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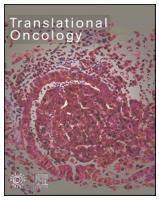

Low expression of lncRNA APTR promotes gastric cancer progression

Aigang Ren [a,1], Qianqian Li [b,1], Yuntong Guo [a], Xiaolong Cui [a], Liwei Wang [a], Yaoliang Huo [a], Hao Chen [a], Hui Liu [a], He Huang [a,*]

[a] Department of Gastrointestinal Surgery, The First Hospital of Shanxi Medical University, Taiyuan, Shanxi 030001, China
[b] Department of Gastroenterology, The Second Hospital of Shanxi Medical University, Taiyuan, Shanxi 030013, China

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ABSTRACT

Background: Gastric cancer (GC) is one of the most common cancers worldwide and the majority of GC patients are diagnosed at advanced stages due to the lack of early detection biomarkers. LncRNAs have been shown to play important roles in various diseases and could be predictive biomarkers and therapeutic targets. Our study demonstrated that low expression of lncRNA APTR could promote gastric cancer progression.
Methods: Differentiated expressed lncRNAs were identified through analyzing TCGA paired GC RNA sequencing data. LncRNA APTR's clinical relevance was analyzed using the TCGA dataset and GEO datasets. APTR expression in patient samples was detected through qPCR. The proliferation, colony formation, and migration of GC cells were tested. Bioinformatic analyses were performed to explore APTR-affected signaling pathways in GC.
Results: LncRNA APTR is lower expressed in gastric tumor samples and low expression of APTR predicts a poor diagnosis and outcome in GC patients. Silencing APTR promotes gastric cancer proliferation and invasiveness. APTR expression is negatively correlated with inflammatory signaling in the gastric tumor microenvironment.
Conclusion: Our study showed that low expression of lncRNA APTR in gastric cancer is correlated with tumorigenesis and poor diagnosis and prognosis, which is a potential biomarker for gastric cancer patients' diagnosis and treatment.

Introduction

Gastric cancer (GC) is one of the most common cancers worldwide, with an estimation of 1089,103 new cases and 768,793 mortality in 2020 [1]. Several factors could affect the incidence of GC, such as *Helicobacter pylori infection,* tobacco smoking, high salt intake, and other dietary factors [2–4]. More than 80% of patients are diagnosed at advanced stages due to occult onset [5,6]. Even though multimodal therapies after surgery have improved the survival rates of patients, the prognosis for advanced GC patients remains poor. Thus, there is a critically urgent need to identify effective biomarkers for the diagnosis and treatment of GC.

Long noncoding RNAs (lncRNAs) are a class of RNA transcripts longer than 200 nt that are not translated into proteins and are now emerging as key regulators with essential roles in the cellular process and disease progression through various molecular mechanisms. Recently, several studies revealed that lncRNAs could participate in GC development via playing oncogenic or repressive roles. LncRNA LINC00342 promotes GC progression by sponging miR-545–5p and regulating CNPY2 expression [7]. LncRNA SNHG22 could interact with EZH2 and then promote GC progression [8]. These findings imply that lncRNAs could be promising therapeutic targets for the treatment of GC.

LncRNA Alu-mediated p21 transcriptional regulator (APTR), which suppresses the CDKN1A/p21 promoter, was found to regulate cell proliferation in several cancers, such as human glioma [9], osteosarcoma [10], breast cancer [11], thyroid cancer [12] and hepatic stellate cells [13]. In this study, we investigated lncRNA APTR's role in GC, which was lower expressed in gastric tumor tissues than in gastric normal tissues. High expression of APTR in gastric cancer is associated with favorable clinical characteristics. Further investigation showed that high expression of APTR in gastric cancer is related to low levels of CAFs in the tumor tissues, and is correlated with suppressed immune signalings. Taken together, our study revealed the important role of APTR in gastric progression, which might provide insight into GC prognosis and treatment.

* Corresponding author.
E-mail address: hh93003@163.com (H. Huang).
[1] These authors contributed equally to this work.

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Materials and methods

Patient tissue samples

Paired gastric cancer tissue specimens and adjacent non-tumor tissue specimens were obtained from 37 patients who were recruited for gastric cancer surgery at the First Hospital of Shanxi Medical University from May 2021 to August 2021. The tissues were collected and stored in a –80 °C freezer. Informed consent was obtained from all individuals included in the study. The research procedures were approved by the Clinical Research Ethics Committee in the First Hospital of Shanxi Medical University. The ethical approval code is 2022-K052.

Cell culture

Gastric cancer cell line AGS was obtained from the cell bank of the Chinese Academy of Sciences (Shanghai, China). AGS cells were cultured in F-12 K Nutrient Mixture (Gibco by life technologies 21,127–022) containing 10% FBS (fetal bovine serum; Thermo Fisher Scientific) and 0.5% penicillin/streptomycin (Sigma Aldrich, St Louis, MO, USA) at 37 °C in a humidified chamber containing 5% CO2.

Short hairpin rna (shRNA) oligos

ShRNA oligos targeting APTR were constructed in pLKO-vector from Addgene (#10,878). ShRNA oligo targeted sequence: 1# 5′-CCACTGTCGCTGGCGTGAA-3′, 2# 5′-GGGGAGGGCTTTCCACTGT-3′.

Lentiviral constructs

Lentivirus was packaged by co-transfection of constructs with second-generation packaging plasmids pMD2.G and psPAX2 into HEK293T cells on 6-well plates by using Lipofectamine 2000 Transfection Reagent (Thermo Fisher Scientific) according to the manufacturer's instructions. After the first 24 h of transfection (250 ng of pMD2. G, 750 ng of psPAX2, 1 μg of target plasmid), the medium was changed to DMEM, and 48 and 72 h after transfection, the supernatants were pooled, filtered through a 0.45-μm filter, and used for infection.

Cell proliferation, migration, and colony formation

Cell Counting kit 8 (Beyotime, Shanghai, China) was used to measure AGS cell proliferation according to the manufacturer's instructions. Briefly, 3×10^3 AGS cells/100 ul medium were seeded into 96-well plates. The cells were incubated with CCK-8 solution at 37 °C for 2 h. Finally, the proliferation rates of gastric cancer cells were quantitated by measuring the OD450 value (absorbance at 456 nm) of each well with a microplate reader. At least three biological replicates were assayed for each sample. AGS cell migration was evaluated by a Transwell system (Corning, Corning, NY, USA) as instructed by the manufacturer. 5×10^4 cells were seeded into the upper chambers with 200 ul serum-free medium while the lower chambers were filled with 800 ul complete medium containing 10% FBS. After 48 h culture, the migrated cells in the lower chamber were then stained with 0.1% crystal violet after fixation with methanol for 5 mins and the non-migrated cells in the upper surface of the chamber were removed by scrubbing. Randomly 5 fields were captured under a microscope. The numbers of cells were counted. For colony formation assay, 1×10^3 AGS cells after shRNA infection were seeded into 6-well plates. After culturing for 2 weeks, cells were stained with 0.1% crystal violet after fixation with methanol for 5 mins. The colonies in 3 wells were captured and counted.

RNA extraction, cDNA synthesis, and qPCR

The total RNA was extracted from cultured cells using RNeasy plus Kit (Qiagen, 74,134) according to the manufacturer's instruction, and then cDNA was synthesized using PrimeScriptTM RT Master Mix (TaKaRa, RR036A). The amplification of cDNA was performed in 10 μl reactions on a real-time PCR system via SYBR Premix Ex Taq II (TaKaRa, RR820A). The mean cycle threshold (Ct) was determined by triplicate PCR runs, and the relative expression was normalized to β-actin as internal control via the 2–ΔΔCt method. The qPCR primer for APTR is APTR-F: AGTAGCAGGAGACAGCAT and APTR-R: TGACAGCCTTCCACAATC. The qPCR primer for β-actin is β-actin-F: CACCATTGGCAATGAGCGGTTC and β-actin-R: AGGTCTTTGCGGATGTCCACGT.

Bioinformatics

CHIPseq data of gastric cancer cells were downloaded from the GEO dataset, GSE162420, and visualized by the Integrative Genomics Viewer. The correlation between the expression of APTR and various genes was analyzed by TCGA Gastric Nature 2014. The clinical relevance of APTR in gastric cancer was analyzed using data from TCGA Gastric Nature 2014 and the GEO dataset, GSE66229. The Kaplan-Meier survival plotter was performed by using data from GSE66229 and GSE15459 and by KM-plotter online tool [14]. The percentage of immune cells in each gastric tumor tissue were analyzed through the EPIC tool [15]. We first downloaded gastric cancer RNAseq data from TCGA datasets. And then we ranked the samples based on the expression of APTR and divided the samples into the high APTR group and the low APTR group. Then we uploaded the organized RNAseq data to the EPIC application to analyze the percentage of immune cells in different groups. APTR-related signaling pathways were analyzed by using the LncGSEA tool [16].

Statistical analysis

All experimental data were reported as mean ± standard error of the mean (SEM) from three independent experiments. All statistical analyses were conducted with GraphPad Prism software 8.3.0 (GraphPad Software, USA). The student's *t*-test was used when two cases were compared. One-way ANOVA was performed for multi-group comparison. Two-tailed Spearman's correlation analysis was used to analyze the correlation between APTR and other genes' expression. Differences in distributions between clinical-pathological and APTR expression were assessed with Chi-square or Fisher's exact test. $P < 0.05$ was considered statistically significant. ns, $p>0.05$. * $p<0.05$. ** $p<0.01$. *** $p<0.001$.

Results

1 Identification of APTR as a differentiated expressed lncRNA in gastric cancer

It is well accepted that lncRNA is an important regulatory factor in the cellular process and disease progression. In order to identify new functional lncRNAs in gastric cancer, we comprehensively analyzed the RNAseq data from paired gastric tumor tissues and adjacent normal tissues using TCGA gastric cancer expression data. We downloaded the lncRNA annotation list from the LNCipedia database (version 5.2), which is a public database for lncRNA sequence and annotation and contains 127, 802 transcripts and 56,946 genes. we compared the gene lists in both TCGA gastric RNAseq data and the LNCipedia database and found a total of 11, 025 lncRNAs are included in TCGA gastric RNAseq data. Unlike coding genes, most lncRNAs are lowly expressed in tissue and cells, which is unlikely to play critical roles in cells. We thus chose to analyze 1, 270 lncRNAs with expression level FPKM>0.5 in our study. Next, we compared the expression level of these 1,270 lncRNAs between gastric tumor tissues and adjacent normal tissues, resulting in the discovery of 35 lncRNAs that are significantly higher expressed in adjacent normal tissues, and 406 lncRNAs that are higher expressed in gastric tumor tissues. Among these differentiated expressed lncRNAs, some lncRNAs have already been reported in gastric cancer, such as

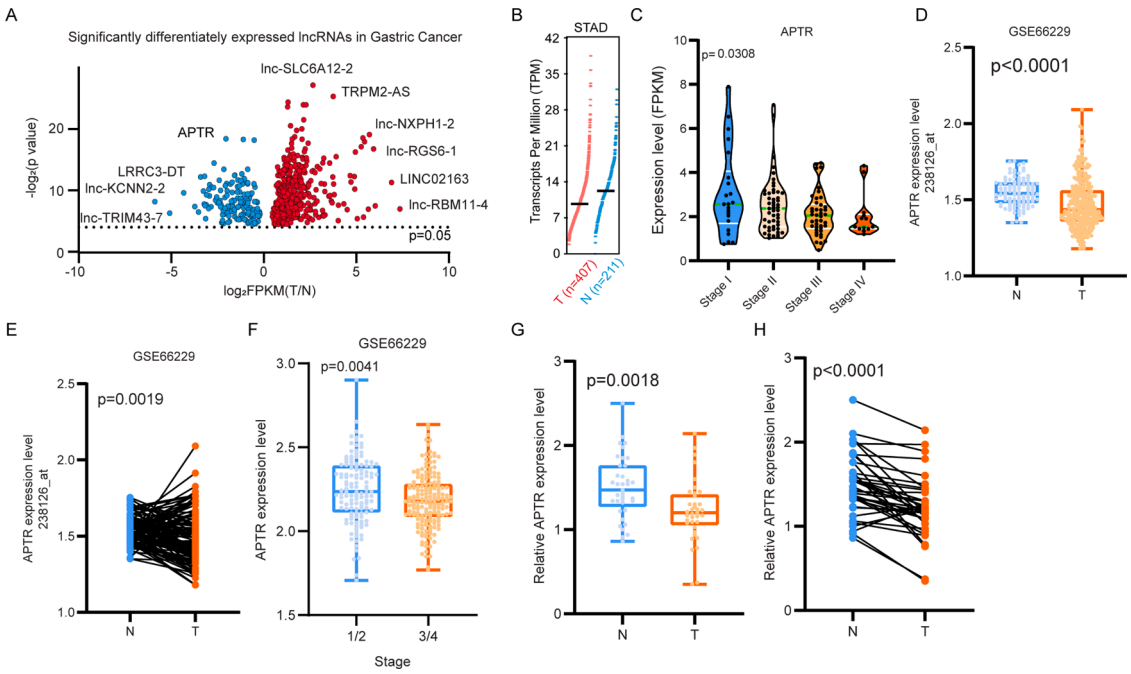
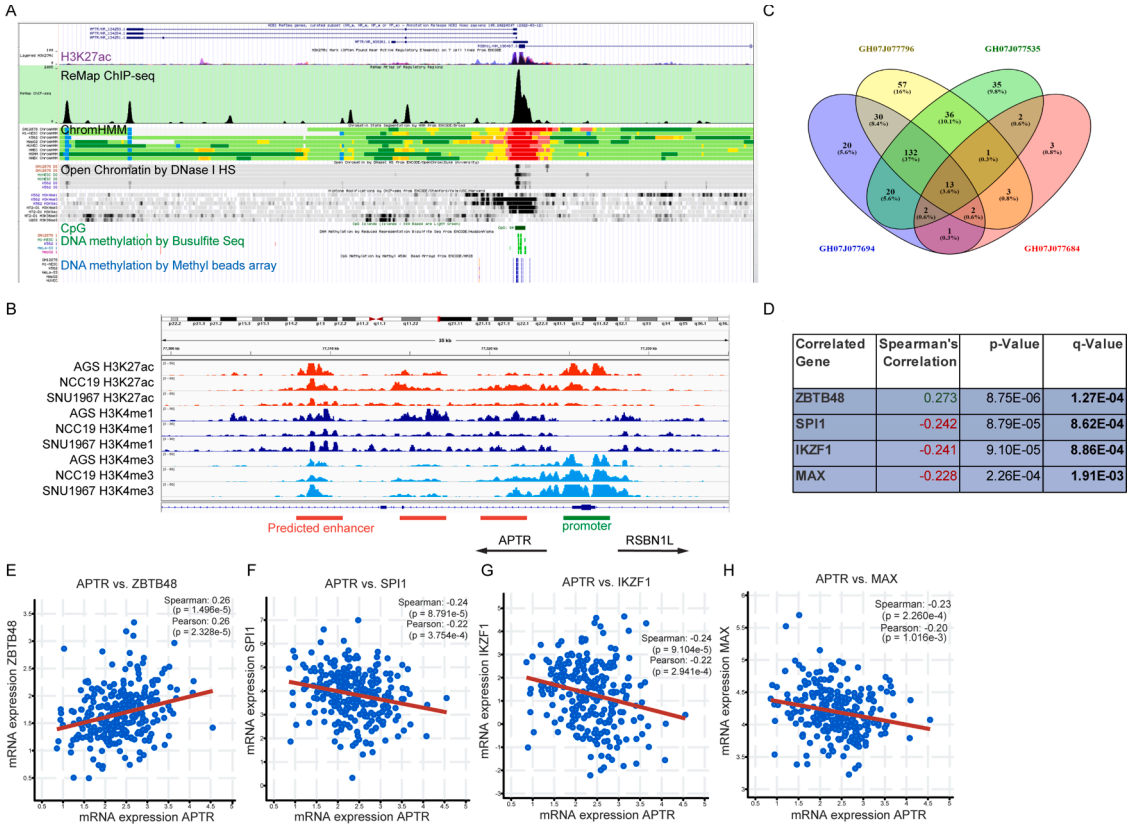


Fig. 1. APTR is lower expressed in gastric tumor samples. A. Differentially expressed lncRNAs with FPKM>0.5 in gastric cancer. B. APTR is higher expressed in gastric normal tissues. Data from the GEPIA server. C. APTR's expression level is negatively correlated with gastric tumor stage in TCGA data. D. and E. APTR is lower expressed in gastric tumor samples in the GSE66229 dataset. F. APTR's expression level is negatively correlated with gastric tumor stage in the GSE66229 dataset. G. and H. qPCR results showed that APTR is higher expressed in collected gastric adjacent normal tissues than gastric tumor tissues.



Correlated Gene	Spearman's Correlation	p-Value	q-Value
ZBTB48	0.273	8.75E-06	**1.27E-04**
SPI1	-0.242	8.79E-05	**8.62E-04**
IKZF1	-0.241	9.10E-05	**8.86E-04**
MAX	-0.228	2.26E-04	**1.91E-03**

Fig. 2. APTR is actively expressed in gastric cancer cells. A. APTR promoter is enriched with H3K27ac, H3K4me3, CpG island, and transcription factors. B. Predicted distal enhancers enriched for H3K27ac, H3K4me1, and H3K4me3, and > 2.5 kb distant from APTR TSS are observed. ChIPseq data from GSE162420. C. Venny analysis of predicted transcription factors of APTR from GeneHancer. D-H. Correlation analysis of predicted transcription factors and APTR in TCGA GC RNA sequencing dataset.

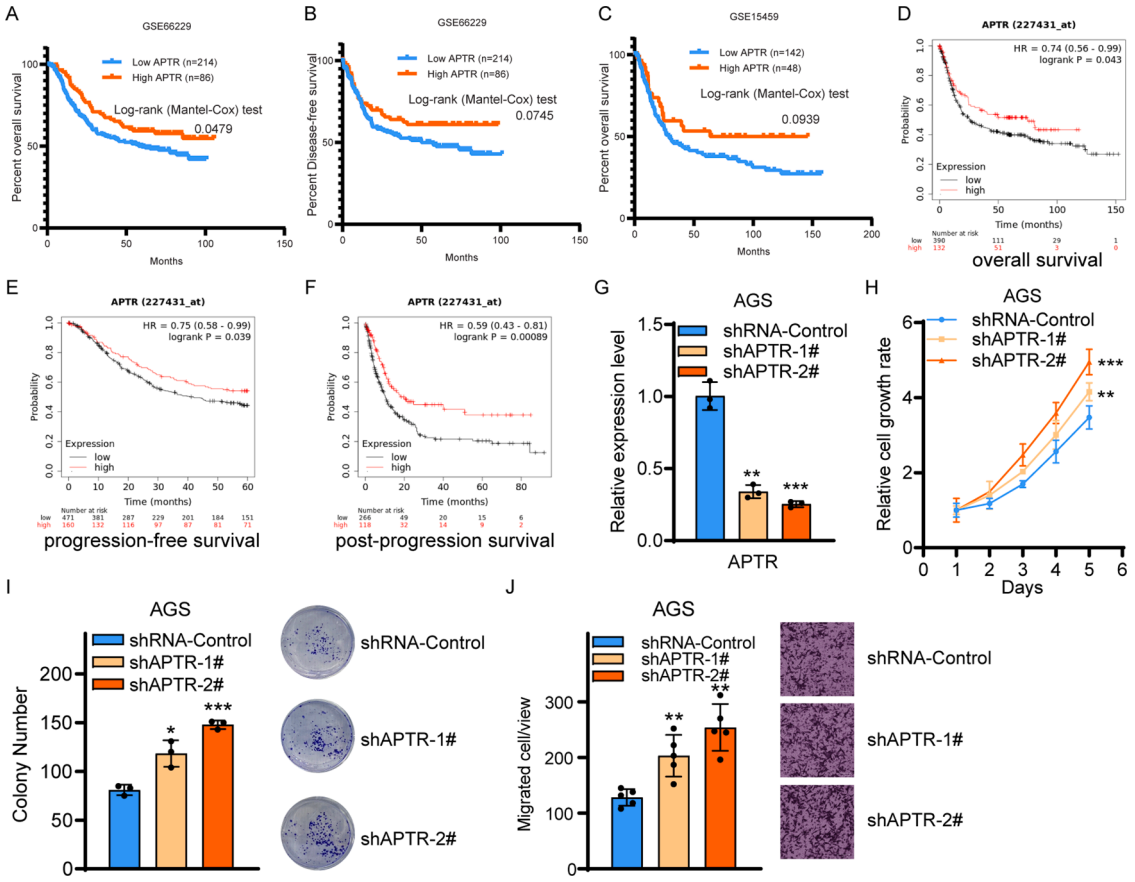


Fig. 3. APTR plays a tumor suppressor role in gastric cancer. A-C, APTR-related survival analysis in GEO datasets. D-F, APTR-related survival analysis by Kaplan-Meier Plotter online server. G. qPCR showed APTR stable knockdown efficiency in AGS cells. H. AGS cell proliferation curve after knocking down APTR. I. AGS colony formation assay after knocking down APTR. J. AGS cell migration assay after knocking down APTR.

LINC01614 [17], TRPM2-AS [18], FOXD2-AS1 [19], and MAFG-AS1 [20], confirming that the identified differentiated lncRNAs are convincing (Fig. 1A).

In the list, we found there are very few studies about lncRNA APTR, which was previously reported to promote the activation of hepatic stellate cells and the progression of liver fibrosis [13] and promote glioma cell proliferation through repressing the p21 promoter [9]. However, APTR's role in gastric cancer has not been investigated yet. In TCGA RNAseq data, APTR is lower expressed in gastric tumor tissues (Fig. 1B) and is negatively correlated with tumor grade (Fig. 1C). We also analyzed the expression of APTR in a published gastric cancer RNA microarray dataset, GSE66229. Similar to the result in the TCGA dataset, APTR is higher expressed in gastric normal tissues than tumor tissues (Fig. 1D and 1E), and APTR is also higher expressed in low-grade gastric tumor tissues than high-grade in tumor tissues (Fig. 1F). We also detected APTR's expression through qPCR in collected 37 pairs of paired tumors and normal gastric tissues, which also showed lower expression of APTR in gastric tumor tissues than in paired normal tissues (Fig. 1G and 1H). These results demonstrated that APTR is a differentially expressed lncRNA in gastric cancer.

2 APTR is actively expressed in gastric cancer cells.

Gene transcription initiation site and enhancer regions are normally enriched with histone modification H3k27ac, which indicates active transcription of the regulated genes. We find there is a significant enrichment of the H3K27ac marker at the initiation site of APTR on 7 cell lines from ENCODE ChIPseq data [21] (Fig. 2A). Remap ChIPseq data showed significant binding of transcription factors at the promoter region of APTR with CpG islands, and the open chromatin status of this region as indicated by chromHMM and DNase I HS suggested APTR is actively transcripted in cells. Besides, these CpG islands can be specifically methylated to suppress the expression of APTR as indicated by DNA methylation sequencing/array (Fig. 2A). Except for the enrichment of H3K27ac and H3K4me3 in the APTR promoter region in gastric cell lines, AGS, NCC19, and SNU1967, we also identified enhancer regions that are distant from the APTR TSS site, as indicated by the enrichment of H3K27ac, H3K4me1, and H3K4me3, implying the active expression of APTR in these cells (Fig. 2B).

GeneHancer is a database of genome-wide enhancer-to-gene and promoter-to-gene associations, embedded in GeneCards [22]. We thus analyzed the potential transcription factors that could regulate the expression of APTR based on the indication from 4 GeneHancer identifier results, each of which includes the predicted transcription factors that may regulate the expression of APTR. Only 13 factors are included in all 4 GeneHancer identifier results (Fig. 2C). We further concluded that 4 factors, ZBTB48, SPI1, IKZF1, and MAX are very likely to regulate APTR transcription based on their differential expression between gastric tumor tissues and normal tissues, and the correlation analysis results (Fig. 2D-H).

3 Low expression of APTR is associated with poor clinical characteristics in patients with gastric cancer

We next explored APTR's clinical relevance in gastric cancer. Based on the survival analysis with data from GEO datasets and through Kaplan-Meier Plotter, we found that lower expression of APTR is related to worse overall survival (Fig. 3A, 3C and 3D), worse disease-free survival (Fig. 3B), worse progression-free survival (Fig. 3E), and worse post-progression survival (Fig. 3F). In TCGA gastric cancer RNAseq data,

Table 1
Correlation between APTR expression and different clinicopathological features in patients with gastric cancer in TCGA gastric cancer dataset.

Characteristics		APTR expression level		Total	p Value
		High (n = 82) No. (%)	Low (n = 59) No. (%)		
Sex					0.1536
	F	25 (51%)	24 (49%)	49	
	M	57 (62%)	35 (38%)	92	
Age					0.3187
	≤ 55	14 (52%)	13 (48%)	27	
	>55	68 (60%)	46 (40%)	114	
Stage					0.0226
	I/II	47 (64%)	26 (36%)	73	
	III/IV	26 (47%)	29 (53%)	55	
	unknown			13	
T					<0.0001
	T1	4 (80%)	1 (20%)	5	
	T2	27 (57%)	20 (43%)	47	
	T3	39 (63%)	23 (37%)	62	
	T4	12 (46%)	14 (54%)	26	
	unknown			1	
N					0.001
	N0	34 (65%)	18 (35%)	52	
	N1	21 (62%)	13 (38%)	34	
	N2	11 (55%)	9 (45%)	20	
	N3	12 (39%)	19 (61%)	31	
	unknown			4	
M					0.0886
	M0	74 (60%)	50 (40%)	124	
	M1	8 (47%)	9 (53%)	17	

low expression of APTR is related to high tumor stage (p <0.0001), tumor invasion (p = 0.001), and distant metastasis (p = 0.0886) (Table 1). Further analysis in GSE66229 dataset showed that low expression of APTR is associated with the mesenchymal phenotype subgroup (p<0.0001), EMT group (p<0.0001), diffuse group (p<0.0001), high tumor stage (p = 0.0336), tumor invasion (p = 0.0716), and distant metastasis (p = 0.0638) (Table 2). These results strongly indicated that low expression of APTR predicts a poor diagnosis and outcome in GC patients.

4 Silencing APTR promotes gastric cancer proliferation and invasiveness

To investigate the role of APTR in gastric cancer, we stably infected the gastric cancer cell line, AGS, with 2 shRNAs (Fig. 3G). Knocking down APTR increased the cell proliferation rate (Fig. 3H) and colony formation in AGS cells (Fig. 3I). Furthermore, the cell migration rate was also increased by silencing APTR (Fig. 3J). These data strongly suggest that APTR plays a tumor suppressor role in gastric cancer cells, which is consistent with its expression pattern in gastric tumor samples.

5 APTR expression is negatively associated with inflammatory signaling in the gastric tumor microenvironment

To analyze APTR's effect on the gastric tumor microenvironment, we divided the tumor samples in TCGA data into high APTR and Low APTR. And we analyzed the percentage of immune cells in each gastric tumor tissue through EPIC. The results showed significant differences in cancer-associated fibroblasts (CAFs), CD4, CD8, Endothelial, macrophages, and NK cells (Fig. 4A). CAFs and endothelial cells promote tumor progression through communication with cancer cells, while NK cells suppress tumor progression by killing cancer cells. Low-APTR gastric tumor samples showed a noticeably higher percentage of CAFs and endothelial cells, and a lower percentage of NK cells than high-APTR gastric tumor samples. Moreover, we further validated APTR's association with CAFs and endothelial cells by analyzing APTR's correlation with molecular markers of these specific cell types (Fig. 4B and 4C). We

Table 2
Correlation between APTR expression and different clinicopathological features in patients with gastric cancer in GSE66229 dataset.

Characteristics		APTR expression level		Total	p Value
		High (n = 150) No. (%)	Low (n = 150) No. (%)		
Sex					0.1193
	F	42 (42%)	59 (58%)	101	
	M	108 (54%)	91 (46%)	199	
Age					0.1566
	≤ 55	32 (42%)	44 (58%)	76	
	>55	118 (53%)	106 (47%)	224	
Subgroup					<0.0001
	EP	129 (60%)	87 (40%)	216	
	MP	21 (25%)	63 (75%)	84	
Recurrence					0.5717
	Yes	59 (47%)	66 (53%)	125	
	No	91 (52%)	84 (48%)	175	
MLH1 IHC					0.6714
	Positive	115 (49%)	121 (51%)	236	
	Negative	34 (53%)	30 (47%)	64	
ACRG.sub					<0.0001
	MSI	33 (49%)	35 (51%)	68	
	EMT	7 (15%)	39 (85%)	46	
	TP53 positive	41 (52%)	38 (48%)	79	
	TP53 negative	70 (65%)	37 (35%)	107	
EBV ISH					0.1177
	Negative	132 (51%)	125 (49%)	257	
	Positive	7 (39%)	11 (61%)	18	
	NA	11 (44%)	14 (56%)	25	
LAUREN					<0.0001
	intestinal	92 (61%)	58 (39%)	150	
	diffuse	57 (40%)	85 (60%)	142	
	mixed	1 (13%)	7 (88%)	8	
PNI					>0.9999
	Positive	43 (49%)	45 (51%)	88	
	Negative	79 (50%)	80 (50%)	159	
	NA	28 (53%)	25 (47%)	53	
Venous Invasion					0.0232
	Positive	27 (61%)	17 (39%)	44	
	Negative	57 (44%)	72 (56%)	129	
	NA	66 (52%)	61 (48%)	127	
lymphovascular invasion					0.8876
	Positive	102 (50%)	103 (50%)	205	
	Negative	38 (52%)	35 (48%)	73	
	NA	10 (45%)	12 (55%)	22	
Stage					0.0336
	I/II	75 (59%)	52 (41%)	127	
	III/IV	75 (43%)	98 (57%)	173	
T					0.0716
	T2	102 (54%)	86 (46%)	188	
	T3	40 (44%)	51 (56%)	91	
	T4	8 (38%)	13 (62%)	21	
N					0.1869
	N0	18 (47%)	20 (53%)	38	
	N1	72 (55%)	59 (45%)	131	
	N2	33 (41%)	47 (59%)	80	
	N3	27 (53%)	24 (47%)	51	
M					0.0638
	M0	140 (51%)	133 (49%)	273	
	M1	10 (37%)	17 (63%)	27	
# of positive node (+)					0.8408
	Negative	18 (47%)	20 (53%)	38	
	≤ 10	91 (50%)	90 (50%)	181	
	>10	41 (51%)	40 (49%)	81	

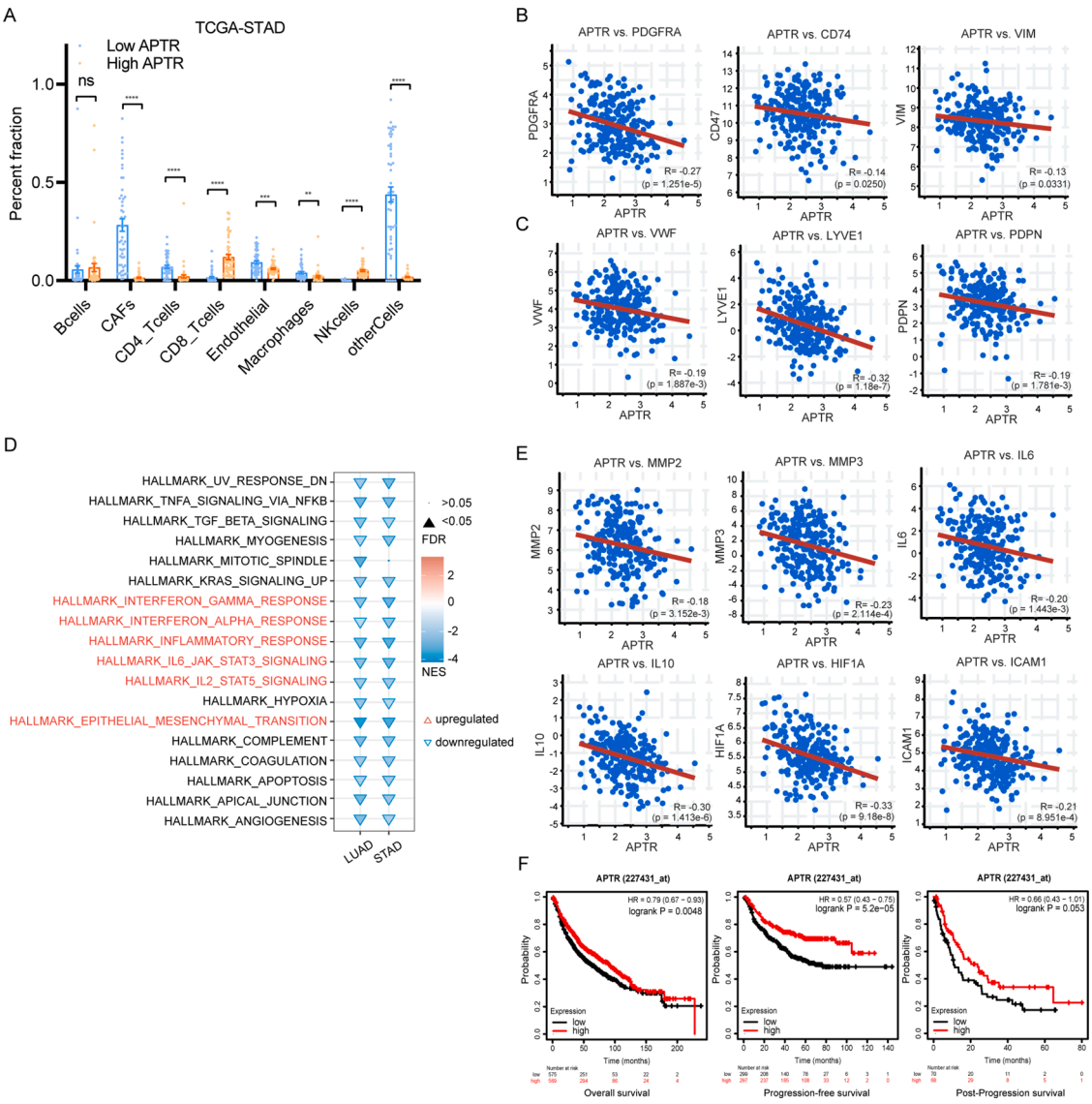


Fig. 4. APTR expression is negatively correlated with inflammatory signaling in the gastric tumor microenvironment. A. analysis of the percentage of immune cells in gastric tumor tissue through EPIC. B. APTR's correlation with molecular markers of CAFs. C. APTR's correlation with molecular markers of endothelial cells. D. analysis of APTR-regulated signaling pathways through lncGSEA. E. APTR's negative correlation with NF-κB and STAT signaling downstream targets in TCGA GC RNAseq data. F. APRT is negatively correlated with overall survival, progression-free survival, and post-progression survival in patients with lung cancer.

next investigated APTR-regulated signaling pathways through lncGSEA [16]. Surprisingly, APTR's expression in the gastric tumor is negatively correlated with inflammatory signalings, such as interferon responses, inflammatory responses, NF-κB, JAK-STAT, and EMT, in both lung tumor and gastric tumor (Fig. 4D), which is consistent with APTR's negative association with CAFs and endothelial cell proportion. We next confirmed APTR's negative correlation with NF-κB and STAT signaling downstream targets (Fig. 4E). As expected, the expression of APRT is also negatively correlated with survival rates in patients with lung cancer by Kaplan-Meier Plotter (Fig. 4F). Taken together, our analysis showed that APTR expression is negatively correlated with inflammatory signaling in the gastric tumor microenvironment.

Discussion

In this study, we comprehensively analyzed the differentially expressed lncRNAs in paired tissues from gastric cancer. We identified 441 lncRNAs differentially expressed in gastric tumor tissues and normal tissues. These deregulated lncRNAs could participate in gastric tumorigenesis. In this study, we mainly focused on APTR which has not been studied in gastric cancer. Our findings showed that low expression of APTR is related to gastric cancer progression and APTR is a favorable biomarker in gastric cancer.

It has been well accepted that lncRNAs regulate multiple signaling pathways and have significant functions in various cellular processes and disease progression. Several lncRNAs identified in our study have been reported to take part in gastric cancer progression. For instance, lncRNA CTBP1-AS2 has been shown to promote hepatocellular carcinoma cell proliferation by sponging miR-623 [23] and miR-195–5p [24] and facilitate colorectal cancer development by inhibiting miR-93–5p [25]. As in gastric cancer, CTBP1-AS2 was shown to increase MMP11 expression via suppressing miR-139–3p [26], leading to gastric cancer progression. In our study, we found lncRNA APTR is significantly lower expressed in gastric tumor tissues and its expression in gastric cancer is negatively correlated with tumor grade, stage, invasion, and metastasis. Lower expression of APTR in gastric cancer is associated with worse survival in GC patients. Our study showed that silencing APTR promoted gastric cancer cell growth and invasiveness. our findings are consistent

with the previous report about APTR's role in thyroid cancer [12], in which APTR is lower expressed in thyroid tumor tissues than in normal tissues, and is negatively related to TNM stages, and distant metastasis. We further discovered that APTR's expression is regulated by enhancers, and transcription factors, such as ZBTB48, SPI1, IKZF1, and MAX, are very likely to regulate APTR's expression in gastric tumor. Since immunotherapy is one of the common treatment options for gastric cancer, analyzing APTR's association with tumor-infiltrating immune cells may provide some indication for the treatment. For example, CAFs are one of the major components of tumor stroma and can stimulate tumor progression through secreting various inflammatory factors [27–29]. Besides, endothelial cells in the tumor microenvironment are known to generate blood vessels and lymph channels, which are hallmarks of malignant disorders [30]. Noticeably, low APTR expression in gastric tumor tissues is significantly correlated with a high percentage of CAFs and endothelial cells, and a low percentage of NK cells in the gastric cancer microenvironment, consistent with low APTR's association with the aggressiveness in gastric cancer, suggesting that APTR may participate in gastric tumor progression through influencing gastric tumor microenvironment. Immune checkpoint inhibitors, such as PD-L1 inhibitor pembrolizumab has been granted by USFDA for the treatment of advanced gastric cancer [31]. It has been shown that interferon receptor signaling pathways and the JAK-STAT pathway could regulate PD-L1 expression, therefore affecting the clinical responses to PD-1 blockade therapy [32]. We analyzed APTR-related signaling pathways in both lung adenocarcinoma and gastric cancer, in both of which APTR is surprisingly negatively correlated with multiple inflammatory signaling pathways, such as interferon responses, inflammatory responses, NF-κB, JAK-STAT, implying that gastric cancer patients with low APTR expression may have a better PD-1 blockade therapy response than these with high APTR expression. Additionally, we also find that low APTR expression is correlated with the EMT signaling pathway, which is consistent with APTR's clinical relevance.

In summary, our study showed that low expression of lncRNA APTR in gastric cancer is correlated with tumorigenesis and poor diagnosis and prognosis, which is a potential biomarker for gastric cancer patients' diagnosis and prognosis, and could be a potential therapeutic target.

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Declaration of Competing Interest

The authors declare no conflicts of interest.

Acknowledgments

Not applicable.

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