International Journal of General Medicine

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Polymorphisms in miRNA Genes Targeting the AMPK Signaling Pathway are Associated with Cervical Cancer Susceptibility in a Han Chinese Population

Xueya Chen[1,*], Zhiling Yan[2,*], Weipeng Liu[1], Lili Guo[1], Jinmei Xu[2], Li Shi[3], Yufeng Yao[1]

[1]Institute of Medical Biology, Chinese Academy of Medical Sciences & Peking Union Medical College, Kunming, People's Republic of China; [2]Department of Gynaecologic Oncology, The No. 3 Affiliated Hospital of Kunming Medical University, Kunming, People's Republic of China; [3]Department of Immunogenetics, Institute of Medical Biology, Chinese Academy of Medical Sciences & Peking Union Medical College, Kunming, People's Republic of China

*These authors contributed equally to this work

Correspondence: Li Shi, Department of Immunogenetics, Institute of Medical Biology, Chinese Academy of Medical Sciences & Peking Union Medical College, Kunming, Yunnan, 650118, People's Republic of China, Email shili.imb@gmail.com; Yufeng Yao, Institute of Medical Biology, Chinese Academy of Medical Sciences & Peking Union Medical College, Kunming, Yunnan, 650118, People's Republic of China, Email leoyyf@gmail.com; yufeng_yao@imbcams.com.cn

Purpose: Cervical cancer (CC) poses a significant threat to women's health worldwide, and multiple signaling pathways have been confirmed to be involved in its development. The AMPK signaling pathway plays a central role in maintaining energy homeostasis, and its dysregulation is closely associated with the occurrence of CC. Changes in microRNA (miRNA) expression levels might be related to the AMPK signaling pathway. Single nucleotide polymorphisms (SNPs) can affect the function of miRNA and result in the development of CC. To investigate the association between the SNPs of AMPK pathway-associated miRNAs and CC in a Han Chinese population, we selected eight miRNA genes located in the AMPK pathway and analyzed nine SNP loci within these genes to explore whether they are associated with genetic susceptibility to cervical intraepithelial neoplasia (CIN) and CC.

Methods: A total of 2,220 subjects were included in this study, including 928 healthy controls, 421 CIN patients, and 871 CC patients. Nine candidate SNPs (rs895819 in miR-27a, rs10061133 in miR-449b, rs41291179 in miR-216a, rs76481776 in miR-182, rs10406069 in miR-5196, rs12803915 and rs550894 in miR-612, rs66683138 in miR-3622b, and rs2620381 in miR-627) were genotyped using the TaqMan method.

Results: The results showed significant differences in the allele distribution of rs41291179 and rs12803915 between the control group and the CIN group, as well as between the control group and the CC group (all P values < 0.005). The A allele of rs41291179 and the G allele of rs12803915 were associated with decreased risk of CIN (OR = 0.05, 95% CI: 0.01–0.39; OR = 0.61, 95% CI: 0.49–0.76) and CC (OR = 0.08, 95% CI: 0.01–0.66; OR = 0.71, 95% CI: 0.59–0.86), respectively.

Conclusion: Our results suggest that polymorphisms in miRNA genes of the AMPK signaling pathway are associated with the development of CC.

Keywords: genotyping, SNP, gene enrichment, genetic susceptibility

Introduction

Cervical cancer (CC) ranks as the fourth most prevalent cancer among women worldwide.[1] If no action is taken, the World Health Organization (WHO) estimates that the annual number of new CC cases will rise from 570,000 to 700,000 between 2018 and 2030, with deaths increasing from 311,000 to 400,000 per year.[1,2] China has a heavy burden of CC, and the incidence and mortality of CC have shown an increasing trend over the past 20 years,[3] with 109,000 new CC cases and 59,000 deaths in 2020, accounting for 18.2% and 17.3% of global cases, respectively.[4]

Received: 10 June 2024
Accepted: 8 September 2024
Published: 16 September 2024

The occurrence, development, metastasis, and drug resistance of CC have been linked to various cell signaling pathways, including the PI3K/AKT, Wnt, Notch, NF-κB, MAPK-ERK, Hedgehog, and JAK/STAT pathways.[5–11] AMP-activated protein kinase (AMPK) serves as a receptor for cellular energy status, monitoring changes in ATP levels and phosphorylating substrates accordingly to regulate ATP precisely.[12,13] ATP is essential for cellular activities,[14,15] and the occurrence and development of malignant tumors rely heavily on an abundant supply of cellular energy. Thus, abnormal regulation of AMPK is closely associated with tumor development.[16,17]

miRNAs play a crucial role in cell differentiation and proliferation by binding to complementary target mRNAs, inhibiting mRNA translation, or degrading it.[18,19] miRNA undergoes two processing steps in the nucleus and cytoplasm for maturation, forming a complex and precise regulatory network. This network allows for a single miRNA to regulate expression of multiple genes and achieve fine regulation of gene expression through the combined regulation of multiple miRNAs.[20–23]

Numerous studies have reported the involvement of miRNAs in regulating components of the AMPK signaling pathway. These miRNAs may lead to abnormal expression of their target genes, resulting in signaling pathway disorders and related diseases. For instance, miR-27a,[24] miR-449a,[25] miR-216a,[26] miR-96,[27] and miR-5196[28] have been implicated in the occurrence of diseases by targeting the AMPK signaling pathway. Additionally, miR-612,[29] miR-3622b,[30] and miR-627[31] have been linked to the occurrence of tumors in the human reproductive system, with their putative target genes potentially playing a role in the AMPK pathway.

Genetic variation plays a significant role in miRNA maturation, with SNPs being the most common human genome genetic polymorphisms.[32,33] Pathway-associated miRNA gene profile expression changes caused by SNPs might be important for explaining the pathomechanism driving cancers.[34] For example, our previous studies reported that rs4636297 in pri-miR-126, rs11614913 in miR-196a2, rs107822 in miR-219a and rs2292832 in miR-149 are associated with CC risk.[8,35] In addition, Gilam A et al reported that rs1071738 in miR-96 is associated with the risk of breast cancer metastasis.[36] Therefore, SNPs are notable factors in regulating miRNAs, which have been shown to be associated with diseases.[37]

In the current study, we first employed bioinformatics tools to identify miRNAs associated with CC. Then, we predicted their target genes and conducted pathway enrichment analysis of the genes. Finally, a total of nine SNPs located in eight miRNAs of the AMPK pathway were selected, and the association between SNPs and the risk of CC in a Han Chinese population was investigated. Our results provide more data for further elucidating the molecular genetic mechanisms of CC.

Methods

Subjects

A total of 421 patients with CIN and 871 patients with CC were randomly selected from the Third Affiliated Hospital of Kunming Medical University. The inclusion criteria were as follows: diagnosis of cervical malignant tumor or CIN II or CIN III confirmed by biopsy or surgery, complete clinical data, good compliance, absence of other concurrent tumors, no previous history of other tumors, and no prior antitumor therapy such as radiotherapy or chemotherapy. Diagnostic criteria were based on Clinical Diagnosis and Treatment Guidelines for Obstetrics and Gynecology and National Guidelines for Diagnosis and Treatment of Cervical Cancer 2022 in China.[38] Simultaneously, 928 healthy individuals were randomly selected as the healthy control group. All subjects belonged to the Han Chinese population. All samples were collected with informed consent from the patients and their families, and the study was approved by the Ethics Committee of the Third Affiliated Hospital of Kunming Medical University (No. KYCS2021193).

DNA Extraction

DNA extraction was performed using QIAamp DNA Blood Mini Kit (Qiagen NV, Venlo, Netherlands). The concentration and purity of the extracted DNA were assessed using a Multiskan™ GO full-wavelength spectrophotometer.

miRNA Selection, Target Gene Prediction and Signal Pathway Enrichment

The miRSNP database (http://bioinfo.life.hust.edu.cn/miRNASNP/#!) was used to retrieve miRNAs associated with CC, as were the HMDD database (http://www.cuilab.cn/hmdd) and the miRWalk database (http://129.206.7.150/). By integrating the data from these three databases, a total of 151 miRNAs were obtained. The target genes of these miRNAs were predicted using the TargetScan 8.0 database (https://www.targetscan.org/vert_80/) and TarBase v8 database (https://bio.tools/tarbase#!). Pathway enrichment analysis was performed using the mirPath database (https://dianalab.e-ce.uth.gr/html/mirpathv3/index.php?R=mirpath#mirnas=hsa-miR-101-3p=Tarbase&selection=0) and the DAVID database (https://david.ncifcrf.gov/).

SNP Selection and Genotyping

Based on the results of pathway analysis, miRNA genes located in the AMPK signaling pathway and SNPs in the mature sequence, precursor sequence, or transcriptional regulatory sequence of these miRNAs were selected. A minor allele frequency (MAF) higher than 0.05 was used as a screening criterion. Following the screening process, a total of nine SNPs were chosen as candidates for this study: rs895819 located in the promoter region of miR-27a, rs10061133 located in the mature sequence of miR-449b, rs41291179 located in the enhancer region of miR-216a, rs76481776 located in the enhancer region of miR-182, rs10406069 located in the non-coding transcript exon region of miR-5196, rs12803915 located in the miR-612 precursor sequence (pre-miRNA), rs550894 in the non-coding transcript exon region, rs66683138 located in the mature sequence of miR-3622b, and rs2620381 located in the mature sequence of miR-627 (Table 1 and Figure 1).

The genotyping reagent TaqMan Genotyping Master Mix and the genotyping probe TaqManSNP Genotyping Assays were purchased from ABI, USA. For the genotyping process, please refer to our previous study.[39] In brief, add 2.5 μL of 2× Master Mix, 0.25 μL of 40× probe, 1.25 μL of nuclease-free water, and 1 μL of template DNA to each well of PCR plate, resulting in a final reaction volume of 5 μL. The reaction is then carried out on the LightCycler®480 instrument with the following program settings: PCR initial heat activation at 95°C for 2min, denaturation at 95°C for 5s, combined annealing/extension at 60°C for 30s and the number of cycles is 40. For quality control, 14.6% of the samples were randomly selected for repeated detection, and 1.7% of the samples were randomly selected for DNA sequencing analysis to verify the accuracy of the TaqMan probe method.

Statistical Analysis

The genotyping results of all SNP loci were summarized using Microsoft Excel software. To assess the age bias, we conducted one-way ANOVA using GraphPad Prism 9.4 software. The Hardy–Weinberg equilibrium (HWE), distribution of alleles and genotypes of the nine SNPs among different groups, and odds ratio were calculated using SHEsis software[40] through the chi-square test and Fischer's exact test. The inheritance model of the nine SNPs was analyzed using SNPstats[41] under five different models: codominant, dominant, recessive, overdominant, and log-additive models. The optimal inheritance model for each SNP was determined based on the Akaike information criterion (AIC) and Bayesian information criterion (BIC), whereby smaller AIC and BIC values indicate lower expected entropy and higher

Table 1 The Basic Information of the SNPs

SNPs	Genes	Function Consequence	Location	Alleles	MAF
rs895819	MIR27A	Promotor region	Chr 19:13836478	T>C	0.36
rs10061133	MIR449B	Mature miRNA variant	Chr 5:55170716	A>G	0.12
rs41291179	MIR216A	Enhancer region	Chr 2:55988955	A>T	0.08
rs76481776	MIR182	Enhancer region	Chr 7:129770387	C>T	0.06
rs10406069	MIR5196	Non coding transcript exon variant	Chr 19:35345627	G>A	0.12
rs12803915	MIR612	Pre-miRNA variant	Chr 11:65444508	G>A	0.14
rs550894	MIR612	Non coding transcript exon variant	Chr 11:65444469	C>A	0.22
rs66683138	MIR3622B	Mature miRNA variant	Chr 8:27701697	G>A	0.30
rs2620381	MIR627	Mature miRNA variant	Chr 15:42199650	A>C	0.08

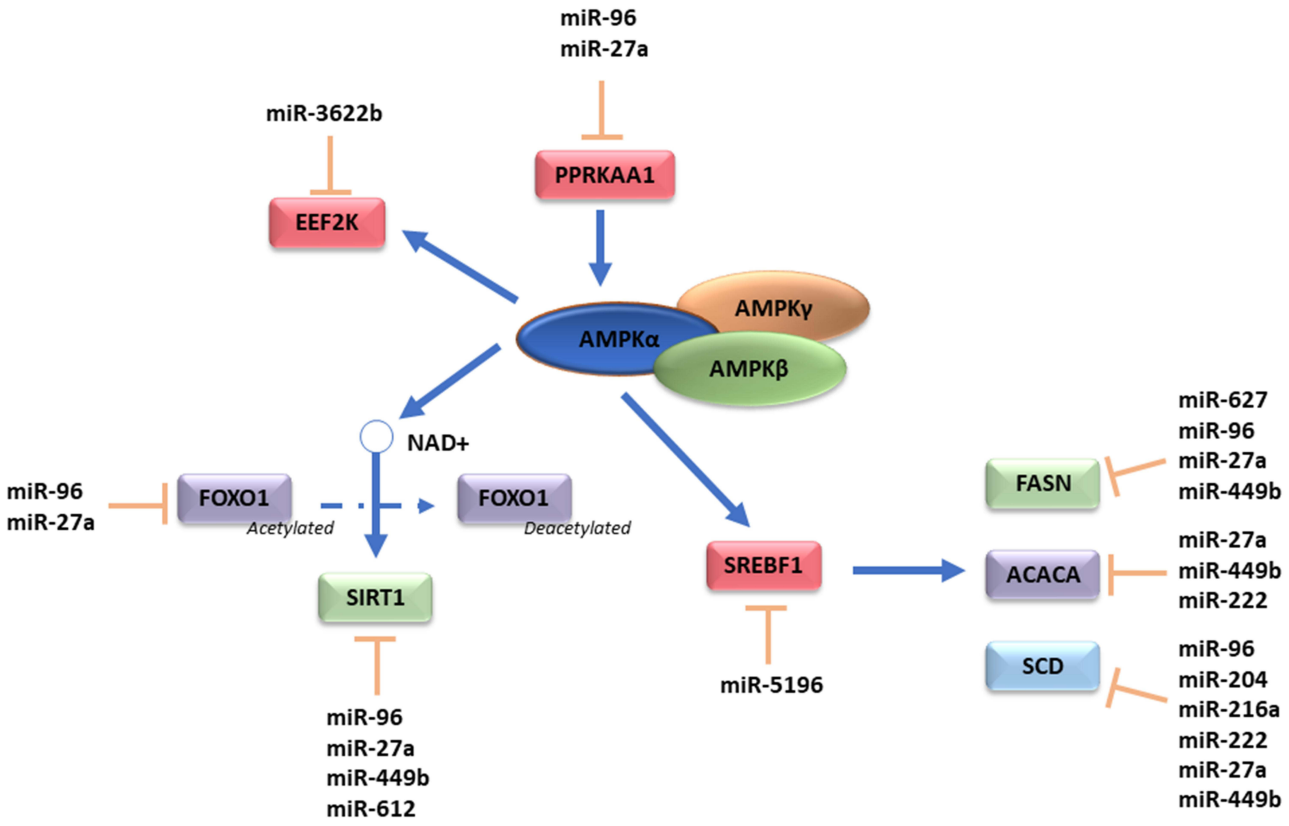

Figure 1 The miRNAs associated with AMPK signaling pathways in the current study.

model accuracy.[42] To account for multiple comparisons, Bonferroni correction was applied to the *P* value, which the *Pc* value was set at 0.05 ($P = 0.05/n$, n = 9).

Results

Clinical Characteristics

A total of 2,220 participants were enrolled in this study. There were 421 patients in the CIN group, whose average age was 45.61±9.60, including 48 patients in grade II and 373 in grade III. The CC group comprised 871 patients, with an average age of 46.28±9.88 years, including 155 patients with adenocarcinoma (AC) and 716 patients with squamous cell carcinoma (SCC). According to CC staging, 563 patients were in stage I, 248 in stage II and 60 in stage III–IV. There were 928 participants in the healthy control group, whose average age was 46.37±9.28. There was no statistically significant difference in age observed among the three groups (F = 0.958, *P* = 0.384) (Table 2).

Table 2 The Clinical Characteristics of Subjects Enrolled in Current Study

		Control	CIN	CC	F	*P*
Cases		928	421	871		
Ages (year)		46.37±9.28	45.61±9.60	46.28±9.88	0.958	0.384
Grades of CIN	II (n)		48			
	III (n)		373			
Pathological types	AC (n)			155		
	SCC (n)			716		
Stages of CC	I (n)			563		
	II (n)			248		
	III–IV (n)			60		

Signaling Pathway Enrichment

Signaling pathway analysis of 151 miRNAs revealed that 41 miRNAs, which may be associated with the development of CC, were significantly enriched in the AMPK signaling pathway (hsa04152). The enrichment analysis yielded an adjusted P value of 5.09×10^{-5}.

Association of Nine SNPs in Eight miRNAs with CIN and CC

Except for rs76481776, the genotypic distribution of the other eight SNPs was consistent with HWE ($P > 0.05$), indicating that the sample selection of this study was representative. The distribution and comparison of alleles and genotypes for each SNP locus in different groups are presented in Table 3. Notably, significant differences were observed in the distribution of alleles and genotypes for the rs41291179 locus in miR-216a and the rs12803915 locus in miR-612 between the control group and the CIN group, as well as between the control group and the CC group (control group vs CIN group, P values were 5.88×10^{-5} and 1.30×10^{-5}, respectively; control group vs CC group, P values were 0.003 and 3.73×10^{-4}, respectively). Furthermore, statistically significant differences were found in the distribution of alleles and genotypes for rs550894 between the control group and the CC group (P value = 0.001). Additionally, the A allele of rs41291179 and the G allele of rs12803915 were associated with a reduced risk of CIN (OR = 0.05, 95% CI: 0.01–0.39; OR = 0.61, 95% CI: 0.49–0.76, respectively) and CC (OR = 0.08, 95% CI: 0.01–0.66; OR = 0.71, 95% CI: 0.59–0.86, respectively). Conversely, the C allele of rs550894 was associated with an increased risk of CC (OR = 1.29, 95% CI: 1.11–1.50).

The results of the inheritance model analysis indicated that the optimal model for rs12803915 and rs550894 in comparison between the control group and the CC group was the log-additive model. Specifically, compared to the GG genotype, the rs12803915 genotype 2AA+AG may be a risk factor for cervical cancer (OR = 1.39, 95% CI: 1.15–1.67); the rs550894 genotype 2AA+AC may act as a protective factor against CC compared to the CC genotype (OR = 0.77, 95% CI: 0.66–0.90). The optimal model for rs12803915 in the control group compared with the CIN group was dominant, with the AG+AA genotype relative to the GG genotype being a potential risk factor for CIN (OR = 1.85, 95% CI: 1.43–2.38). Due to the absence of the T/T genotype of rs41291179 in this study, analysis of the five inheritance models could not be conducted. However, compared with the A/A genotype, the T/A genotype was found to be associated with an increased risk of CC and CIN (OR = 12.50, 95% CI: 1.54–100.00; OR = 20.00, 95% CI: 2.56–100.00, respectively). See Tables 4 and 5.

Association of Nine SNPs in Eight miRNAs with Different Pathological Types of CC

To ascertain whether the selected SNPs are associated with different pathological types of CC, the distribution of the nine SNPs in CC patients was analyzed by stratifying them according to pathological type. As shown in Table 6, significant differences were observed in the distribution of alleles and genotypes of rs12803915 and rs550894 between the SCC group and the control group (P values were 0.001). The results of the inheritance model analysis are displayed in Table 7, revealing that the optimal model for both loci is the log-additive model. Based on this model, compared to the GG genotype, the 2AA+AG genotype of rs12803915 may act as a risk factor for SCC (OR = 1.37, 95% CI: 1.14–1.67); relative to the CC genotype, the 2AA+AC genotype of rs550894 may serve as a protective factor against SCC (OR = 0.75, 95% CI: 0.64–0.88). Furthermore, the distribution of alleles and genotypes of rs41291179 exhibited statistically significant differences between the AC group and the control group, with the A allele and A/A genotype of this locus potentially acting as protective factors for AC (OR = 0.03, 95% CI: 0.003–0.28). However, there were no significant differences in the distribution of alleles and genotypes between the AC group and the SCC group.

Association of Nine SNPs in Eight miRNAs with Different Stages of CC and CIN Groups

To investigate whether the selected SNPs are associated with disease progression, the CIN group was categorized into grade I and grade II, while the CC group was divided into stage I and stage II+III+IV. As shown in Table 8, significant

Table 3 The Allele and Genotype Distribution of 9 SNPs in Control, CIN and CC Groups

SNPs	Allele/Genotype	Control n (%)	CIN n (%)	CC n (%)	CIN vs Ctrl		CC vs Ctrl		CC VS CIN	
					OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
rs895819	T	1360 (73.3)	590 (70.1)	1299 (74.6)	0.85 (0.71–1.02)	0.085	1.07 (0.92–1.24)	0.377	1.25 (1.04–1.50)	0.016
	C	496 (26.7)	252 (29.9)	443 (25.4)						
	T/T	493 (53.1)	209 (49.6)	488 (56.0)						
	C/T	374 (40.3)	172 (40.9)	323 (37.1)		0.134		0.375		0.058
	C/C	61 (6.6)	40 (9.5)	60 (6.9)						
rs10061133	A	1338 (72.1)	614 (72.9)	1261 (72.4)	1.04 (0.87–1.25)	0.654	1.01 (0.88–1.17)	0.842	0.97 (0.81–1.17)	0.776
	G	518 (27.9)	228 (27.1)	481 (27.6)						
	A/A	488 (52.6)	212 (50.4)	449 (51.5)						
	A/G	362 (39.0)	190 (45.1)	363 (41.7)		0.011		0.293		0.195
	G/G	78 (8.4)	19 (4.5)	59 (6.8)						
rs41291179	A	1855 (99.9)	833 (98.9)	1731 (99.4)	0.05 (0.01–0.39)	5.88×10^{-5}	0.08 (0.01–0.66)	0.003	1.70 (0.70–4.12)	0.234
	T	1 (0.1)	9 (1.1)	11 (0.6)						
	A/A	927 (99.9)	412 (97.9)	860 (98.7)		5.72×10^{-5}		0.003		0.233
	A/T	1 (0.1)	9 (2.1)	11 (1.3)						
rs76481776	C	1798 (96.9)	834 (99.0)	1696 (97.4)	3.36 (1.60–7.07)	0.001	1.19 (0.80–1.76)	0.386	0.35 (0.17–0.75)	0.005
	T	58 (3.1)	8 (1.0)	46 (2.6)						
	C/C	886 (95.5)	413 (98.1)	828 (95.1)						
	C/T	26 (2.8)	8 (1.9)	40 (4.6)		0.015		0.002		0.027
	T/T	16 (1.7)	0	3 (0.3)						
rs10406069	G	1422 (76.6)	618 (73.4)	1340 (76.9)	0.84 (0.70–1.01)	0.071	1.02 (0.87–1.19)	0.828	1.21 (1.00–1.46)	0.050
	A	434 (23.4)	224 (26.6)	402 (23.1)						
	G/G	541 (58.3)	227 (53.9)	518 (59.5)						
	A/G	340 (36.6)	164 (39.0)	304 (34.9)		0.170		0.688		0.146
	A/A	47 (5.1)	30 (7.1)	49 (5.6)						
rs12803915	G	1635 (88.1)	689 (81.8)	1463 (84.0)	0.61 (0.49–0.76)	1.30×10^{-5}	0.71 (0.59–0.86)	3.73×10^{-4}	1.16 (0.94–1.45)	0.169
	A	221 (11.9)	153 (18.2)	279 (16.0)						
	G/G	722 (77.8)	276 (65.6)	622 (71.5)						
	A/G	191 (20.6)	137 (32.5)	219 (25.1)		9.92×10^{-6}		0.002		0.009
	A/A	15 (1.6)	8 (1.9)	30 (3.4)						
rs550894	C	1359 (73.2)	620 (73.6)	1357 (77.9)	1.02 (0.85–1.23)	0.822	1.29 (1.11–1.50)	0.001	1.26 (1.04–1.53)	0.017
	A	497 (26.8)	222 (26.4)	385 (22.1)						
	C/C	498 (53.7)	229 (54.4)	522 (59.9)						
	A/C	363 (39.1)	162 (38.5)	313 (35.9)		0.969		0.003		0.031
	A/A	67 (7.2)	30 (7.1)	36 (4.2)						

rs66683138	G	1103 (59.4)	484 (57.5)	1040 (59.7)	0.92 (0.78–1.09)	0.341	1.01 (0.88–1.16)	0.868	1.10 (0.93–1.29)	0.282
	A	753 (40.6)	358 (42.5)	702 (40.3)						
	G/G	316 (34.0)	139 (33.0)	324 (37.2)						
	A/G	471 (50.8)	206 (48.9)	392 (45.0)		0.416		0.045		0.310
	A/A	141 (15.2)	76 (18.1)	155 (17.8)						
rs2620381	A	1697 (91.4)	772 (91.7)	1593 (91.4)	1.03 (0.77–1.39)	0.827	1.00 (0.79–1.26)	0.989	0.97 (0.72–1.30)	0.837
	C	159 (8.6)	70 (8.3)	149 (8.6)						
	A/A	775 (83.5)	352 (83.6)	731 (83.9)						
	A/C	147 (15.8)	68 (16.2)	131 (15.0)		0.621		0.606		0.279
	C/C	6 (0.7)	1 (0.2)	9 (1.1)						

Note: The statistical significant threshold was set at $P < 0.005$ after Bonferroni correction.

https://doi.org/10.2147/IJGM.S473133

Table 4 The Inheritance Model Analysis of 9 SNPs Between CC and Control Groups

SNPs	Model	Genotypes	CC (n%)	Control (n%)	OR (95% CI)	*P* Value	AIC	BIC
rs895819	Codominant	T/T	488 (56.0)	493 (53.1)	1.00	0.370	2496.2	2512.7
		C/T	323 (37.1)	374 (40.3)	0.87 (0.72–1.06)			
		C/C	60 (6.9)	61 (6.6)	0.99 (0.68–1.45)			
	Dominant	T/T	488 (56.0)	493 (53.1)	1.00	0.220	2494.6	2505.6
		C/T-C/C	383 (44.0)	435 (46.9)	0.89 (0.74–1.08)			
	Recessive	T/T-C/T	811 (93.1)	867 (93.4)	1.00	0.790	2496.1	2507.1
		C/C	60 (6.9)	61 (6.6)	1.05 (0.72–1.52)			
	Overdominant	T/T-C/C	548 (62.9)	554 (59.7)	1.00	0.160	2494.2	2505.2
		C/T	323 (37.1)	374 (40.3)	0.87 (0.72–1.05)			
	Log-additive	—	—	—	0.93 (0.81–1.09)	0.380	2495.4	2506.3
rs10061133	Codominant	A/A	449 (51.5)	488 (52.6)	1.00	0.290	2495.7	2512.2
		G/A	363 (41.7)	362 (39.0)	1.09 (0.90–1.32)			
		G/G	59 (6.8)	78 (8.4)	0.82 (0.57–1.18)			
	Dominant	A/A	449 (51.5)	488 (52.6)	1.00	0.660	2495.9	2506.9
		G/A-G/G	422 (48.5)	440 (47.4)	1.04 (0.87–1.25)			
	Recessive	A/A-G/A	812 (93.2)	850 (91.6)	1.00	0.190	2494.4	2505.4
		G/G	59 (6.8)	78 (8.4)	0.79 (0.56–1.12)			
	Overdominant	A/A-G/G	508 (58.3)	566 (61.0)	1.00	0.250	2494.8	2505.8
		G/A	363 (41.7)	362 (39.0)	1.11 (0.93–1.35)			
	Log-additive	—	—	—	0.98 (0.85–1.14)	0.840	2496.1	2507.1
rs41291179	—	A/A	860 (98.7)	927 (99.9)	1.00	0.001	2485.7	2496.7
		T/A	11 (1.3)	1 (0.1)	12.50 (1.54–100.00)			
rs76481776	Codominant	C/C	828 (95.1)	886 (95.5)	1.00	0.002	2485.2	2501.7
		C/T	40 (4.6)	26 (2.8)	1.64 (1.00–2.70)			
		T/T	3 (0.3)	16 (1.7)	0.20 (0.06–0.69)			
	Dominant	C/C	828 (95.1)	886 (95.5)	1.00	0.680	2496.0	2507.0
		C/T-T/T	43 (4.9)	42 (4.5)	1.10 (0.71–1.69)			
	Recessive	C/C-C/T	868 (99.7)	912 (98.3)	1.00	0.003	2487.1	2498.1
		T/T	3 (0.3)	16 (1.7)	0.20 (0.06–0.68)			
	Overdominant	C/C-T/T	831 (95.4)	902 (97.2)	1.00	0.043	2492.0	2503.0
		C/T	40 (4.6)	26 (2.8)	1.67 (1.01–2.78)			
	Log-additive	—	—	—	0.88 (0.63–1.23)	0.450	2495.6	2506.6
rs10406069	Codominant	G/G	518 (59.5)	541 (58.3)	1.00	0.690	2497.4	2513.9
		A/G	304 (34.9)	340 (36.6)	0.93 (0.77–1.14)			
		A/A	49 (5.6)	47 (5.1)	1.09 (0.72–1.67)			
	Dominant	G/G	518 (59.5)	541 (58.3)	1.00	0.610	2495.9	2506.9
		A/G-A/A	353 (40.5)	387 (41.7)	0.95 (0.79–1.15)			
	Recessive	G/G-A/G	822 (94.4)	881 (94.9)	1.00	0.600	2495.9	2506.8
		A/A	49 (5.6)	47 (5.1)	1.12 (0.74–1.69)			
	Overdominant	G/G-A/A	567 (65.1)	588 (63.4)	1.00	0.440	2495.5	2506.5
		A/G	304 (34.9)	340 (36.6)	0.93 (0.76–1.12)			
	Log-additive	—	—	—	0.98 (0.84–1.15)	0.830	2496.1	2507.1
rs12803915	Codominant	G/G	622 (71.5)	722 (77.8)	1.00	0.002	2485.5	2502.0
		A/G	219 (25.1)	191 (20.6)	1.33 (1.06–1.67)			
		A/A	30 (3.4)	15 (1.6)	2.33 (1.23–4.35)			
	Dominant	G/G	622 (71.4)	722 (77.8)	1.00	0.002	2486.4	2497.4
		A/G-A/A	249 (28.6)	206 (22.2)	1.41 (1.14–1.72)			
	Recessive	G/G-A/G	841 (96.6)	913 (98.4)	1.00	0.012	2489.9	2500.9
		A/A	30 (3.4)	15 (1.6)	2.17 (1.16–4.00)			
	Overdominant	G/G-A/A	652 (74.9)	737 (79.4)	1.00	0.021	2490.8	2501.8
		A/G	219 (25.1)	191 (20.6)	1.30 (1.04–1.61)			

(*Continued*)

Table 4 (Continued).

SNPs	Model	Genotypes	CC (n%)	Control (n%)	OR (95% CI)	*P* Value	AIC	BIC
	Log-additive	—	—	—	1.39 (1.15–1.67)	5.00×10^{-4}	2484.0	2495.0
rs550894	Codominant	C/C	522 (59.9)	498 (53.7)	1.00	0.003	2486.2	2502.7
		A/C	313 (35.9)	363 (39.1)	0.82 (0.68–1.00)			
		A/A	36 (4.2)	67 (7.2)	0.51 (0.34–0.78)			
	Dominant	C/C	522 (59.9)	498 (53.7)	1.00	0.007	2488.9	2499.9
		A/C-A/A	349 (40.1)	430 (46.3)	0.78 (0.64–0.93)			
	Recessive	C/C-A/C	835 (95.9)	861 (92.8)	1.00	0.004	2488.1	2499.1
		A/A	36 (4.1)	67 (7.2)	0.56 (0.36–0.84)			
	Overdominant	C/C-A/A	558 (64.1)	565 (60.9)	1.00	0.160	2494.2	2505.2
		A/C	313 (35.9)	363 (39.1)	0.87 (0.72–1.05)			
	Log-additive	—	—	—	0.77 (0.66–0.90)	0.001	2485.3	2496.3
rs66683138	Codominant	G/G	324 (37.2)	316 (34.0)	1.00	0.045	2491.9	2508.4
		G/A	392 (45.0)	471 (50.8)	0.81 (0.66–1.00)			
		A/A	155 (17.8)	141 (15.2)	1.08 (0.81–1.41)			
	Dominant	G/G	324 (37.2)	316 (34.0)	1.00	0.160	2494.2	2505.2
		G/A-A/A	547 (62.8)	612 (66.0)	0.87 (0.72–1.05)			
	Recessive	G/G-G/A	716 (82.2)	787 (84.8)	1.00	0.140	2493.9	2504.9
		A/A	155 (17.8)	141 (15.2)	1.20 (0.94–1.56)			
	Overdominant	G/G-A/A	479 (55.0)	457 (49.2)	1.00	0.015	2490.2	2501.2
		G/A	392 (45.0)	471 (50.8)	0.79 (0.66–0.95)			
	Log-additive	—	—	—	0.99 (0.86–1.12)	0.870	2496.1	2507.1
rs2620381	Codominant	A/A	731 (83.9)	775 (83.5)	1.00	0.610	2497.1	2513.6
		C/A	131 (15.0)	147 (15.8)	0.94 (0.73–1.22)			
		C/C	9 (1.1)	6 (0.7)	1.59 (0.56–4.55)			
	Dominant	A/A	731 (83.9)	775 (83.5)	1.00	0.810	2496.1	2507.1
		C/A-C/C	140 (16.1)	153 (16.5)	0.97 (0.76–1.25)			
	Recessive	A/A-C/A	862 (99.0)	922 (99.3)	1.00	0.370	2495.3	2506.3
		C/C	9 (1.0)	6 (0.7)	1.61 (0.57–4.55)			
	Overdominant	A/A-C/C	740 (85.0)	781 (84.2)	1.00	0.640	2495.9	2506.9
		C/A	131 (15.0)	147 (15.8)	0.94 (0.73–1.22)			
	Log-additive	—	—	—	1.00 (0.79–1.27)	0.990	2496.1	2507.1

Notes: AIC, Akaike information criterion; BIC, Bayesian information criterion. AIC = −2 ln(L) + 2 k, BIC = −2 ln(L) + ln(n)*k. The statistical significant threshold was set at $P < 0.005$ after Bonferroni correction.

differences were observed in the distribution of alleles and genotypes of rs41291179 and rs12803915 between the CIN II group and the CIN III group (*P* values were 0.002 and 1.39×10^{-4}, respectively). The A allele of rs41291179 and the G allele of rs12803915 were associated with a reduced risk of CIN II progressing to CIN III (OR = 0.16, 95% CI: 0.04–0.59; OR = 0.41, 95% CI: 0.26–0.66, respectively). There were no statistically significant differences observed in the distribution of all SNP loci among different stages of CC, $Pc > 0.05$ (see Table S1).

Discussion

Aberrant activation of the AMPK signaling pathway has been implicated in various cancers, contributing to tumor cell proliferation, invasion, metastasis, and other malignant behaviors.[43,44] Any change in miRNA expression level or function may be related to signaling pathways.[34] Abnormal expression of miRNAs has been identified as a significant cause of dysregulated signaling pathways, and SNPs in these miRNA genes are known to influence their expression.[37] In the current study, we investigated the association between SNPs located in miRNA genes of the AMPK signaling pathway and the risk of CC in a Han Chinese population. Our results revealed that the target genes of miRNAs were significantly enriched in the AMPK signaling pathway and that three SNPs of AMPK pathway-associated miRNAs,

Table 5 The Inheritance Model Analysis of 9 SNPs Between CIN and Control Groups

SNPs	Model	Genotypes	CIN (n%)	Control (n%)	OR (95% CI)	*P* Value	AIC	BIC
rs895819	Codominant	T/T	209 (49.6)	493 (53.1)	1.00	0.140	1676.9	1692.5
		C/T	172 (40.9)	374 (40.3)	1.09 (0.85–1.39)			
		C/C	40 (9.5)	61 (6.6)	1.54 (1.01–2.38)			
	Dominant	T/T	209 (49.6)	493 (53.1)	1.00	0.240	1677.4	1687.8
		C/T-C/C	212 (50.4)	435 (46.9)	1.15 (0.91–1.45)			
	Recessive	T/T-C/T	381 (90.5)	867 (93.4)	1.00	0.063	1675.4	1685.8
		C/C	40 (9.5)	61 (6.6)	1.49 (0.98–2.27)			
	Overdominant	T/T-C/C	249 (59.1)	554 (59.7)	1.00	0.850	1678.8	1689.2
		C/T	172 (40.9)	374 (40.3)	1.02 (0.81–1.30)			
	Log-additive	—	—	—	1.18 (0.98–1.41)	0.084	1675.8	1686.2
rs10061133	Codominant	A/A	212 (50.4)	488 (52.6)	1.00	0.008	1671.3	1686.9
		G/A	190 (45.1)	362 (39.0)	1.20 (0.95–1.54)			
		G/G	19 (4.5)	78 (8.4)	0.56 (0.33–0.95)			
	Dominant	A/A	212 (50.4)	488 (52.6)	1.00	0.450	1678.2	1688.6
		G/A-G/G	209 (49.6)	440 (47.4)	1.10 (0.87–1.37)			
	Recessive	A/A-G/A	402 (95.5)	850 (91.6)	1.00	0.008	1671.7	1682.1
		G/G	19 (4.5)	78 (8.4)	0.52 (0.31–0.86)			
	Overdominant	A/A-G/G	231 (54.9)	566 (61.0)	1.00	0.034	1674.3	1684.7
		G/A	190 (45.1)	362 (39.0)	1.28 (1.02–1.61)			
	Log-additive	—	—	—	0.96 (0.79–1.15)	0.650	1678.6	1689.0
rs41291179	—	A/A	412 (97.9)	927 (99.9)	1.00	1.00×10^{-4}	1663.5	1673.9
		T/A	9 (2.1)	1 (0.1)	20.00 (2.56–100.00)			
rs76481776	Codominant	C/C	413 (98.1)	886 (95.5)	1.00	0.001	1667.6	1683.3
		C/T	8 (1.9)	26 (2.8)	0.66 (0.30–1.47)			
		T/T	0	16 (1.7)				
	Dominant	C/C	413 (98.1)	886 (95.5)	1.00	0.012	1672.5	1682.9
		C/T-T/T	8 (1.9)	42 (4.5)	0.41 (0.19–0.88)			
	Recessive	C/C-C/T	421 (100.0)	912 (98.3)	1.00	5.00×10^{-4}	1666.7	1677.2
		T/T	0	16 (1.7)				
	Overdominant	C/C-T/T	413 (98.1)	902 (97.2)	1.00	0.320	1677.8	1688.2
		C/T	8 (1.9)	26 (2.8)	0.67 (0.30–1.49)			
	Log-additive	—	—	—	0.42 (0.22–0.81)	0.002	1669.3	1679.7
rs10406069	Codominant	G/G	227 (53.9)	541 (58.3)	1.00	0.180	1677.3	1693.0
		A/G	164 (39.0)	340 (36.6)	1.15 (0.90–1.47)			
		A/A	30 (7.1)	47 (5.1)	1.52 (0.93–2.44)			
	Dominant	G/G	227 (53.9)	541 (58.3)	1.00	0.130	1676.5	1687.0
		A/G-A/A	194 (46.1)	387 (41.7)	1.19 (0.94–1.52)			
	Recessive	G/G-A/G	391 (92.9)	881 (94.9)	1.00	0.140	1676.6	1687.0
		A/A	30 (7.1)	47 (5.1)	1.43 (0.89–2.33)			
	Overdominant	G/G-A/A	257 (61.0)	588 (63.4)	1.00	0.420	1678.1	1688.6
		A/G	164 (39.0)	340 (36.6)	1.10 (0.87–1.41)			
	Log-additive	—	—	—	1.19 (0.99–1.43)	0.071	1675.5	1685.9
rs12803915	Codominant	G/G	276 (65.6)	722 (77.8)	1.00	<0.0001	1658.5	1674.1
		A/G	137 (32.5)	191 (20.6)	1.89 (1.45–2.44)			
		A/A	8 (1.9)	15 (1.6)	1.39 (0.58–3.33)			
	Dominant	G/G	276 (65.6)	722 (77.8)	1.00	<0.0001	1656.9	1667.3
		A/G-A/A	145 (34.4)	206 (22.2)	1.85 (1.43–2.38)			
	Recessive	G/G-A/G	413 (98.1)	913 (98.4)	1.00	0.710	1678.7	1689.1
		A/A	8 (1.9)	15 (1.6)	1.18 (0.50–2.78)			
	Overdominant	G/G-A/A	284 (67.5)	737 (79.4)	1.00	<0.0001	1657.0	1667.4
		A/G	137 (32.5)	191 (20.6)	1.85 (1.43–2.44)			

(*Continued*)

Table 5 (Continued).

SNPs	Model	Genotypes	CIN (n%)	Control (n%)	OR (95% CI)	*P* Value	AIC	BIC
	Log-additive	—	—	—	1.67 (1.32–2.08)	<0.0001	1660.1	1670.5
rs550894	Codominant	C/C	229 (54.4)	498 (53.7)	1.00	0.970	1680.7	1696.4
		A/C	162 (38.5)	363 (39.1)	0.97 (0.76–1.23)			
		A/A	30 (7.1)	67 (7.2)	0.97 (0.62–1.54)			
	Dominant	C/C	229 (54.4)	498 (53.7)	1.00	0.800	1678.7	1689.2
		A/C-A/A	192 (45.6)	430 (46.3)	0.97 (0.77–1.22)			
	Recessive	C/C-A/C	391 (92.9)	861 (92.8)	1.00	0.950	1678.8	1689.2
		A/A	30 (7.1)	67 (7.2)	0.99 (0.63–1.54)			
	Overdominant	C/C-A/A	259 (61.5)	565 (60.9)	1.00	0.820	1678.8	1689.2
		A/C	162 (38.5)	363 (39.1)	0.97 (0.77–1.23)			
	Log-additive	—	—	—	0.98 (0.81–1.18)	0.820	1678.8	1689.2
rs66683138	Codominant	G/G	139 (33.0)	316 (34.0)	1.00	0.420	1679.1	1694.7
		G/A	206 (48.9)	471 (50.8)	0.99 (0.77–1.28)			
		A/A	76 (18.1)	141 (15.2)	1.22 (0.87–1.72)			
	Dominant	G/G	139 (33.0)	316 (34.0)	1.00	0.710	1678.7	1689.1
		G/A-A/A	282 (67.0)	612 (66.0)	1.05 (0.82–1.33)			
	Recessive	G/G-G/A	345 (81.9)	787 (84.8)	1.00	0.190	1677.1	1687.5
		A/A	76 (18.1)	141 (15.2)	1.23 (0.91–1.67)			
	Overdominant	G/G-A/A	215 (51.1)	457 (49.2)	1.00	0.530	1678.4	1688.8
		G/A	206 (48.9)	471 (50.8)	0.93 (0.74–1.18)			
	Log-additive	—	—	—	1.09 (0.92–1.28)	0.330	1677.9	1688.3
rs2620381	Codominant	A/A	352 (83.6)	775 (83.5)	1.00	0.580	1679.7	1695.3
		C/A	68 (16.2)	147 (15.8)	1.02 (0.75–1.39)			
		C/C	1 (0.2)	6 (0.7)	0.37 (0.04–3.03)			
	Dominant	A/A	352 (83.6)	775 (83.5)	1.00	0.960	1678.8	1689.2
		C/A-C/C	69 (16.4)	153 (16.5)	0.99 (0.73–1.35)			
	Recessive	A/A-C/A	420 (99.8)	922 (99.3)	1.00	0.300	1677.7	1688.1
		C/C	1 (0.2)	6 (0.7)	0.37 (0.04–3.03)			
	Overdominant	A/A-C/C	353 (83.8)	781 (84.2)	1.00	0.880	1678.8	1689.2
		C/A	68 (16.2)	147 (15.8)	1.02 (0.75–1.41)			
	Log-additive	—	—	—	0.97 (0.72–1.30)	0.820	1678.8	1689.2

Note: The statistical significant threshold was set at $P < 0.005$ after Bonferroni correction.

rs41291179, located in the enhancer region of miR-216a, and rs12803915 and rs550894, located in the pre-miRNA sequence of miR-612, are associated with the occurrence and development of CC.

miR-216a has been implicated in the development of various human tumors, with demonstrated antitumor properties.[45–47] Predictions suggest that miR-216a regulates the AMPK signaling pathway by targeting the gene stearoyl-CoA desaturase (*SCD*). Studies have shown that *SCD* promotes the proliferation, migration, and metastasis of cancer cells.[48–50] Xing et al reported that *SCD* expression was significantly upregulated in patients with cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC) compared to the control group.[51] In the current study, our results showed that the SNP rs41291179 in miR-216a is associated with the development of CC and CIN. According to the Ensembl database (https://asia.ensembl.org/index.html), this variant is located in an enhancer binding site. Hence, this SNP might enhance miR-216a expression, reducing *SCD* expression and being associated with the occurrence and progression of CC. However, no association between this SNP and disease susceptibility was reported in previous studies.[52–54] Considering the low MAF of this SNP, at only 0.08, the sample size had a significant impact on the statistical results, which might be an important reason for the discrepancies between our study and previous studies. Furthermore, we analyzed the association between rs41291179 and different pathological types of CC, with significant differences found between the control group and the AC group in the distribution of alleles and genotypes. Growing

Table 6 The Allele and Genotype Distribution of 9 SNPs in Different Pathological Types of CC

SNPs	Allele/Genotype	Control n (%)	SCC n (%)	AC n (%)	SCC vs Ctrl		AC vs Ctrl		SCC vs AC	
					OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
rs895819	T	1360 (73.3)	1068 (74.6)	231 (74.5)	1.07 (0.91–1.25)	0.398	1.07 (0.81–1.40)	0.647	1.00 (0.76–1.33)	0.981
	C	496 (26.7)	364 (25.4)	79 (25.5)						
	T/T	493 (53.1)	406 (56.7)	82 (52.9)						
	C/T	374 (40.3)	256 (35.8)	67 (43.2)		0.160		0.397		0.094
	C/C	61 (6.6)	54 (7.5)	6 (3.9)						
rs10061133	A	1338 (72.1)	1038 (72.5)	223 (71.9)	1.02 (0.87–1.19)	0.802	0.99 (0.76–1.30)	0.955	1.03 (0.78–1.35)	0.844
	G	518 (27.9)	394 (27.5)	87 (28.1)						
	A/A	488 (52.6)	371 (51.8)	78 (50.3)						
	A/G	362 (39.0)	296 (41.3)	67 (43.2)		0.392		0.509		0.908
	G/G	78 (8.4)	49 (6.9)	10 (6.5)						
rs41291179	A	1855 (99.9)	1426 (99.6)	305 (98.4)	0.13 (0.02–1.07)	0.024	0.03 (0.003–0.28)	1.37×10^{-6}	3.90 (1.18–12.85)	0.016
	T	1 (0.1)	6 (0.4)	5 (1.6)						
	A/A	927 (99.9)	710 (99.2)	150 (96.8)		0.024		1.33×10^{-6}		0.016
	A/T	1 (0.1)	6 (0.8)	5 (3.2)						
rs76481776	C	1798 (96.9)	1393 (97.3)	303 (97.7)	1.15 (0.76–1.74)	0.500	1.40 (0.63–3.09)	0.408	0.82 (0.37–1.86)	0.643
	T	58 (3.1)	39 (2.7)	7 (2.3)						
	C/C	886 (95.5)	680 (95.0)	148 (95.5)						
	C/T	26 (2.8)	33 (4.6)	7 (4.5)		0.008		0.139		0.721
	T/T	16 (1.7)	3 (0.4)	0						
rs10406069	G	1422 (76.6)	1110 (77.5)	230 (74.2)	1.05 (0.89–1.24)	0.544	0.88 (0.67–1.16)	0.353	1.20 (0.90–1.59)	0.208
	A	434 (23.4)	322 (22.5)	80 (25.8)						
	G/G	541 (58.3)	431 (60.2)	87 (56.1)						
	A/G	340 (36.6)	248 (34.6)	56 (36.1)		0.702		0.394		0.380
	A/A	47 (5.1)	37 (5.2)	12 (7.8)						
rs12803915	G	1635 (88.1)	1205 (84.1)	258 (83.2)	0.72 (0.59–0.88)	0.001	0.67 (0.48–0.93)	0.017	1.07 (0.77–1.49)	0.688
	A	221 (11.9)	227 (15.9)	52 (16.8)						
	G/G	722 (77.8)	512 (71.5)	110 (71.0)						
	A/G	191 (20.6)	181 (25.3)	38 (24.5)		0.005		0.026		0.718
	A/A	15 (1.6)	23 (3.2)	7 (4.5)						
rs550894	C	1359 (73.2)	1122 (78.4)	235 (75.8)	1.32 (1.13–1.56)	0.001	1.15 (0.87–1.52)	0.339	1.15 (0.86–1.54)	0.327
	A	497 (26.8)	310 (21.6)	75 (24.2)						
	C/C	498 (53.7)	433 (60.5)	89 (57.4)						
	A/C	363 (39.1)	256 (35.8)	57 (36.8)		0.002		0.634		0.469
	A/A	67 (7.2)	27 (3.7)	9 (5.8)						

rs66683138	G	1103 (59.4)	870 (60.8)	170 (54.8)	1.06 (0.92–1.22)	0.442	0.83 (0.65–1.06)	0.129	1.28 (0.99–1.63)	0.054
	A	753 (40.6)	562 (39.2)	140 (45.2)						
	G/G	316 (34.0)	275 (38.4)	49 (31.6)						
	A/G	471 (50.8)	320 (44.7)	72 (46.5)		0.051		0.108		0.175
	A/A	141 (15.2)	121 (16.9)	34 (21.9)						
rs2620381	A	1697 (91.4)	1310 (91.5)	283 (91.3)	1.01 (0.79–1.29)	0.962	0.98 (0.64–1.51)	0.934	1.02 (0.66–1.58)	0.914
	C	159 (8.6)	122 (8.5)	27 (8.7)						
	A/A	775 (83.5)	601 (83.9)	130 (83.9)						
	A/C	147 (15.8)	108 (15.1)	23 (14.8)		0.698		0.660		0.939
	C/C	6 (0.7)	7 (1.0)	2 (1.3)						

Note: SCC, Squamous cell carcinoma; AC, adenocarcinoma. The statistical significant threshold was set at $P < 0.005$ after Bonferroni correction.

https://doi.org/10.2147/IJGM.S473133

Table 7 The Inheritance Model Analysis of rs12803915 and rs550894 Between SCC and Control Groups

SNPs	Model	Genotypes	SCC (n%)	Control (n%)	OR (95% CI)	*P* Value	AIC	BIC
rs12803915	Codominant	G/G	512 (71.5)	722 (77.8)	1.00	0.005	2247.2	2263.4
		A/G	181 (25.3)	191 (20.6)	1.33 (1.06–1.69)			
		A/A	23 (3.2)	15 (1.6)	2.17 (1.11–4.17)			
	Dominant	G/G	512 (71.5)	722 (77.8)	1.00	0.003	2247.1	2258.0
		A/G-A/A	204 (28.5)	206 (22.2)	1.39 (1.11–1.75)			
	Recessive	G/G-A/G	693 (96.8)	913 (98.4)	1.00	0.034	2251.1	2261.9
		A/A	23 (3.2)	15 (1.6)	2.00 (1.04–3.85)			
	Overdominant	G/G-A/A	535 (74.7)	737 (79.4)	1.00	0.024	2250.6	2261.4
		A/G	181 (25.3)	191 (20.6)	1.30 (1.03–1.64)			
	Log-additive	—	—	—	1.37 (1.14–1.67)	0.001	2245.4	2256.2
rs550894	Codominant	C/C	433 (60.5)	498 (53.7)	1.00	0.001	2244.4	2260.6
		A/C	256 (35.8)	363 (39.1)	0.81 (0.66–1.00)			
		A/A	27 (3.7)	67 (7.2)	0.46 (0.29–0.74)			
	Dominant	C/C	433 (60.5)	498 (53.7)	1.00	0.006	2248.0	2258.8
		A/C-A/A	283 (39.5)	430 (46.3)	0.76 (0.62–0.93)			
	Recessive	C/C-A/C	689 (96.2)	861 (92.8)	1.00	0.002	2246.4	2257.2
		A/A	27 (3.8)	67 (7.2)	0.50 (0.32–0.79)			
	Overdominant	C/C-A/A	460 (64.2)	565 (60.9)	1.00	0.160	2253.7	2264.5
		A/C	256 (35.8)	363 (39.1)	0.87 (0.71–1.06)			
	Log-additive	—	—	—	0.75 (0.64–0.88)	6.00×10^{-4}	2243.9	2254.7

Note: The statistical significant threshold was set at $P < 0.005$ after Bonferroni correction.

evidence reveals that AC has hidden lesions, an unclear starting site, and shorter overall survival than SCC.[55–57] Thus, the polymorphisms of this SNP in miR-126a should be further studied, especially regarding the pathogenic mechanism for AC.

Recent studies have highlighted involvement of miR-612 as a tumor suppressor in progression of various human tumors, such as CC,[29] hepatocellular carcinoma[58] and colorectal cancer.[59] In the current study, based on the results of TarBase,[60,61] we predict that Sirtuin-1 (*SIRT1*), a target gene of miR-612, is involved in the AMPK signaling pathway and influences disease progression. *SIRT1* plays various roles in biological processes, including gene regulation, proliferation, and tumorigenesis.[62–64] Our current results showed that the distribution of the rs12803915 allele, located in the precursor sequence of miR-612, differed between the control group and the CIN group, as well as between the control group and the CC group. Therefore, the G allele of rs12803915 may act as a protective factor for CC. In 2012, Kim et al observed an allelic effect of rs12803915 on expression of mature miR-612 in a series of cell lines, and the A allele increased expression in most prostate cancer cell lines but decreased it in five colon cancer cell lines,[65] indicating that the A allele of this SNP affects expression of *SIRT1* by regulating miR-612 maturation and thus might play roles in CIN and CC.

Moreover, our results illustrated differences in the rs550894 located in miR-612 between the control and CC groups, suggesting its association with cervical lesions. The C allele of rs550894 may act as a risk factor for CC, which is similar to the conclusion of Zhao et al with regard to lung cancer, whereby the A allele of rs550894 is associated with good prognosis in patients with advanced non-small cell lung cancer.[66] Kim et al also reported that the A allele of rs550894 can increase expression of mature miR-612 in most prostate cancer cell lines while decreasing it in most colon cancer cell lines and prostate cancer cell lines, even though the effect was absent in four breast cancer cell lines.[65] Currently, there is no research about rs550894 in CC cell lines. Therefore, analysis of effects of this SNP in the context of CC cell lines is needed.

Table 8 The Allele and Genotype Distribution of 9 SNPs in CIN II and CIN III Groups

SNPs	Allele/Genotype	CIN III n (%)	CIN II n (%)	CIN II vs CIN III	
				OR (95% CI)	*P*
rs895819	T	526 (70.5)	64 (66.7)	0.84 (0.53–1.32)	0.439
	C	220 (29.5)	32 (33.3)		
	T/T	183 (49.1)	26 (54.2)		
	C/T	160 (42.9)	12 (25.0)		0.004
	C/C	30 (8.0)	10 (20.8)		
rs10061133	A	540 (72.4)	74 (77.1)	1.28 (0.78–2.12)	0.330
	G	206 (27.6)	22 (22.9)		
	A/A	185 (49.6)	27 (56.2)		
	A/G	170 (45.6)	20 (41.7)		0.542
	G/G	18 (4.8)	1 (2.1)		
rs41291179	A	741 (99.3)	92 (95.8)	0.16 (0.04–0.59)	0.002
	T	5 (0.7)	4 (4.2)		
	A/A	368 (98.7)	44 (91.7)		0.002
	A/T	5 (1.3)	4 (8.3)		
rs76481776	C	738 (98.9)	96 (100.0)	-	0.308
	T	8 (1.1)	0		
	C/C	365 (97.9)	48 (100.0)		
	C/T	8 (2.1)	0		0.306
rs10406069	G	548 (73.5)	70 (72.9)	0.97 (0.60–1.57)	0.910
	A	198 (26.5)	26 (27.1)		
	G/G	204 (54.7)	23 (47.9)		
	A/G	140 (37.5)	24 (50.0)		0.136
	A/A	29 (7.8)	1 (2.1)		
rs12803915	G	624 (83.6)	65 (67.7)	0.41 (0.26–0.66)	1.39×10^{-4}
	A	122 (16.4)	31 (32.3)		
	G/G	258 (69.2)	18 (37.5)		
	A/G	108 (28.9)	29 (60.4)		6.09×10^{-5}
	A/A	7 (1.9)	1 (2.1)		
rs550894	C	555 (74.4)	65 (67.7)	0.72 (0.46–1.14)	0.162
	A	191 (25.6)	31 (32.3)		
	C/C	211 (56.5)	18 (37.5)		
	A/C	133 (35.7)	29 (60.4)		0.003
	A/A	29 (7.8)	1 (2.1)		
rs66683138	G	440 (59.0)	44 (45.8)	0.59 (0.38–0.90)	0.014
	A	306 (41.0)	52 (54.2)		
	G/G	129 (34.6)	10 (20.8)		
	A/G	182 (48.8)	24 (50.0)		0.046
	A/A	62 (16.6)	14 (29.2)		
rs2620381	A	682 (91.4)	90 (93.8)	1.41 (0.59–3.34)	0.437
	C	64 (8.6)	6 (6.2)		
	A/A	310 (83.1)	42 (87.5)		
	A/C	62 (16.6)	6 (12.5)		0.714
	C/C	1 (0.3)	0		

Note: The statistical significant threshold was set at $P < 0.005$ after Bonferroni correction.

Conclusions

miRNAs associated with the development of CC were identified using bioinformatics tools and enriched in the AMPK signaling pathway. Our current study systematically analyzed the role of miRNA gene polymorphisms targeting the AMPK signaling pathway in CC and found that such polymorphisms may impact the development of CC through the

AMPK/SCD (rs41291179 in miR-216a) and AMPK/SIRT1 (rs12803915 and rs550894 in miR-612) pathways. However, there are some limitations in this study. We only collected HPV infection status for some of the patients, as well as other clinical information, which prevented us from conducting relevant analyses of SNP genotyping and HPV infection status. Future research should focus on how SNP alleles affect the maturation of miRNAs and are associated with CC development in vitro and in vivo.

Abbreviations

CC, cervical cancer; CIN, cervical intraepithelial neoplasia; WHO, World Health Organization; miRNA, microRNA; SNP, Single nucleotide polymorphisms; AMPK, AMP-activated protein kinase; AIC, Akaike information criterion; BIC, Bayesian information criterion; AC, adenocarcinoma; SCC, squamous cell carcinoma.

Ethical Approval and Informed Consent

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Ethics Committee of Third Affiliated Hospital of Kunming Medical University. Samples were collected after obtaining written informed consent from all patients.

Funding

The current study was supported by grants from the CAMS Innovation Fund for Medical Sciences (CIFMS) (2021-I2M-1-004), the National Science Foundation of China (82103190), the Association Foundation Program of Yunnan Provincial Science and Technology Department and Kunming Medical University (202201AY070001-139), and Yunnan Province "Xingdian Talent Support Program" (XDYC-CYCX-2023-0074). The funders had no role in the study design, data collection and analysis, decision to publish or preparation of the manuscript.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Sung H, Ferlay J, Siegel RL. et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin*. 2021;71(3):209–249. doi:10.3322/caac.21660
2. Canfell K, Kim JJ, Brisson M, et al. Mortality impact of achieving WHO cervical cancer elimination targets: a comparative modelling analysis in 78 low-income and lower-middle-income countries. *Lancet*. 2020;395(10224):591–603. doi:10.1016/s0140-6736(20)30157-4
3. Chen F, Hu SY, Li Y, et al. Expert consensus on the path construction toward comprehensive prevention and control for cervical cancer in China. *Chin Prevent Med*. 2022;23(10):721–726. doi:10.16506/j.1009-6639.2022.10.001
4. Singh D, Vignat J, Lorenzoni V, et al. Global estimates of incidence and mortality of cervical cancer in 2020: a baseline analysis of the WHO Global Cervical Cancer Elimination Initiative. *Lancet Glob Health*. 2023;11(2):e197–e206. doi:10.1016/s2214-109x(22)00501-0
5. Bhattacharjee R, Das SS, Biswal SS, et al. Mechanistic role of HPV-associated early proteins in cervical cancer: molecular pathways and targeted therapeutic strategies. *Crit Rev Oncol Hematol*. 2022;174:103675. doi:10.1016/j.critrevonc.2022.103675
6. Gutiérrez-Hoya A, Soto-Cruz I. Role of the JAK/STAT Pathway in Cervical Cancer: its Relationship with HPV E6/E7 Oncoproteins. *Cells*. 2020;9(10):2297. doi:10.3390/cells9102297
7. Liu C, Wang R. The Roles of Hedgehog Signaling Pathway in Radioresistance of Cervical Cancer. *Dose Response*. 2019;17(4):1559325819885293. doi:10.1177/1559325819885293
8. Chen K, Yan Z, Dong X, et al. Genetic Polymorphisms in microRNA Genes Targeting PI3K/Akt Signal Pathway Modulate Cervical Cancer Susceptibility in a Chinese Population. *Front Genet*. 2022;13:856505. doi:10.3389/fgene.2022.856505
9. Castro-Muñoz LJ, Manzo-Merino J, Muñoz-Bello JO, et al. The Human Papillomavirus (HPV) E1 protein regulates the expression of cellular genes involved in immune response. *Sci Rep*. 2019;9(1):13620. doi:10.1038/s41598-019-49886-4
10. Kim SH, Juhnn YS, Kang S, et al. Human papillomavirus 16 E5 up-regulates the expression of vascular endothelial growth factor through the activation of epidermal growth factor receptor, MEK/ ERK1,2 and PI3K/Akt. *Cell Mol Life Sci*. 2006;63(7–8):930–938. doi:10.1007/s00018-005-5561-x
11. Gupta S, Kumar P, Das BC. HPV: molecular pathways and targets. *Curr Probl Cancer*. 2018;42(2):161–174. doi:10.1016/j.currproblcancer.2018.03.003
12. Herzig S, Shaw RJ. AMPK: guardian of metabolism and mitochondrial homeostasis. *Nat Rev Mol Cell Biol*. 2018;19(2):121–135. doi:10.1038/nrm.2017.95
13. Carling D. AMPK signalling in health and disease. *Curr Opin Cell Biol*. 2017;45:31–37. doi:10.1016/j.ceb.2017.01.005
14. Boyer PD, Chance B, Ernster L, Mitchell P, Racker E, Slater EC. Oxidative phosphorylation and photophosphorylation. *Annu Rev Biochem*. 1977;46(1):955–966. doi:10.1146/annurev.bi.46.070177.004515
15. Bonora M, Patergnani S, Rimessi A, et al. ATP synthesis and storage. *Purinergic Sig*. 2012;8(3):343–357. doi:10.1007/s11302-012-9305-8

16. Hsu CC, Peng D, Cai Z, Lin HK. AMPK signaling and its targeting in cancer progression and treatment. *Semin Cancer Biol*. 2022;85:52–68. doi:10.1016/j.semcancer.2021.04.006
17. Lin SC, Hardie DG. AMPK: sensing Glucose as well as Cellular Energy Status. *Cell Metab*. 2018;27(2):299–313. doi:10.1016/j.cmet.2017.10.009
18. Hill M, Tran N. miRNA interplay: mechanisms and consequences in cancer. *Dis Model Mech*. 2021;14(4). doi:10.1242/dmm.047662
19. Fabian MR, Sonenberg N. The mechanics of miRNA-mediated gene silencing: a look under the hood of miRISC. *Nat Struct Mol Biol*. 2012;19(6):586–593. doi:10.1038/nsmb.2296
20. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*. 2004;116(2):281–297. doi:10.1016/s0092-8674(04)00045-5
21. Lee Y, Jeon K, Lee JT, Kim S, Kim VN. MicroRNA maturation: stepwise processing and subcellular localization. *EMBO j*. 2002;21(17):4663–4670. doi:10.1093/emboj/cdf476
22. Lee Y, Kim M, Han J, et al. MicroRNA genes are transcribed by RNA polymerase II. *EMBO j*. 2004;23(20):4051–4060. doi:10.1038/sj.emboj.7600385
23. Lee Y, Ahn C, Han J, et al. The nuclear RNase III Drosha initiates microRNA processing. *Nature*. 2003;425(6956):415–419. doi:10.1038/nature01957
24. Liu J, Li X, Lu S, Zheng X, Zhang X, Zhao W. Glucagon-like peptide-1 (GLP-1) improved diabetic lung fibrosis via AMPK and microRNA-27a (miR-27a). *Ann Transl Med*. 2021;9(6):492. doi:10.21037/atm-21-869
25. Gupta S, Panda PK, Hashimoto RF, et al. Dynamical modeling of miR-34a, miR-449a, and miR-16 reveals numerous DDR signaling pathways regulating senescence, autophagy, and apoptosis in HeLa cells. *Sci Rep*. 2022;12(1):4911. doi:10.1038/s41598-022-08900-y
26. Li R, Shi TT, Wang Q, Zhang YX. Elevated lncRNA MIAT in peripheral blood mononuclear cells contributes to post-menopausal osteoporosis. *Aging*. 2022;14(7):3143–3154. doi:10.18632/aging.204001
27. Yue C, Chen J, Li Z, Li L, Chen J, Guo Y. microRNA-96 promotes occurrence and progression of colorectal cancer via regulation of the AMPKα2-FTO-m6A/MYC axis. *J Exp Clin Cancer Res*. 2020;39(1):240. doi:10.1186/s13046-020-01731-7
28. Gutierrez-Camino A, Richer C, St-Onge P, et al. Role of rs10406069 in miR-5196 in hyperdiploid childhood acute lymphoblastic leukemia. *Epigenomics*. 2020;12(22):1949–1955. doi:10.2217/epi-2020-0152
29. Jin Y, Zhou X, Yao X, Zhang Z, Cui M, Lin Y. MicroRNA-612 inhibits cervical cancer progression by targeting NOB1. *J Cell Mol Med*. 2020;24(5):3149–3156. doi:10.1111/jcmm.14985
30. Bucay N, Sekhon K, Majid S, et al. Novel tumor suppressor microRNA at frequently deleted chromosomal region 8p21 regulates epidermal growth factor receptor in prostate cancer. *Oncotarget*. 2016;7(43):70388–70403. doi:10.18632/oncotarget.11865
31. Enwald M, Lehtimäki T, Mishra PP, Mononen N, Murtola TJ, Raitoharju E. Human Prostate Tissue MicroRNAs and Their Predicted Target Pathways Linked to Prostate Cancer Risk Factors. *Cancers*. 2021;13(14):3537. doi:10.3390/cancers13143537
32. Defo J, Awany D, Ramesar R. From SNP to pathway-based GWAS meta-analysis: do current meta-analysis approaches resolve power and replication in genetic association studies? *Brief Bioinform*. 2023;24(1). doi:10.1093/bib/bbac600
33. Wu MC, Kraft P, Epstein MP, et al. Powerful SNP-set analysis for case-control genome-wide association studies. *Am J Hum Genet*. 2010;86(6):929–942. doi:10.1016/j.ajhg.2010.05.002
34. Asl ER, Amini M, Najafi S, et al. Interplay between MAPK/ERK signaling pathway and MicroRNAs: a crucial mechanism regulating cancer cell metabolism and tumor progression. *Life Sci*. 2021;278:119499. doi:10.1016/j.lfs.2021.119499
35. Yan Z, Zhou Z, Li C, et al. Polymorphisms in miRNA genes play roles in the initiation and development of cervical cancer. *J Cancer*. 2019;10(20):4747–4753. doi:10.7150/jca.33486
36. Gilam A, Conde J, Weissglas-Volkov D, et al. Local microRNA delivery targets Palladin and prevents metastatic breast cancer. *Nat Commun*. 2016;7(1):12868. doi:10.1038/ncomms12868
37. Cai Y, Yu X, Hu S, Yu J. A brief review on the mechanisms of miRNA regulation. *Genomics Proteomics Bioinf*. 2009;7(4):147–154. doi:10.1016/s1672-0229(08)60044-3
38. National Health Commission Of The People's Republic Of China. National guidelines for diagnosis and treatment of cervical cancer 2022 in China (English version). *Chin J Cancer Res*. 2022;34(3):256–269. doi:10.21147/j.issn.1000-9604.2022.03.06.
39. Yang J, Wang Y, Zhang S, et al. The Association of TNF-α Promoter Polymorphisms with Genetic Susceptibility to Cervical Cancer in a Chinese Han Population. *Int J Gen Med*. 2022;15:417–427. doi:10.2147/ijgm.S350263
40. Shi YY, He L. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. *Cell Res*. 2005;15(2):97–98. doi:10.1038/sj.cr.7290272
41. Solé X, Guinó E, Valls J, Iniesta R, Moreno V. SNPStats: a web tool for the analysis of association studies. *Bioinformatics*. 2006;22(15):1928–1929. doi:10.1093/bioinformatics/btl268
42. Vrieze SI. Model selection and psychological theory: a discussion of the differences between the Akaike information criterion (AIC) and the Bayesian information criterion (BIC). *Psychol Methods*. 2012;17(2):228–243. doi:10.1037/a0027127
43. Giordanetto F, Karis D. Direct AMP-activated protein kinase activators: a review of evidence from the patent literature. *Expert Opin Ther Pat*. 2012;22(12):1467–1477. doi:10.1517/13543776.2012.743994
44. Xiao B, Sanders MJ, Carmena D, et al. Structural basis of AMPK regulation by small molecule activators. *Nat Commun*. 2013;4(1):3017. doi:10.1038/ncomms4017
45. Hamidi AA, Taghehchian N, Zangouei AS, et al. Molecular mechanisms of microRNA-216a during tumor progression. *Cancer Cell Int*. 2023;23(1):19. doi:10.1186/s12935-023-02865-2
46. Woo SM, Kim S, Seo SU, et al. Inhibition of USP1 enhances anticancer drugs-induced cancer cell death through downregulation of survivin and miR-216a-5p-mediated upregulation of DR5. *Cell Death Dis*. 2022;13(9):821. doi:10.1038/s41419-022-05271-0
47. Zhao J, Li L, Yang T. MiR-216a-3p suppresses the proliferation and invasion of cervical cancer through downregulation of ACTL6A-mediated YAP signaling. *J Cell Physiol*. 2020;235(12):9718–9728. doi:10.1002/jcp.29783
48. Han S, Wang Y, Ma J, Wang Z, Wang HD, Yuan Q. Sulforaphene inhibits esophageal cancer progression via suppressing SCD and CDH3 expression, and activating the GADD45B-MAP2K3-p38-p53 feedback loop. *Cell Death Dis*. 2020;11(8):713. doi:10.1038/s41419-020-02859-2
49. Tesfay L, Paul BT, Konstorum A, et al. Stearoyl-CoA Desaturase 1 Protects Ovarian Cancer Cells from Ferroptotic Cell Death. *Cancer Res*. 2019;79(20):5355–5366. doi:10.1158/0008-5472.Can-19-0369
50. Peck B, Schulze A. Lipid desaturation - The next step in targeting lipogenesis in cancer? *Febs j*. 2016;283(15):2767–2778. doi:10.1111/febs.13681

51. Xing C, Yin H, Yao ZY, Xing XL. Prognostic Signatures Based on Ferroptosis- and Immune-Related Genes for Cervical Squamous Cell Carcinoma and Endocervical Adenocarcinoma. *Front Oncol*. 2021;11:774558. doi:10.3389/fonc.2021.774558
52. Quach H, Barreiro LB, Laval G, et al. Signatures of purifying and local positive selection in human miRNAs. *Am J Hum Genet*. 2009;84(3):316–327. doi:10.1016/j.ajhg.2009.01.022
53. Duan S, Mi S, Zhang W, Dolan ME. Comprehensive analysis of the impact of SNPs and CNVs on human microRNAs and their regulatory genes. *RNA Biol*. 2009;6(4):412–425. doi:10.4161/rna.6.4.8830
54. Kyriakidis I, Kyriakidis K, Tsezou A. MicroRNAs and the Diagnosis of Childhood Acute Lymphoblastic Leukemia: systematic Review, Meta-Analysis and Re-Analysis with Novel Small RNA-Seq Tools. *Cancers*. 2022;14(16):3976. doi:10.3390/cancers14163976
55. Wilailak S, Kengsakul M, Kehoe S. Worldwide initiatives to eliminate cervical cancer. *Int J Gynaecol Obstet*. 2021;155(Suppl 1):102–106. doi:10.1002/ijgo.13879
56. Stolnicu S, Barsan I, Hoang L, et al. International Endocervical Adenocarcinoma Criteria and Classification (IECC): a New Pathogenetic Classification for Invasive Adenocarcinomas of the Endocervix. *Am J Surg Pathol*. 2018;42(2):214–226. doi:10.1097/pas.0000000000000986
57. Roma AA, Mistretta TA, Diaz De Vivar A, et al. New pattern-based personalized risk stratification system for endocervical adenocarcinoma with important clinical implications and surgical outcome. *Gynecol Oncol*. 2016;141(1):36–42. doi:10.1016/j.ygyno.2016.02.028
58. Liu Y, Lu LL, Wen D, et al. MiR-612 regulates invadopodia of hepatocellular carcinoma by HADHA-mediated lipid reprogramming. *J Hematol Oncol*. 2020;13(1):12. doi:10.1186/s13045-019-0841-3
59. Sheng L, He P, Yang X, Zhou M, Feng Q. miR-612 negatively regulates colorectal cancer growth and metastasis by targeting AKT2. *Cell Death Dis*. 2015;6(7):e1808. doi:10.1038/cddis.2015.184
60. Karagkouni D, Paraskevopoulou MD, Chatzopoulos S, et al. DIANA-TarBase v8: a decade-long collection of experimentally supported miRNA-gene interactions. *Nucleic Acids Res*. 2018;46(D1):D239–d245. doi:10.1093/nar/gkx1141
61. Balakrishnan I, Yang X, Brown J, et al. Genome-wide analysis of miRNA-mRNA interactions in marrow stromal cells. *Stem Cells*. 2014;32(3):662–673. doi:10.1002/stem.1531
62. Alves-Fernandes DK, Jasiulionis MG. The Role of SIRT1 on DNA Damage Response and Epigenetic Alterations in Cancer. *Int J Mol Sci*. 2019;20(13):3153. doi:10.3390/ijms20133153
63. An Y, Wang B, Wang X, Dong G, Jia J, Yang Q. SIRT1 inhibits chemoresistance and cancer stemness of gastric cancer by initiating an AMPK/FOXO3 positive feedback loop. *Cell Death Dis*. 2020;11(2):115. doi:10.1038/s41419-020-2308-4
64. Dilmac S, Kuscu N, Caner A, et al. SIRT1/FOXO Signaling Pathway in Breast Cancer Progression and Metastasis. *Int J Mol Sci*. 2022;23(18):10227. doi:10.3390/ijms231810227
65. Kim HK, Prokunina-Olsson L, Chanock SJ. Common genetic variants in miR-1206 (8q24.2) and miR-612 (11q13.3) affect biogenesis of mature miRNA forms. *PLoS One*. 2012;7(10):e47454. doi:10.1371/journal.pone.0047454
66. Zhao Y, Wei Q, Hu L, et al. Polymorphisms in MicroRNAs are associated with survival in non-small cell lung cancer. *Cancer Epidemiol Biomarkers Prev*. 2014;23(11):2503–2511. doi:10.1158/1055-9965.Epi-14-0389