

A functional variant at miR-132-3p, miR-212-3p, and miR-361-5p binding site in *CD80* gene alters susceptibility to gastric cancer in a Chinese Han population

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Abstract A number of single-nucleotide polymorphisms within the 3'-UTR of genes have been shown to relate to the occurrence of cancers. In this study, by using polymerase chain reaction–restriction fragment length analysis method, we determined an SNP rs1599795 in the 3'-UTR of *CD80* gene in 183 gastric cancer patients and 348 healthy controls. Statistical analysis results showed that SNP rs1599795 genotypes were significantly correlated with the risk of gastric cancer. Compared with the AA homozygotes, the TA heterozygotes were significantly more prevalent in the patients (OR 1.44, 95 % CI 0.98–2.11) with a larger tumor size ($P = 0.001$), deeper infiltration ($P = 1.5 \times 10^{-5}$), higher possibility of lymph node metastasis ($P = 0.003$), and more in the late stage (TNM stage III and IV; $P = 0.003$); the TT homozygotes had larger tumor size ($P = 0.001$) and lower degree of differentiation ($P = 2.2 \times 10^{-4}$). Dual-luciferase reporter assays showed that miR-132-3p, miR-212-3p, and miR-361-5p inhibited the expression of *CD80* through binding with the *CD80* 3'-UTR, and this inhibitory role of miR-

132-3p, miR-212-3p, and miR-361-5p was impacted by rs1599795. Our findings have shown that the SNP rs1599795 in *CD80* 3'-UTR, through disrupting the regulatory role of miR-132-3p, miR-212-3p, and miR-361-5p in *CD80* expression, contributed to the occurrence of gastric cancer.

Keywords *CD80* · Single-nucleotide polymorphism · microRNA · Gastric cancer

Introduction

Although with the continuous improvement of diagnostic techniques and surgical treatment, the survival rate of patients with gastric cancer has been improved [1]; gastric cancer is still the second cause of deaths from cancers worldwide [2]. It is estimated that 40 % of gastric cancer occurred in China [2]. The occurrence and development of gastric cancer is a multiple-factor and multiple-stage process involving genetic and epigenetic alterations of protein-coding proto-oncogenes and tumor suppressor genes [3].

CD80, an important co-stimulatory ligand, binding to its corresponding receptor *CD28* or *CTLA-4* [4], is essential for the activation and regulation of T-cell immunity [5]. *CD80* is expressed on a variety of normal epithelial tissue, such as skin, respiratory tract, gastrointestinal tract, and genitourinary tract [6]. However, low expression of *CD80* has been found in the majority of tumors, which cannot induce effective anti-tumor immunity response, resulting in tumor immune escape [7]. Almost all patients with benign gastric mucosa and primary gastric carcinoma showed high levels of expression of the *CD80*; however, the percentage of *CD80*-positive cells of well-differentiated carcinoma and normal mucosa was significantly higher than that of

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poorly differentiated primary carcinomas. Furthermore, almost all of the metastatic carcinoma cells revealed consistently very low or undetectable levels of expression of the *CD80* molecule [8]. The expression of the *CD80* mRNA was observed in the process of the development of cancer, but the protein level of *CD80* decreased along with the development of cancer [9], indicating that there are some posttranscriptional regulation mechanism mediated in the expression of *CD80* gene.

MicroRNA (miRNAs), a class of small noncoding RNAs of about 22 nucleotides, play a posttranscriptional regulation role in gene expression by binding to the 3'-untranslated regions (UTRs) of target messenger RNAs (mRNAs), leading to translational repression or gene silencing [9, 10]. miRNA genes represent only a small part of human genome, but they can regulate almost one-third of all the human genes [11]. Recent studies have shown that miRNAs were associated with a variety of physiological and pathological processes, including cell differentiation, value-added, apoptosis, insulin secretion, cholesterol synthesis, and tumorigenesis [12]. miRNA can inhibit the translation of a target gene by binding to the target mRNA with 6–8 complementary nucleotides, which is usually restricted to the 2–8 nucleotides of the 5'-end of the miRNA, and this sequence is referred to as “seed region” [13]. If variation occurs in this region, it will seriously affect the regulatory role of miRNA. Single-nucleotide polymorphisms (SNPs), the most common human genetic variations, have been proved to be significantly related to the occurrence of diseases including gastric cancer. More and more studies have provided evidences that SNPs located in the miRNA (miRSNPs) binding sites through affecting the binding of miRNAs with the target genes resulted in reduction or increase in the target mRNA translation, and thus being associated with the susceptibility to gastric cancers [10]. Wu et al. [14] pointed out that the rs2910164 polymorphism in the sequence of miR-146a precursor may influence the susceptibility to gastric cancer in the Chinese population. We have also found that SNPs rs4143815 and rs4819388 in the 3'-UTRs of *B7-H1* and *B7-H2* genes were significantly related to the occurrence of gastric cancer [15, 16]. In this study, we used the case-control method to investigate the relationship between miRSNPs in the 3'-UTR of *CD80* gene and the risk of gastric cancer. In addition, we also assessed the effect of the miRSNPs on the regulatory role of miRNAs in the expression of the *CD80* protein.

Materials and methods

Study subjects

A total of 531 genetically unrelated subjects including 348 normal controls and a total of 183 gastric cancer patients

Table 1 Characteristics and clinicopathologies of the patients

Characteristics	Cases, <i>n</i> (%)	Controls, <i>n</i> (%)
Gender		
Male	135 (73.8)	223 (64.1)
Female	48 (26.2)	125 (35.9)
Age		
≤60	87 (47.5)	289 (83.0)
>60	96 (52.5)	59 (17.0)
Degree of differentiation		
Low	104 (56.8)	
Middle	56 (43.2)	
Site		
Antral	92 (50.3)	
Others	91 (49.7)	
Tumor size		
≤25	117 (63.9)	
>25	66 (36.1)	
Maximal diameter of tumor		
≤5	117 (63.9)	
>5	66 (36.1)	
TNM stage		
I + II	43 (23.5)	
III + IV	140 (76.5)	

participated in this study after giving written informed consent. They were recruited from the First Affiliated Hospital of Soochow University between March 2007 and May 2009. All subjects were Han Chinese and were raised in Suzhou. They were histologically confirmed by two pathologists. Individuals those were previously cancerous or had metastasizing cancer from other origins were excluded. For gastric cancer patients, the clinicopathological variables, including tumor markers, tumor location, histological type, tumor size, tumor area, differentiation grade, depth of tumor infiltration, lymph node metastasis, distant metastasis, and TNM stage, were obtained from the medical records. None of the patients had undergone radiotherapy or chemotherapy before the surgery. The variables of depth in tumor infiltration, lymph node metastasis, distant metastasis, and TNM stage were examined and staged according to the American Joint Commission for Cancer Staging in 2002. The controls have no gastrointestinal disorders and personal or familial history of cancers, which was traced back ≥3 generations and laterally to second- and third-degree relatives. They were obtained from the hospital of patients' routine health examinations. The research protocol was approved by the institutional review board of Soochow University.

In the patients with gastric cancer, 92 and 91 cancers were occurred at the antrum and others, respectively; 56 and 104 cases were moderately and poorly differentiated,

respectively; 11, 19, 145, and 8 tumors were at T1, T2, T3, and T4 stages, respectively; 140 and 32 patients were with lymph node metastasis and distant metastasis; 19, 24, 100, and 40 patients were at TNM I, II, III, and IV stages, respectively (Table 1).

Genomic DNA samples

The whole blood samples from patients and controls were collected and stored in Vacutainer® tubes (BD Franklin Lakes, NJ) containing anticoagulant of EDTA. Total genomic DNA was extracted from the whole blood according to the phenol/chloroform method. The purity and concentration of the extracted DNA were determined by UV-Vis spectrophotometer. The extracted DNA was stored at 4 °C in TE buffer (10 mmol/l Tris-HCl, 1 mmol/l EDTA, pH 8.0).

Selection of miRSNPs

First, the SNPs in the 3'-UTR of the *CD80* gene were obtained from the published databases of NCBI dbSNP BUILED 129 (<http://www.ncbi.nlm.nih.gov/SNP>) and ENSEMBL v58 (<http://www.ensembl.org/>). From the HapMap, the reported SNP allele frequency selected a minor allele frequency (MAF) of not less than 10 % of the SNPs. Then, the online softwares of Diana-Micro (<http://diana.cslab.ece.ntua.gr/microT/>), MicroInspector (<http://miRNA.imbb.forth.gr/microinspector/>), miR-anda (<http://www.microrna.org/>), miRNAMap (<http://miRNAMap.mbc.nctu.edu.tw/>), RNAhybrid (<http://bibiserv.techfak.uni-bielefeld.de/rnahybrid/>), and Target Scan v5.1 (<http://targets.can.org/>) were used to predict the possible miRNA binding sites in *CD80* 3'-UTR. The SNPs located in putative miRNA binding sites were regarded as candidate miRSNPs.

SNP genotyping

The genotypes of the SNP rs1599795 (A>T) were determined by using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis, which were validated by Sanger's DNA sequencing method (Gene Script, Nanjing, China). Specific measurement procedure is as follows:

The PCR was performed in 25 µl containing 10 mM Tris-HCl (pH 8.0), 50 mM KCl, 1.5 mM MgCl₂, 0.2 mM of each dNTP, 0.4 µM of forward primer (5'-GGTGTT TACCCAGTATTC C-3') and reverse primer (5'-CA AATGAGGTCAAACCTAG C-3'), 1 µl of genomic DNA (~100 ng), and 0.5 U *Taq* DNA Polymerase (MBI). The PCR cycling was performed in a PTC-200 DNA Tetrad thermal cycler (Bio-Rad) as follows: 94 °C for 5 min,

followed by 30 cycles of 94 °C for 20 s, 56 °C for 30 s, and 72 °C for 30 s. Final extension was completed at 72 °C for 7 min. Then, 10 µl of the PCR products was digested with 10 U of restriction enzyme *TaqI* for 8 h at 37 °C in 20 µl of mixture. After heat inactivation for 15 min at 70 °C, 10 µl of the digested DNA fragments was analyzed at room temperature by electrophoresis on a 2 % agarose (LP0028A, Oxoid, Hampshire, England) gel containing 0.5 mg/ml ethidium bromide at 100 V for 20 min in 1× TAE buffer. Images of the gels were taken using GeneGenius BioImaging Systems (SynGene, Cambridge, England). The SNP genotypes of TT, TA, and AA were determined according to the size of the electrophoretic band patterns of 196/43, 239/196/43, and 239 bp, respectively.

Cell culture

The CHO cells were purchased from American Type Culture Collection (Manassas, VA). They were cultured in RPMI 1640 medium (Invitrogen) supplemented with 10 % fetal bovine serum (GBICO BRL, Rockville, MD, USA) at 37 °C in a humidified incubator with 5 % CO₂. Cells in the logarithmic growth phase were used for experiments.

Luciferase reporter assays

To generate *CD80*/3'-UTR/pGL3 luciferase reporter constructs, the *CD80* 3'-UTR was amplified with the primers of 5'-GGCTAGTCTAGATAGCTCTGGTGACCTTGATC-3' (forward) and 5'-GGCTAGTCTAGACTTCCCTTAGTA TTGCTGAC-3' (reverse). They were then cloned in the downstream of the firefly luciferase gene in pGL3-Control vector (Promega) according to the manufacturer's protocol. Positive clones were selected by restriction digestion using *XbaI* (MBI) and confirmed by DNA sequencing method (GenScript, Nanjing, China).

To generate luciferase reporter constructs containing the potential binding site for miR-132-3p, miR-212-3p, and miR-361-5p, two pairs of nucleotide oligos of 5'-CATA TTGGACTGTAATCTCTTTAAAT G-3' (forward; the allele is underlined) and 5'-CATTTAAAGAGATTATTCA GTCCAATAT G-3' (reverse), and 5'-CATATTGGACTG TTAAATCTCTTTAAAT G-3' (forward; the allele is underlined) and 5'-pCATTTAAAGAGATTAACAGTCCA ATAT G-3' (reverse) were annealed and cloned downstream of the firefly luciferase gene in pGL3-Control vector according to the manufacturer's protocol, respectively. Positive clones were confirmed by DNA sequencing method.

For luciferase reporter assays, the CHO cells were plated at 5 × 10⁴ cells per well in 24-well plate and were transfected with lipofectamine 2000 (Invitrogen) according to

the manufacturer's recommendations after 24 h. Each co-transfection reaction contained 200 ng of pGL3 construct, 200 ng of pRL-TK plasmid (Promega) that served as a normalizing control, and 50 μ M of miR-132-3p, miR-212-3p, or miR-361-5p mimic (GenePharma Inc., Shanghai, China). The negative controls were performed with an unrelated miRNA sequence (5'-UUCUCCGAACGUGU CACGUTT-3'). The transfected CHO cells were maintained in RPMI 1640 medium with 10 % FBS. Each transfection was carried out in triplicate. After 24 h of incubation, cells were collected and analyzed for luciferase activity with the dual-luciferase reporter assay system (Promega).

Statistical analysis

Chi-square test was used to evaluate the differences in frequency distributions of each allele and genotype of the miRSNPs between the cases and controls. Chi-square test was also used to assess the difference between the miRSNPs and the clinicopathological features. The crude and adjusted odds ratios (ORs) and 95 % confidence intervals (CIs) were obtained to assess the association of the miRSNPs with gastric cancer risk using unconditional univariate and multivariate logistic regression models, respectively. Hardy–Weinberg equilibrium of the genotype distribution among control groups was tested by a goodness-of-fit chi-square test. The genotypes were further stratified by subgroups of the clinicopathological variables. All of the statistical analyses were carried out by using STATA software (version 10.0; StataCorp LP, TX, USA) and were done by two persons independently in a blind fashion. For all tests, $P < 0.05$ was considered statistically significant.

Results

The miRSNPs in the CD80 gene 3'-UTR

First, we found 21 SNPs from the SNP databases NCBI db SNP BUILED 129 and ENSEMBL v58 in the 3'-UTR of *CD80* gene, as shown in Table 2. The allele frequencies in the Chinese population (<http://www.hapmap.org/index.html.en>) are shown in Table 2. Among them, the minor allele frequencies (MAF) of the SNPs rs1599795, rs1599796, rs17281703, rs57271503, and rs7628626 are greater than 10 %.

Then, we used bioinformatic softwares Diana-Micro, MicroInspector, miR-anda, miRNAMap, RNAhybrid, and Target Scan to predict miRNAs that can bind to the *CD80* 3'-UTR. In the SNPs with MAF of more than 10 %, we found that the SNPs rs1599795 and rs1599796 were

Table 2 SNPs located in the *CD80* gene 3'-UTR and the predicted miRNAs

SNP	Nucleotide change	MAF ^a	Position on 3'-UTR	miRNA ^b
rs114679726	C>G	NA	702	
rs115823839	C>T	NA	326	
rs11916344	C>T	T = 0.012	1,483	miR-553
rs138498602	A>G	NA	797	
rs142483532	insCT	NA	1,300	
rs144353012	C>T	NA	153	
rs149639969	C>T	NA	464	
rs1599795	A>T	T = 0.267	780	miR-361, miR-132, miR-212
rs1599796	G>A	A = 0.289	701	miR-550
rs17281703	G>A	A = 0.156	1,086	NA
rs180804720	C>T	NA	35	
rs182875317	A>G	NA	789	
rs182900553	A>C	NA	559	
rs185399972	A>G	NA	1,447	
rs187767204	C>T	NA	717	
rs190655233	C>G	NA	1,444	
rs193014753	A>G	NA	612	
rs57271503	G>A	A = 0.258	42	NA
rs74592427	A>G	NA	1,265	
rs7628626	C>A	A = 0.105	214	NA
rs79179279	insT	NA	1,443	

^a Minor allele frequency

^b Confirmed by more than two softwares

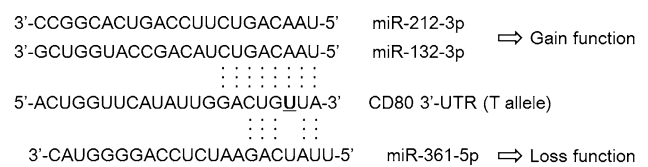


Fig. 1 Schematic of CD80 3'-UTR (T allele) harbors putative binding sites of miR-132-3p and miR-212-3p, and loss of the binding site of miR-361-5p. The SNP rs1599795 A>T is located within the “seed region” of the miR-132-3p, miR-212-3p, and miR-361-5p

located in the miRNA binding sites. Based on the genotype distribution of these two SNPs in 45 healthy Chinese published by HapMap (<http://www.hapmap.com>), statistical analysis results showed approximately full linkage disequilibrium between the two SNPs. Furthermore, we found that the SNP rs1599795 was located in the binding sites of miR-132-3p, miR-212-3p, and miR-361-5p (Fig. 1). Thus, we investigated the association of this SNP with the risk of gastric cancer.

To study the relationship between the SNP and gastric cancer, we used polymerase chain reaction–restriction fragment length polymorphism analysis method for the

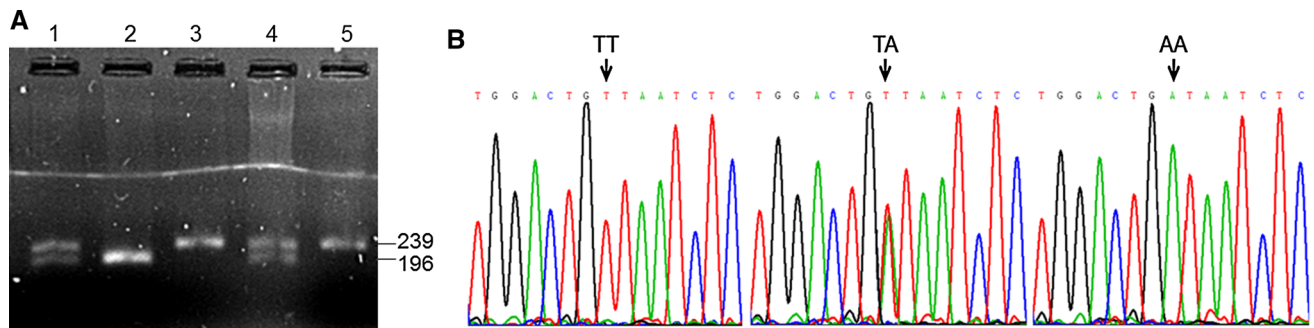


Fig. 2 Typical genotyping results. **a** The PCR–RFLP results of 5 samples. The genotypes of samples 1–5 are TA, TT, AA, TA, and AA, respectively. **b** The DNA sequencing results of 3 genotypes. *Arrows* indicate the location of SNP sites

Table 3 Rs1599795 genotype distribution in patients and controls and their correlation with gastric cancer

Genotype	Case, n (%)	Control, n (%)	P value	OR (95 % CI)
AA	78 (42.6)	182 (52.3)		1.00
TA	82 (44.8)	133 (38.2)	0.065	1.44 (0.98–2.11)
TT	23 (12.5)	33 (9.5)	0.116	1.63 (0.90–2.95)
T-allele carrier	105 (57.4)	166 (47.7)	0.036	1.48 (1.03–2.12)

determination of the SNP genotype distribution in 183 gastric cancer patients and 348 controls. The genotyping results of 5 samples and typical DNA sequencing results are shown in Fig. 2a, b.

Correlation of rs1599795 with gastric cancer

The SNP rs1599795 genotype frequencies and its correlation with gastric cancer are shown in Table 3. Chi-square (χ^2) statistical analysis results showed that the genotypes of rs1599795 were in Hardy–Weinberg equilibrium distribution pattern in the healthy control group ($P = 0.48$). Further, logistic regression analysis results revealed that a significant increased risk of gastric cancer was associated with the T-allele carriers (OR 1.48, 95 % CI 1.03–2.12; $P = 0.036$) as compared with the AA genotype.

Then, we did stratified analysis of the association of the rs1599795 genotypes with the clinicopathological parameters of gastric cancer (Table 4). We found a significant association of the rs1599795 genotypes with the tumor size, tumor differentiation, tumor location, depth of invasion, lymph node metastasis, distant metastasis, and TNM staging. Compared with the AA homozygote, the carriers of TA genotype were more likely to have the gastric cancer in the antrum outside parts ($P = 0.019$), with larger tumor sizes ($P = 0.001$), more in the late TNM stages (stages III

and IV; $P = 0.003$), deeper infiltration ($P = 1.5 \times 10^{-5}$), and higher possibility of lymph node metastasis ($P = 0.003$). Furthermore, the TT homozygous patients had larger tumor sizes ($P = 0.001$), lower differentiation degree of tumor cells ($P = 2.2 \times 10^{-4}$), and less possibility of distant metastases ($P = 0.006$) than the AA homozygous patients.

In addition, statistical analysis results showed that the A-allele frequency was significantly higher in the patients than that in the control group (35 vs. 28.6 %; $P = 0.036$), indicating that the A allele is associated with the incidence of gastric cancer.

The effect of rs1599795 on the regulatory role of miR-132-3p, miR-212-3p, and miR-361-5p in CD80 expression

Since the SNP rs1599795 was predicted to locate in the binding site of miR-132-3p, miR-212-3p, and miR-361-5p, we hypothesized that the expression of CD80 is regulated by these microRNAs, which can be impacted by rs1599795. To test whether or not the inhibitory role of miR-132-3p, miR-212-3p, and miR-361-5p is impacted by rs1599795, we constructed pGL3 vectors including A-allele- and T-allele-specific binding sequences, and then co-transfected it with miR-132-3p, miR-212-3p, or miR-361-5p in CHO cells. We found that the expression of T-allele-specific pGL3 construct was significantly suppressed by miR-132-3p ($P = 0.003$; Fig. 3b) and miR-212-3p ($P = 0.018$; Fig. 3b). However, the expression of A-allele-specific pGL3 construct was not inhibited by miR-132-3p ($P = 0.458$) and miR-212-3p ($P = 0.659$; Fig. 3a). In addition, the miR-132-3p significantly suppressed the expression of A-allele-specific pGL3 construct ($P = 0.006$; Fig. 3a), instead of T-allele-specific pGL3 construct ($P = 0.092$; Fig. 3a). These findings suggested that the inhibitory roles of miR-132-3p, miR-212-3p, and miR-361-5p in the expression of CD80 were affected by rs1599795.

Table 4 Stratified analysis of rs1599795 genotype with clinicopathological parameters of gastric cancer

Pathological parameters	Genotype				TA versus AA		TT versus AA		T carrier versus AA	
	AA	TA	TT	T carrier	P value	OR (95 % CI)	P value	OR (95 % CI)	P value	OR (95 % CI)
Tumor site										
Antral/Others	48/30	35/47	9/14	44/61	0.019	2.149 (1.142–4.043)	0.093	2.489 (0.959–6.459)	0.011	2.218 (1.219–4.036)
Tumor size										
>25/≤ 25	16/62	37/45	13/10	55/50	0.001	3.186 (1.581–6.422)	0.001	5.038 (1.87–13.568)	<0.001	0.235 (0.120–0.458)
Degree of differentiation										
Low/Middle	39/24	42/32	23/0	65/32	0.603	1.238 (0.624–2.458)	<0.001	0.629 (0.520–0.762)	0.611	0.800 (0.413–1.550)
Maximal diameter of tumor										
>5/≤ 5	26/52	31/51	9/14	65/40	0.621	1.216 (0.635–2.326)	0.625	1.286 (0.492–3.360)	<0.001	0.308 (0.167–0.568)
TNM stage										
I + II/III + IV	27/51	11/71	5/18	16/89	0.003	3.417 (1.554–7.514)	0.312	1.906 (0.638–5.698)	0.003	2.945 (1.451–5.976)
Depth of tumor invasion										
pT1 + pT2/pT3 + pT4	22/56	3/79	5/18	8/97	<0.001	10.345 (2.952–36.25)	0.604	1.414 (0.468–4.278)	<0.001	4.763 (1.989–11.41)
Lymph node metastasis										
No/Yes	27/51	11/71	5/18	16/89	0.003	3.417 (1.554–7.514)	0.312	1.906 (0.638–5.698)	0.003	2.945 (1.451–5.976)
Distant metastases										
No/Yes	58/20	70/12	23/0	93/12	0.113	0.497 (0.224–1.102)	0.006	0.716 (0.624–0.821)	0.017	0.374 (0.170–0.822)

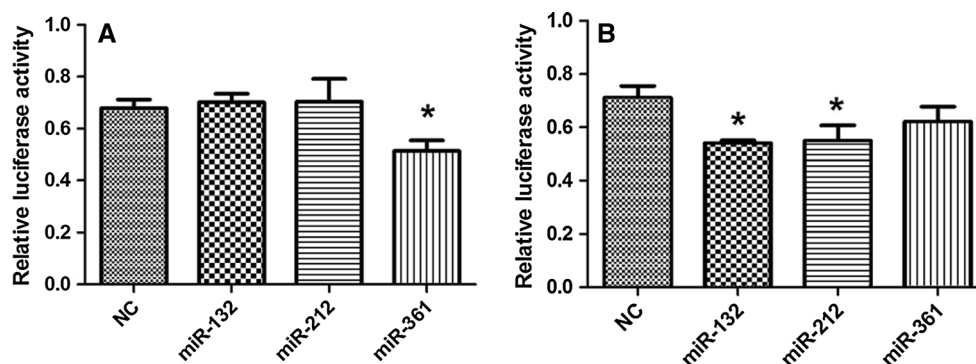


Fig. 3 Effect of rs1599795 on the inhibitory role of miR-132-3p, miR-212-3p, and miR-361-5p in CD80 expression. **a** The expression of A-allele-specific pGL3 construct was obviously inhibited by miR-361-5p ($P = 0.006$). **b** The expression of T-allele-specific CD80/3'-UTR/pGL3 luciferase was significantly suppressed by miR-132-3p

($P = 0.003$) and miR-212-3p ($P = 0.018$), but not by miR-361-5p ($P = 0.092$). The negative controls (NC) were performed with an unrelated miRNA sequence (5'-UUCUCCGAACGUGUCACGUTT-3'), respectively

Discussion

In the present study, we investigated the relationship between miRSNPs within the 3'-UTR of *CD80* gene and the risk of gastric cancer. We found that an SNP rs1599795, locating in the binding sites of miR-132-3p, miR-212-3p, and miR-361-5p, through disrupting the inhibitory role of miR-132-3p, miR-212-3p, and miR-361-5p on *CD80* expression, played an important role in the development of gastric cancer. Compared with the AA genotype, both the TA genotype and T-allele carriers had a substantially higher risk of gastric cancer. Furthermore, the TA heterozygotes were significantly more prevalent in the patients (OR 1.44, 95 % CI 0.98–2.11) with a larger tumor

size ($P = 0.001$), deeper infiltration ($P = 1.5 \times 10^{-5}$), higher possibility of lymph node metastasis ($P = 0.003$), and more in the late stage (TNM stages III and IV; $P = 0.003$); the TT homozygotes had larger tumor size ($P = 0.001$) and lower degree of differentiation ($P = 2.2 \times 10^{-4}$). These findings indicated that the T allele might be a risk factor for the occurrence and development of gastric cancer.

CD80, as an important co-stimulatory ligand, by binding with its receptor *CD28*, plays an important role in the cell-mediated immune responses [17]. Studies have demonstrated that the transfection of *CD80* into tumor cells resulted in remarkable reduction of tumor metastasis [18]. However, most tumors including gastric cancer had no or

low expression of *CD80*, leading to unresponsive T cells for the specific antigens and ineffective anti-tumor immune response [19, 20].

Numerous microRNAs have been reported to be deregulated in cancers; however, the consequence of miRNA deregulation in cancer is still unknown for many miRNAs [21]. As for the miRNAs of interest in this study, miR-132 expression was found to be increased in lung cancer [22–24], endocrine pancreatic tumors [22], and colorectal carcinoma [25]. Increased miR-212 expression was reported in colorectal carcinoma [25] and gastric cancer [26], but it was also shown to be down-regulated in gastric cancers [27, 28]. miR-361 expression is increased in bladder cancer [29]. In this study, we also found T allele of SNP rs1599795 that lead to *CD80* lost one miRNA of miR-361-5p inhibition, but intensify inhibition by two miRNAs miR-132-3p and miR-212-3p, thus lead to low expression of *CD80*, which may be involved in cancer immune evasion. That may be an important part in number of mechanisms regulating expression in *CD80*.

In summary, we reported the first evidence that the SNP rs1599795 in *CD80* 3'-UTR was involved in the occurrence of gastric cancer. This SNP was also found to be related to the clinicopathological features of gastric cancer, suggesting it may have important roles in tumor development. Furthermore, we provided a novel insight into the regulatory mechanism of *CD80* expression mediated by miR-132-3p, miR-212-3p, and miR-361-5p in gastric cancer.

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Conflict of interest The authors declare no conflict of interest.

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