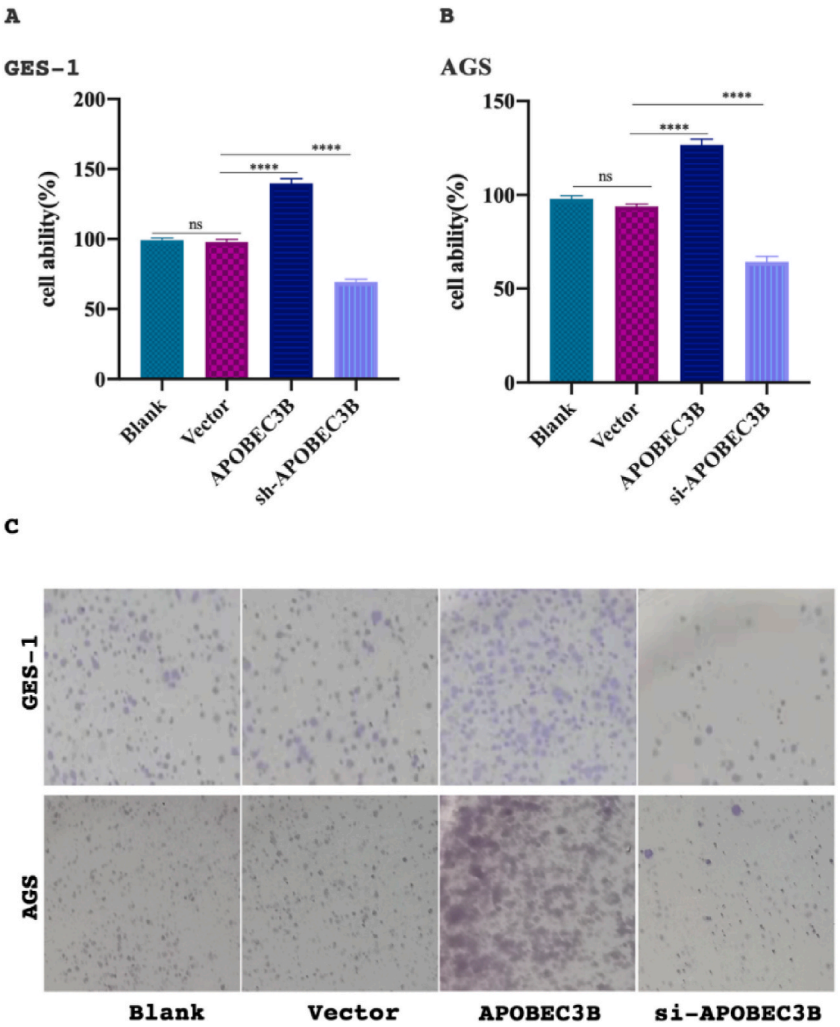


Fig. 3. APOBEC3B promotes gastric cancer cell proliferation. (**a, b**) Effect of APOBEC3B expression on GES-1 and AGS cell proliferation. (**c**) Effect of APOBEC3B expression on the clonal formation rate of GES-1 and AGS cells. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

4. Discussion

Cancer has a significant impact on human health, and its occurrence is linked to various factors, including bacteria, viruses, smoking, obesity, and environmental factors [30,31]. GC is a prevalent malignant tumor, ranking fifth in incidence among solid tumors worldwide, and has a high fatality rate. Despite the reduction in incidence and mortality through early screening and planned prevention strategies, GC remains a major obstacle to increasing human life expectancy in the 21st century. Due to the absence of symptoms during its initial stages, most patients with GC are diagnosed and treated at an advanced stage, leading to an unfavorable prognosis [32]. Consequently, there is an urgent need for novel diagnostic biomarkers and targeted therapies for GC.

APOBEC3B, as a cytidine deaminase, is upregulated in various malignant tumors with a poor prognosis. Here, it alters the expression of key cancer genes and is, therefore, involved in tumor development [8,33]. Using bioinformatics analysis, previous reports have shown that APOBEC3B plays a key role in GC; however, little is known about the mechanism by which *APOBEC3B* gene expression is regulated. In this study, we showed that APOBEC3B was overexpressed in GC. APOBEC3B upregulation enhanced the proliferation of GC cells *in vitro* and tumorigenicity *in vivo*. Regarding the underlying mechanism, APOBEC3B upregulated P53, MCM2, and cyclin D1 and affected cell cycle progression. Furthermore, APOBEC3B expression positively correlated with P53, MCM2, and cyclin D1 expression in a mouse model and in GC cells.

Few reports have shown a positive correlation between APOBEC3B expression and the genes associated with cell proliferation. David et al. [34] reported that APOBEC3B expression was correlated with adverse clinicopathological features, as well as proliferative characteristics, such as cell cycle-related genes and mitosis, in breast cancer. Du et al. [35] reported that APOBEC3B knockdown affected ovarian cancer cell viability and, in adrenal cortical cancer [36], knockdown of APOBEC3B reduced cell proliferation and tumor growth in a xenograft mouse model. However, the mechanism by which APOBEC3B affects GC progression remains elusive. In

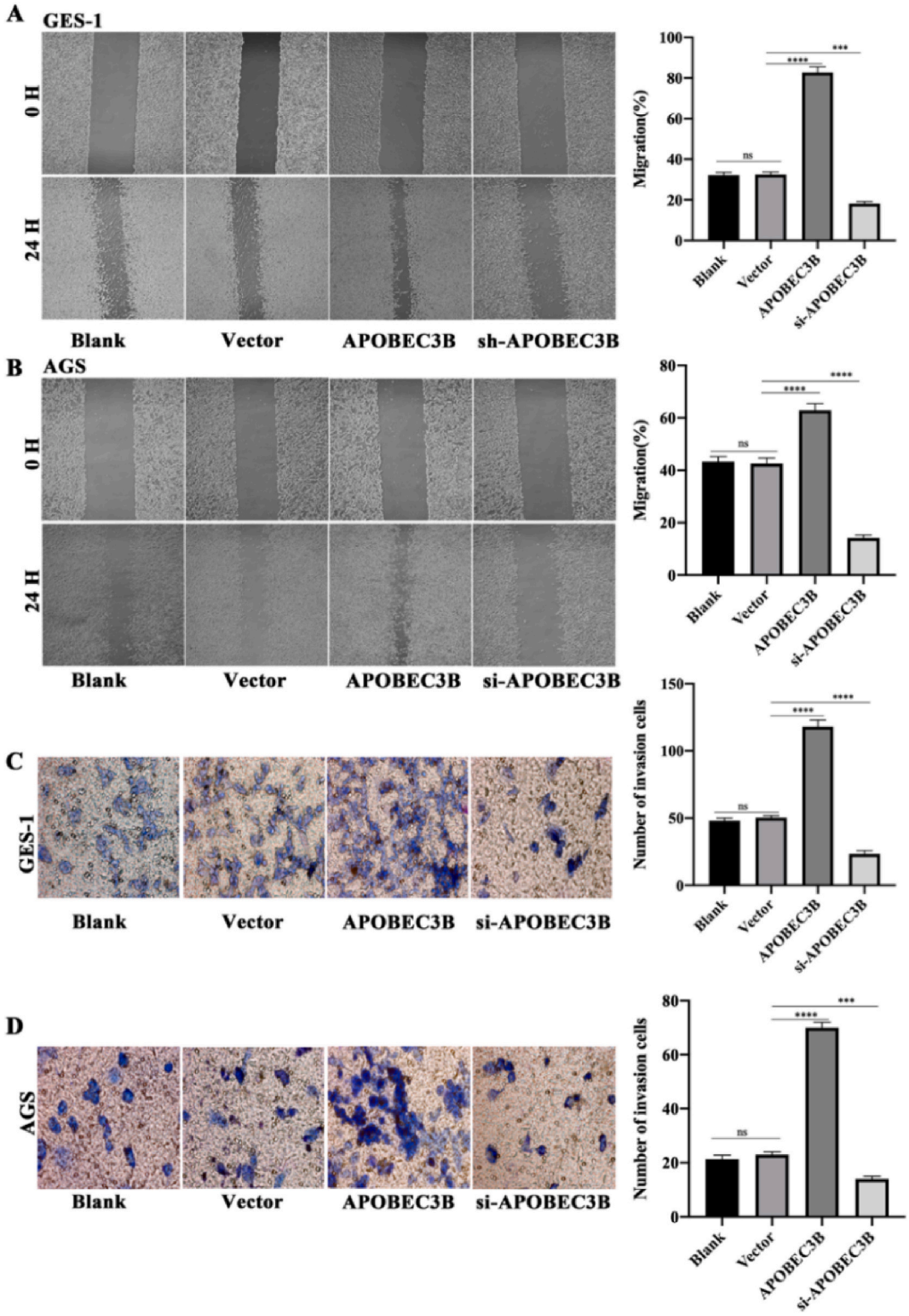


Fig. 4. APOBEC3B promotes gastric cancer cell migration and invasion. (**a, b**) Effect of APOBEC3B expression on the migration of GES-1 and AGS cells. (**c, d**) Effect of APOBEC3B expression on the invasion of GES-1 and AGS cells.

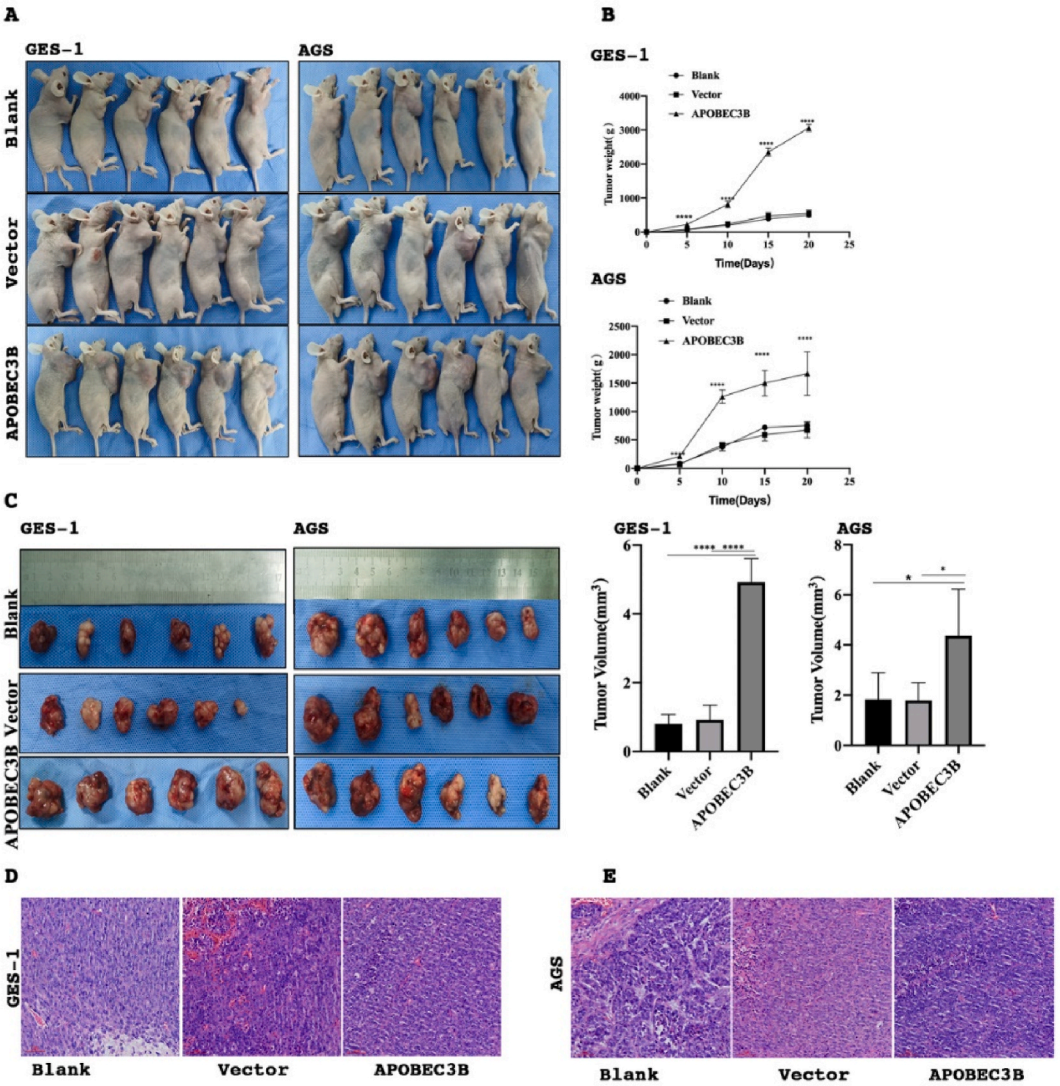


Fig. 5. Effect of APOBEC3B expression on the growth of xenograft tumors in nude mice. (**a**) Effect of APOBEC3B expression on tumor size. (**b**) Effect of APOBEC3B expression on tumor growth. (**c**) Effect of APOBEC3B expression on tumor volume. (**d, e**) H&E staining of APOBEC3B in tumor tissue. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

our study, we observed APOBEC3B overexpression in a mouse model and human tumor tissue. Upregulated APOBEC3B expression significantly enhanced cell proliferation, migration, and invasion, whereas APOBEC3B knockdown significantly inhibited cell proliferation, migration, and invasion. Furthermore, APOBEC3B overexpression in nude mice promoted GC cell growth. Taken together, our findings showed that APOBEC3B plays an oncogenic role in GC and is involved in the development and progression of GC, providing new directions and strategies for the treatment and prevention of GC.

The COVID-19 pandemic has been hard on people living with cancer. Researchers have found that deamination mediated by the APOBEC3 family plays an important role in the evolution of the novel coronavirus. Deamination of the APOBEC3 family accelerated the mutation of the virus and promoted the emergence of new variants of SARS-CoV-2 [37]. Warren et al. [38] reported that APOBEC3B, along with a variety of DNA viruses, such as restricted HBV, HPV, herpes virus, and adeno-associated virus, is a driving factor of a variety of malignant tumors, such as liver cancer, breast cancer, and uterine cancer. APOBEC3B-mediated mutations have been shown [33] to cause cell cycle defects in multiple cell lines. The mechanisms by which APOBEC3B is dysregulated in multiple cancers remain unclear. Gara et al. [36] reported that high APOBEC3B expression results in the increase/loss of chromosomes 4 and 8, as well as an increase in P53 mutation frequency, in adrenal cortical carcinoma. P53 is critically involved in APOBEC3B regulation. Furthermore, loss of P53 activity can activate the tumor mutation mechanism, triggering the transformation of normal cells into cancer cells [39]. Our findings are consistent with those of previous reports. Using bioinformatics analysis, we found that P53 mutations were closely associated with APOBEC3B expression. Immunohistochemical results showed a positive correlation between APOBEC3B and P53 expression in GC tissue, suggesting that P53 inactivation could alter APOBEC3B expression and promote GC progression.

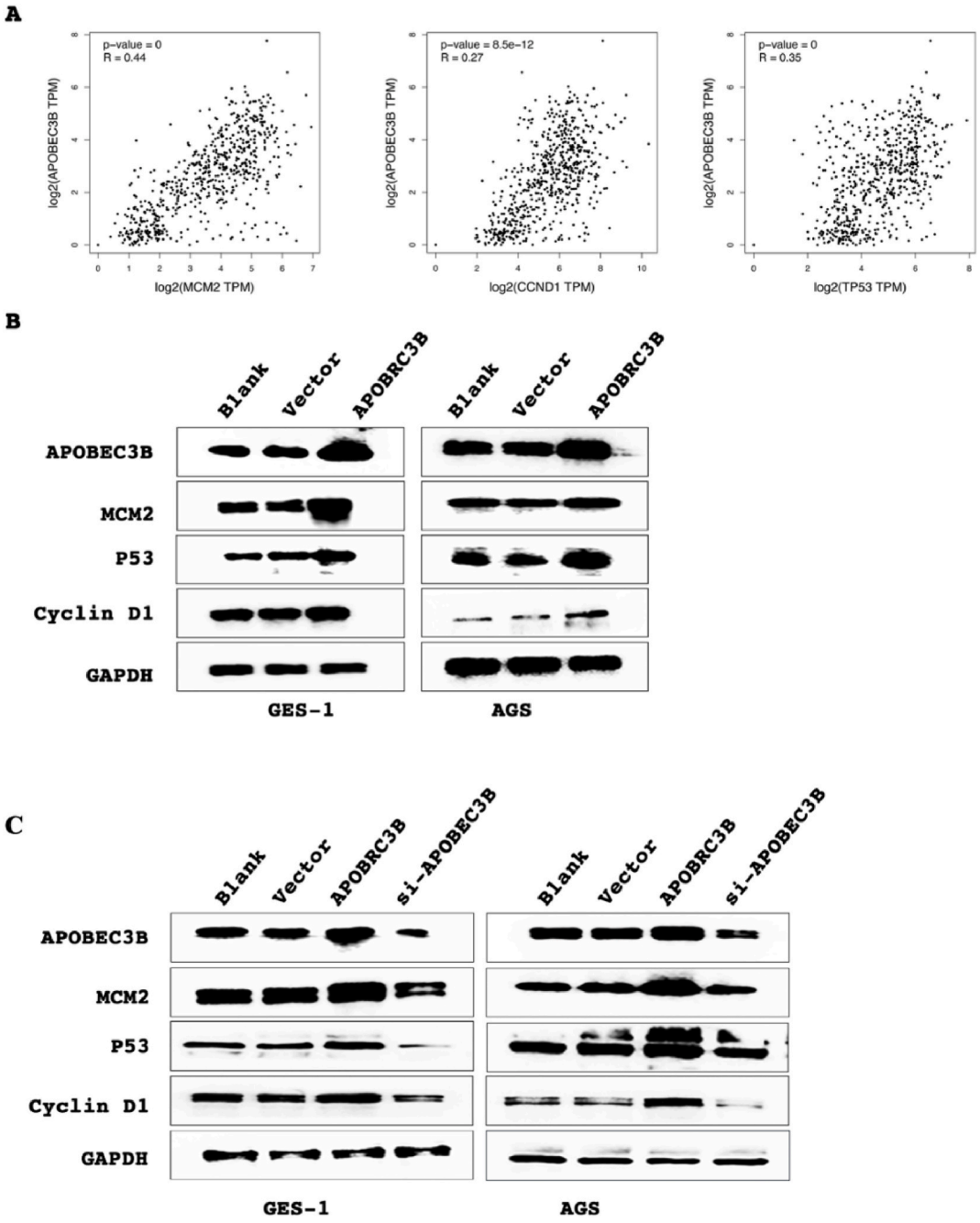


Fig. 6. APOBEC3B promotes gastric cancer development through P53, MCM2, and cyclin D1. (**a**) Correlation between P53, MCM2, cyclin D1, and APOBEC3B expression. (**b**) Effect of APOBEC3B on P53, MCM2, and cyclin D1 expression in mice after subcutaneous injection of GES-1 and AGS cells. (**c**) Effect of APOBEC3B on P53, MCM2, and cyclin D1 expression in GES-1 and AGS cells.

Accelerated cell cycle, uncontrolled cell growth, and an imbalance between cell growth and apoptosis are specific features of tumor cells [40]. It has been established that cyclin D1 overexpression alters cell cycle progression, leads to evasion of normal cell checkpoint regulation, induces cell proliferation, and increases tumor susceptibility [41]. Keyhanian et al. [42] reported increased MCM2 and cyclin D1 expression in leiomyoma, which was closely associated with leiomyoma progression. Furthermore, Li et al. [43] showed that downregulated MCM2 expression significantly inhibited the proliferation of progenitor cells in chronic rhinitis. Several studies focusing on MCM2 [44] have identified molecular events that may be potential markers of cell proliferation. Moreover, DNA replication is crucial for maintaining genomic stability and regulating the cell cycle [45], and all replication processes start from the beginning of DNA replication. Replication fork damage is often the main cause of DNA damage and genomic structural alterations

[46]. MCM2 is critically involved in DNA replication. Ishimi et al. [47] showed that MCM2 promoted cancer cell development by interfering with cellular DNA replication. This suggests that MCM2 has specific effects on APOBEC3B and is among the leading factors promoting genetic mutations. The role of MCM2 and APOBEC3B in the occurrence and development of GC remains unclear. In our cohort, we utilized the correlation analysis function of the GEPIA database and found that MCM2 and cyclin D1 were positively correlated with APOBEC3B expression in GC tissues. Thus, APOBEC3B may induce mutations in GC cells by regulating MCM2 expression. We also confirmed that APOBEC3B overexpression and knockdown promoted and inhibited MCM2 expression in both mouse models and GC cells, respectively. Our study underscored that APOBEC3B has a growth-promoting function in GC, possibly owing to the APOBEC3B-mediated regulation of MCM2 and cyclin D1. However, our findings did not elucidate the correlation between the number of genetic mutations and GC progression *in vitro* and *in vivo*. Thus, future studies are required to explore this correlation further.

5. Conclusions

APOBEC3B, a crucial factor in GC development, is overexpressed in GC and is significantly associated with P53 activity. Mechanistically, APOBEC3B promotes GC cell proliferation by upregulating MCM2 and cyclin D1 *in vitro* and *in vivo*. Further studies are warranted to elucidate the mechanisms underlying the involvement of APOBEC3B in regulating GC cells.

Ethical statement

The study design was approved by the Ethics Committee of Binzhou Medical University Hospital (approval number: 2016–26). All patients provided informed consent. All animal experiments were performed in accordance with the NIH's Guide for the Care and Use of Laboratory Animals.

Clinical trial

Not applicable.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Funding

This work was supported by the Natural Science Foundation of Shandong Province (grant number ZR2020MH243) and the National Natural Science Foundation of China (grant number 81772637).

CRediT authorship contribution statement

Nana Su: Writing – review & editing, Writing – original draft, Visualization, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Erle Zhou:** Visualization, Supervision, Software, Resources, Methodology, Investigation. **Min Cui:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources. **Hong Li:** Visualization, Project administration, Formal analysis, Data curation. **Shuhua Wu:** Software, Project administration, Formal analysis, Conceptualization. **Qian Zhang:** Writing – review & editing, Resources, Project administration, Funding acquisition, Data curation. **Zhang Cao:** Writing – review & editing, Project administration, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e24458.

References

[1] F. Bray, J. Ferlay, I. Soerjomataram, R.L. Siegel, L.A. Torre, A. Jemal, Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, Ca - Cancer J. Clin. 68 (6) (2018) 394–424, https://doi.org/10.3322/caac.21492.

[2] A.P. Thrift, H.B. El-Serag, Burden of gastric cancer, Clin. Gastroenterol. Hepatol. 18 (3) (2020) 534–542, https://doi.org/10.1016/j.cgh.2019.07.045.

[3] J. Chen, T. MacCarthy, The preferred nucleotide contexts of the AID/APOBEC cytidine deaminases have differential effects when mutating retrotransposon and virus sequences compared to host genes, PLoS Comput. Biol. 13 (3) (2017) e1005471, https://doi.org/10.1371/journal.pcbi.1005471.

[4] F. Borzooee, M. Larijani, Pichia pastoris as a host for production and isolation of mutagenic AID/APOBEC enzymes involved in cancer and immunity, N. Biotech. 51 (2019) 67–79, https://doi.org/10.1016/j.nbt.2019.02.006.

[5] R.G. Vile, A. Melcher, H. Pandha, K.J. Harrington, J.S. Pulido, APOBEC and cancer viroimmunotherapy: thinking the unthinkable, Clin. Cancer Res. 27 (12) (2021) 3280–3290, https://doi.org/10.1158/1078-0432.CCR-20-1888.

[6] Y. Mao, M. Lv, Y. Zhang, G. Nie, J. Cui, Y. Wang, Y. Wang, W. Cao, X. Liu, X. Wang, H. Wang, APOBEC3B expression and its prognostic potential in breast cancer, Oncol. Lett. 19 (4) (2020) 3205–3214, https://doi.org/10.3892/ol.2020.11433.

[7] D. Schulz, G. Piontek, U.M. Zissler, G. Multhoff, M. Wirth, A. Pickhard, MEK1/2 regulates APOBEC3B and polymerase iota-induced mutagenesis in head and neck cancer cells, Am. J. Cancer Res. 11 (11) (2021) 5581–5590.

[8] H. Zhang, Z. Chen, Z. Wang, Z. Dai, Z. Hu, X. Zhang, M. Hu, Z. Liu, Q. Cheng, Correlation between APOBEC3B expression and clinical characterization in lower-grade gliomas, Front. Oncol. 11 (2021) 625838, https://doi.org/10.3389/fonc.2021.625838.

[9] D. Wang, X. Li, J. Li, Y. Lu, S. Zhao, X. Tang, X. Chen, J. Li, Y. Zheng, S. Li, R. Sun, M. Yan, D. Yu, G. Cao, Y. Yang, APOBEC3B interaction with PRC2 modulates microenvironment to promote HCC progression, Gut 68 (10) (2019) 1846–1857, https://doi.org/10.1136/gutjnl-2018-317601.

[10] Q. Fan, T. Huang, X. Sun, Y.W. Wang, J. Wang, Y. Liu, T. Ni, S.L. Gu, Y.H. Li, Y.D. Wang, HPV-16/18 E6-induced APOBEC3B expression associates with proliferation of cervical cancer cells and hypomethylation of cyclin D1, Mol. Carcinog. 60 (5) (2021) 313–330, https://doi.org/10.1002/mc.23292.

[11] W. Ma, D.W. Ho, K.M. Sze, Y.M. Tsui, L.K. Chan, J.M. Lee, I.O. Ng, APOBEC3B promotes hepatocarcinogenesis and metastasis through novel deaminase-independent activity, Mol. Carcinog. 58 (5) (2019) 643–653, https://doi.org/10.1002/mc.22956.

[12] C.J. Warren, J.A. Westrich, K.V. Doorslaer, D. Pyeon, Roles of APOBEC3A and APOBEC3B in human papillomavirus infection and disease progression, Viruses 9 (8) (2017) 233, https://doi.org/10.3390/v9080233.

[13] A.M. Sieuwerts, S. Willis, M.B. Burns, M.P. Look, M.E. Meijer-Van Gelder, A. Schlicker, M.R. Heideman, H. Jacobs, L. Wessels, B. Leyland-Jones, K.P. Gray, J. A. Foekens, R.S. Harris, J.W. Martens, Elevated APOBEC3B correlates with poor outcomes for estrogen-receptor-positive breast cancers, Horm. Cancer. 5 (6) (2014) 405–413, https://doi.org/10.1007/s12672-014-0196-8.

[14] M. Kanapathipillai, Treating p53 mutant aggregation-associated cancer, Cancers 10 (6) (2018) 154, https://doi.org/10.3390/cancers10060154.

[15] A. Parrales, T. Iwakuma, Targeting oncogenic mutant p53 for cancer therapy, Front. Oncol. 5 (2015) 288, https://doi.org/10.3389/fonc.2015.00288.

[16] H. Yamazaki, K. Shirakawa, T. Matsumoto, Y. Kazuma, H. Matsui, Y. Horisawa, E. Stanford, A.D. Sarca, R. Shirakawa, K. Shindo, A. Takaori-Kondo, APOBEC3B reporter myeloma cell lines identify DNA damage response pathways leading to APOBEC3B expression, PLoS One 15 (1) (2020) e0223463, https://doi.org/10.1371/journal.pone.0223463.

[17] M. Wang, C. Yang, X. Zhang, X. Li, Characterizing genomic differences of human cancer stratified by the TP53 mutation status, Mol. Genet. Genom. 293 (3) (2018) 737–746, https://doi.org/10.1007/s00438-018-1416-7.

[18] M.B. Burns, L. Lackey, M.A. Carpenter, A. Rathore, A.M. Land, B. Leonard, E.W. Refsland, D. Kotandeniya, N. Tretyakova, J.B. Nikas, D. Yee, N.A. Temiz, D. E. Donohue, R.M. McDougle, W.L. Brown, E.K. Law, R.S. Harris, APOBEC3B is an enzymatic source of mutation in breast cancer, Nature 494 (7437) (2013) 366–370, https://doi.org/10.1038/nature11881.

[19] S. Wang, M. Jia, Z. He, X.S. Liu, APOBEC3B and APOBEC mutational signature as potential predictive markers for immunotherapy response in non-small cell lung cancer, Oncogene 37 (29) (2018) 3924–3936, https://doi.org/10.1038/s41388-018-0245-9.

[20] N. Tuteja, N.Q. Tran, H.Q. Dang, R. Tuteja, Plant MCM proteins: role in DNA replication and beyond, Plant Mol. Biol. 77 (6) (2011) 537–545, https://doi.org/10.1007/s11103-011-9836-3.

[21] N.E. Simon, A. Schwacha, The Mcm2-7 replicative helicase: a promising chemotherapeutic target, BioMed Res. Int. 2014 (2014) 549719, https://doi.org/10.1155/2014/549719.

[22] T. Ubhi, G.W. Brown, Exploiting DNA replication stress for cancer treatment, Cancer Res. 79 (8) (2019) 1730–1739, https://doi.org/10.1158/0008-5472.CAN-18-3631.

[23] F.I. Montalto, F. De Amicis, Cyclin D1 in cancer: a molecular connection for cell cycle control, adhesion and invasion in tumor and stroma, Cells 9 (12) (2020) 2648, https://doi.org/10.3390/cells9122648.

[24] K. Qu, Z. Wang, H. Fan, J. Li, J. Liu, P. Li, Z. Liang, H. An, Y. Jiang, Q. Lin, X. Dong, P. Liu, C. Liu, MCM7 promotes cancer progression through cyclin D1-dependent signaling and serves as a prognostic marker for patients with hepatocellular carcinoma, Cell Death Dis. 8 (2) (2017) e2603, https://doi.org/10.1038/cddis.2016.352.

[25] J. Gao, B.A. Aksoy, U. Dogrusoz, G. Dresdner, B. Gross, S.O. Sumer, Y. Sun, A. Jacobsen, R. Sinha, E. Larsson, E. Cerami, C. Sander, N. Schultz, Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal, Sci. Signal. 6 (269) (2013) pl1, https://doi.org/10.1126/scisignal.2004088.

[26] D.S. Chandrashekar, B. Bashel, S.A.H. Balasubramanya, C.J. Creighton, I. Ponce-Rodriguez, B.V.S.K. Chakravarthi, S. Varambally, UALCAN: a portal for facilitating tumor subgroup gene expression and survival analyses, Neoplasia 19 (8) (2017) 649–658, https://doi.org/10.1016/j.neo.2017.05.002.

[27] Z. Tang, C. Li, B. Kang, G. Gao, C. Li, Z. Zhang, GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses, Nucleic Acids Res. 45 (W1) (2017), https://doi.org/10.1093/nar/gkx247. W98–W102.

[28] U. Rüder, C. Denkert, C.A. Kunze, P. Jank, J. Lindner, K. Jöhrens, H. Kulbe, J. Sehouli, M. Dietel, E. Braicu, S. Darb-Esfahani, APOBEC3B protein expression and mRNA analyses in patients with high-grade serous ovarian carcinoma, Histol. Histopathol. 34 (4) (2019) 405–417, https://doi.org/10.14670/HH-18-050.

[29] Y. Sumiyoshi, Y. Kakeji, A. Egashira, K. Mizokami, H. Orita, Y. Maehara, Overexpression of hypoxia-inducible factor 1alpha and p53 is a marker for an unfavorable prognosis in gastric cancer, Clin. Cancer Res. 12 (17) (2006) 5112–5117, https://doi.org/10.1158/1078-0432.CCR-05-2382.

[30] E.J. Maskouni, T. Jamalvandi, F. Tabatabaei, S. Bourenjan Shirazi, H. Saadati, A. Letafati, M. Hosseini, S. Motlaghzadeh, Z. Khalesi, P. Moradi, S. Saeb, N. Sheikh, E. Fozouni, A. Khatami, A.H. Baker, Z. Keyvanlou, V. Tamrchi, A. Tavakoli, S. Ghorbani, Association between Epstein-Bar virus and colorectal cancer: a systematic review and meta-analysis, Microb. Pathog. 179 (2023) 106087, https://doi.org/10.1016/j.micpath.2023.106087.

[31] S. Ghorbani, A. Letafati, A. Khatami, R. Farzi, S. Shabani, P. Moradi, V. Tambrchi, H. Saadati, S. Papizadeh, M.V. Rad, R. Tabatabaei, Association between human herpesvirus-6 and primary brain tumors: a systematic review and meta-analysis, Future Virol. 17 (5) (2022) 305–314, https://doi.org/10.2217/fvl-2021-0096.

[32] A. Jemal, F. Bray, M.M. Center, J. Ferlay, E. Ward, D. Forman, Global cancer statistics, CA Cancer, J. Clin. 61 (2) (2011) 69–90, https://doi.org/10.3322/caac.20107.

[33] M.B. Burns, L. Lackey, M.A. Carpenter, A. Rathore, A.M. Land, B. Leonard, E.W. Refsland, D. Kotandeniya, N. Tretyakova, J.B. Nikas, D. Yee, APOBEC3B is an enzymatic source of mutation in breast cancer, Nature 494 (7437) (2013) 366–370, https://doi.org/10.1038/nature11881.

[34] D.W. Cescon, B. Haibe-Kains, T.W. Mak, APOBEC3B expression in breast cancer reflects cellular proliferation, while a deletion polymorphism is associated with immune activation, Proc. Natl. Acad. Sci. U.S.A. 112 (9) (2015) 2841–2846, https://doi.org/10.1073/pnas.1424869112.

[35] Y. Du, X. Tao, J. Wu, H. Yu, Y. Yu, H. Zhao, APOBEC3B up-regulation independently predicts ovarian cancer prognosis: a cohort study, Cancer Cell Int. 18 (78) (2018), https://doi.org/10.1186/s12935-018-0572-5.

[36] S.K. Gara, M.V. Tyagi, D.T. Patel, K. Gaskins, J. Lack, Y. Liu, E. Kebebew, GATA3 and APOBEC3B are prognostic markers in adrenocortical carcinoma and APOBEC3B is directly transcriptionally regulated by GATA3, Oncotarget 11 (36) (2020) 3354–3370, https://doi.org/10.18632/oncotarget.27703.

[37] R. Matyášek, A. Kovařík, Mutation patterns of human SARS-CoV-2 and bat RaTG13 coronavirus genomes are strongly biased towards C>U transitions, indicating rapid evolution in their hosts, Genes 1 (7) (2020) 761, https://doi.org/10.3390/genes11070761.

[38] S. Revathidevi, A.K. Murugan, H. Nakaoka, I. Inoue, A.K. Munirajan, APOBEC: a molecular driver in cervical cancer pathogenesis, Cancer Lett. 496 (2021) 104–116, https://doi.org/10.1016/j.canlet.2020.10.004.
[39] M. Periyasamy, A.K. Singh, C. Gemma, C. Kranjec, R. Farzan, D.A. Leach, N. Navaratnam, H.L. Pálinkás, B.G. Vértessy, T.R. Fenton, J. Doorbar, p53 controls expression of the DNA deaminase APOBEC3B to limit its potential mutagenic activity in cancer cells, Nucleic Acids Res. 45 (19) (2017) 11056–11069, https://doi.org/10.1093/nar/gkx721.
[40] G. Kroemer, J. Pouyssegur, Tumor cell metabolism: cancer's Achilles' heel, Cancer Cell 13 (6) (2008) 472–482, https://doi.org/10.1016/j.ccr.2008.05.005.
[41] F.I. Montalto, F. De Amicis, Cyclin D1 in cancer: a molecular connection for cell cycle control, adhesion and invasion in tumor and stroma, Cells 9 (12) (2020) 2648, https://doi.org/10.3390/cells9122648.
[42] K. Keyhanian, J.M. Lage, E. Chernetsova, H. Sekhon, Z. Eslami, S. Islam, Combination of MCM2 with Ki67 and p16 immunohistochemistry can distinguish uterine leiomyosarcomas, Int. J. Gynecol. Pathol. 39 (4) (2020) 354–361, https://doi.org/10.1097/PGP.0000000000000616.
[43] L.Y. Li, Y.T. Zhou, L. Sun, X.Y. Liu, J. Li, Y. Hong, X.Y. Ye, Q. Bao, Q.X. Meng, W.P. Wen, H.X. Chen, Downregulation of MCM2 contributes to the reduced growth potential of epithelial progenitor cells in chronic nasal inflammation, J. Allergy Clin. Immunol. 147 (5) (2021) 1966–1973.e3, https://doi.org/10.1016/j.jaci.2020.11.026.
[44] C. Cheung, C.L. Hsu, K.P. Chen, S.T. Chong, C.H. Wu, H.C. Huang, H.F. Juan, MCM2-regulated functional networks in lung cancer by multi-dimensional proteomic approach, Sci. Rep. 7 (1) (2017) 13302, https://doi.org/10.1038/s41598-017-13440-x.
[45] T. Ubhi, G.W. Brown, Exploiting DNA replication stress for cancer treatment, Cancer Res. 79 (8) (2019) 1730–1739, https://doi.org/10.1158/0008-5472.CAN-18-3631.
[46] J.M. Dewar, J.C. Walter, Mechanisms of DNA replication termination, Nat. Rev. Mol. Cell Biol. 18 (8) (2017) 507–516, https://doi.org/10.1038/nrm.2017.42.
[47] Y. Ishimi, Regulation of MCM2-7 function, Genes Genet. Syst. 93 (4) (2018) 125–133, https://doi.org/10.1266/ggs.18-00026.