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MiR-610 functions as a tumor suppressor in oral squamous cell carcinoma by directly targeting AGK

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Abstract. – OBJECTIVE: MicroRNA-610 (miR-610) functions as a tumor suppressor in various types of cancers. However, whether miR-610 acted as a functional miRNA in oral squamous cell carcinoma (OSCC) is still largely unknown. The current study was designed to explore the expression pattern and function of miR-610 in OSCC and to investigate the possible molecular mechanisms.

PATIENTS AND METHODS: Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR) was performed to detect the expression of miR-610 in OSCC tissues and cells lines. The associations between miR-610 expression and clinicopathologic features and prognosis were analyzed. Proliferation, migration, and invasion capacities of OSCC cells were assessed after overexpressing miR-610. The regulation of acylglycerol kinase (AGK) by miR-610 was confirmed by Western blotting, Dual-Luciferase reporter assays and rescue experiments.

RESULTS: We found that miR-610 expression was significantly down-regulated in both OSCC tissues and cell lines. Low miR-610 expression was associated with advanced T classification, TNM stage and poorer prognosis of OSCC patients. Functionally, the overexpression of miR-610 significantly suppressed OSCC cells proliferation, migration, invasion and EMT process. Mechanistically, AGK was confirmed to be the downstream target of miR-610 in OSCC cells. Furthermore, forced expression of AGK could rescue the inhibiting roles on cell proliferation and metastasis induced by miR-610 in OSCC cells.

CONCLUSIONS: Our findings highlight the important role of miR-610 in regulating OSCC progression by targeting AGK, indicating that miR-610 may represent a novel potential therapeutic target and prognostic marker for OSCC.

Key Words

MiR-610, Proliferation, Metastasis, EMT, AGK, Oral squamous cell carcinoma.

Introduction

Oral and oropharyngeal cancers are the most common malignancies of the head and neck region, and are well-known for their high rate of proliferation and nodal metastasis[1,2]. Oral squamous cell carcinoma (OSCC), the most frequent of all oral cancers, accounts for more than 90% of oral cancers[3]. The pathogenesis of OSCC is related to the rates of cell proliferation and apoptosis[4]. Despite recent advances in therapy, the 5-year survival rate for OSCCs patients remained unchanged at 50-55% from nearly five decades ago[5,6]. Frequent loco-regional recurrences and highly metastatic neck lymph node properties of OSCC become a great challenge for successful treatment[7]. Therefore, a better understanding of the potential mechanism of OSCC may provide novel therapeutic strategies for this disease. MicroRNAs (miRNAs), which are highly conserved, single-stranded and non-coding RNAs of about 22 nucleotides (nt), are now recognized as a very large gene family[8]. It has been confirmed that miRNAs negatively regulate gene expression by imprecisely binding to a complementary sequence in the 3'-UTR of their target mRNAs[9]. More and more evidence[10,11] indicates that miRNAs act as important regulators in many biological processes such as cell proliferation, inflammation, differentiation, apoptosis and invasion. Recently, growing data revealed that miRNAs were abnormally expressed in human tumors, implying an important role for miRNAs during tumor progression[12]. Up to date, several miRNAs have been identified as tumors suppressors or oncogenes, such as miR-142[13], miR-2[14], and miR-338-3p[15]. However, there are many miRNAs

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needed to be further investigated. MiR-610, located at 11p14.1, was a newly identified functional miRNA in tumors. Its dysregulation has been reported in several tumors, such as melanoma[16], osteosarcoma[17], and gastric cancer[18], indicating that miR-610 may be involved in the regulation of tumor progression, which was demonstrated by a series of functional experiments. However, to our best knowledge, there are no reports on the clinical significance and biological function of miR-610 in OSCC. The present work provided the first evidence that miR-610 may serve as a tumor promoter in OSCC afind act as a potential prognostic biomarker for OSCC patients.

Patients and Methods

Patients and Tissue Specimens

Oral squamous cell carcinoma and normal adjacent tissue specimens were collected from 137 patients who underwent surgical resection at the Liaocheng People's Hospital between May 2008 to June 2010 with informed consent. The tissue specimens were immediately frozen in liquid nitrogen and stored at -80°C after being isolated. All the patients enrolled in this study had never subjected to preoperative chemo- or radiotherapy. The clinical information of the patients was summarized in Table II. The Ethics Committee of Liaocheng People's Hospital approved the study documents.

Cell Lines and Cell Transfection

The five human OSCC cell lines (SCC-25, SCC-4, CAL-27, TCA-83, and HN-6) were obtained from the Cell Bank of Type Culture of the Chinese Academy of Sciences (Xuhui, Shanghai, China). The human immortalized oral epithelial cell line, HIOEC, was purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). All the cells were maintained with Dulbecco's Modified Eagle's Medium (DMEM; Thermo Fisher Scientific, Waltham, MA, USA). Besides, 10% fetal bovine serum (FBS; Hyclone, South Logan, UT, USA) and 100 U/mL penicillin/streptomycin (Biodragon, Beijing, China) were also added into the medium. The cells were cultured at 37°C with 5% CO_2. The negative control mimic (control mimic) and miR-610 mimic were all purchased from GenScript Co., Ltd. (Nanjing, Jiangsu, China). The cDNA of AKG was subcloned into pcDNA3.1 vector by FitGene Co., Ltd. (Guangzhou, Guangdong, China). The miRNA mimics or plasmid vectors were transfected into oral squamous cell carcinoma cells by Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) in accordance with the manufacturer's instructions.

Reverse Transcription Quantitative Polymerase Chain Reaction (qRT-PCR)

Whole RNA was extracted from the oral squamous cell carcinoma cell lines by using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and stored at -80°C. The conversion of total RNA to cDNA was conducted using Master Premix kit (Foregene, Chengdu, Sichuan, China). The expression levels of AGK were examined by SYBR Real Time-Polymerase Chain Reaction kit (GenePharma, Shanghai, China), with GAPDH as an internal control. Besides, miRNA Purification kit (HaiGene, Harbin, Heilongjiang, China) and HG SYBR Green miRNA qPCR kit (HaiGene, Harbin, Heilongjiang, China) were applied to determine the expression levels of miR-610, with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal control. The primers were listed in Table I. All qRT-PCR reactions were carried out using the LightCycler 480 system (Roche Molecular Biochemicals, Mannheim, Germany). The relative expression was calculated according to the $2^{-\Delta\Delta Ct}$ method.

Western Blot Analysis

The cells were collected and lysed with radio immunoprecipitation assay (RIPA) buffer (Beyotime, Shanghai, China). Then, an equal amount of protein lysates was separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and subsequently transferred onto polyvinylidene difluoride (PVDF) membranes (Sangong Biotech, Shanghai, China). After blocking with 5% non-fat milk for 1-2 h, the membranes were incubated with primary antibodies overnight at 4°C. Afterward, proteins were examined by antibodies employing enhanced chemiluminescence (ECL;

Table I. The primers for PCR.

Primer name	Primer sequence (5′-3′)
MiR-610 (F)	GCCGAGTGAGCTAAATGTGT
MiR-610 (R)	CTCAACTGGTGTCGTGGA
AGK (F)	GCCATTGGCTCTATGGAAAACA
AGK (R)	TGCATTGGGAGGAATGAGTTG
GAPDH (F)	AGGTCGGTGTGAACGGATTTG
GAPDH (R)	GGGGTCGTTGATGGCAACA

Table II. Correlation between miR-610 expression and different clinicopathological features in OSCC.

Clinicopathological features	No. of cases	miR-610 expression		*p*-value
		High	Low	
Age				NS
< 60	71	31	40	
≥ 60	66	34	32	
Gender				NS
Male	81	41	40	
Female	56	24	32	
Smoking status				NS
Non-smoker	51	21	30	
Smoker	86	44	42	
Histology/differentiation				NS
Well + moderate	76	39	37	
Poor	61	26	35	
Distant organ metastasis				NS
No	97	47	50	
Yes	40	18	22	
Local recurrence				NS
No	89	45	44	
Yes	48	20	28	
T classification				0.003
T1 + T2	88	50	38	
T3 + T4	49	15	34	
TNM stage				0.005
I + II	91	51	40	
III + IV	46	14	32	

Beyotime, Shanghai, China). The primary antibodies against vimentin as well as N-Cadherin were purchased from R&D Systems, Inc. (Minneapolis, MN, USA). The primary antibodies against GAPDH and AKG were obtained from Abcam Co., Ltd. (Cambridge, MA, USA).

Cell Viability and Colony Formation Assays

A Cell Counting Kit-8 (CCK-8; Dojindo Laboratories, Kumamoto, Japan) was applied to assess cell viability. In brief, SCC-25 and CAL-27 cells (about 2000 cells per well) were seeded into 96-well plates and continued to be cultured for 24-48 h at 37°C. Subsequently, cells were transfected with corresponding mimics or plasmids. Then, 10 μl CCK-8 reagent was added into each well and cells were maintained at 37°C for an additional 1 h. Finally, the absorbance was examined at a wavelength of 450 nm using a microplate spectrophotometer system (Molecular Devices, Sunnyvale, CA, USA). For colony formation assays, miRNA mimic or plasmids-transfected SCC-25 and CAL-27 cells (400 cells per well) were placed in 6-well plates and cultured in medium containing 10% FBS. After culturing for two weeks, the cell colonies were first fixed with methanol, and then stained with 0.3% crystal violet solution. Finally, the cell colonies were imaged by a microscope (DYS-809; DianYing, Shanghai, China).

Transwell Assays

The migratory or invasive abilities of SCC-25 and CAL-27 cells were carried out by transwell assays using cell culture inserts with 8-μm pore polycarbonate membrane filters (Corning Incorporated, Corning, NY, USA) pre-coated without (for migration assays) or with (for invasion assays) 50 μl of Matrigel (200 mg/ml; BD Biosciences, Franklin Lakes, NJ, USA). After transfection with miRNA mimics or plasmids, the SCC-25 or CAL-27 cells were plated into the upper side of the inserts, maintained for 24 h, and then allowed to invade through the membranes. Subsequently, the cells on the upper sides of the membranes were removed with cotton swabs. The cells on the lower side of the membranes were first fixed with methanol, and then stained with 0.3% crystal violet solution. Then, the cell colonies were imaged by a microscope (DYS-809; DianYing, Shanghai, China).

Luciferase Reporter Assays

The wild-type 3'-UTR (AGK WT) or the corresponding mutant 3'-UTR (AGK MUT) sequence of AGK was constructed into a pGL3 Basic vector (Promega, Madison, WI, USA) by FitGene Co., Ltd. (Guangzhou, Guangdong, China). Then, SCC-25 or CAL-27 cells were seeded into 24-well plates and continued to culture for 24 h. Afterward, the cells were co-transfected with AGK WT or AGK MUT plasmids and miR-610 mimics. After 24 h, the relative Luciferase activity was examined by the use of a Dual-Luciferase reporter kit (Promega, Madison, WI, USA).

Statistical Analysis

We used the SPSS 20.0 statistics software (SPSS Inc., Chicago, IL, USA) or GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA, USA) for all statistical analyses. The Student's *t*-test was employed for comparisons between groups. The multi-group comparison was performed using one-way analysis of variance (ANOVA). The paired comparison was performed by SNK approach. Survival curves were plotted by the Kaplan-Meier method and compared by the log-rank test. All data were expressed as mean ± the standard error of the mean (SEM). The level of significance was set at $p<0.05$.

Results

MiR-610 Was Downregulated in OSCC Tissues and Cell Lines and Correlated with Unfavorable Prognosis

First, we examined the miR-610 level in the OSCC tissues. As shown in Figure 1A, we found that miR-610 expression levels in OSCC tissues were higher than those in the matched normal tissues ($p<0.01$). In addition, we detected the expression levels of miR-610 in five OSCC cell lines and the human immortalized oral epithelial cell line HIOEC by RT-PCR, finding that the miR-610 expression was significantly down-regulated in all OSCC cell lines compared to HIOEC (Figure 1B). Our results first revealed that miR-610 may act as a functional miRNA in the progression of OSCC.

Then, to explore the clinical significance of miR-610 levels in OSCC patients, we analyzed the relationships between miR-610 expression levels and clinicopathological characteristics. All 137 OSCC patients were divided into the high miR-610 expression group and low miR-610 expression group using the cutoff value of miR-610 expression. As shown in Table II, we found that low expression was significantly associated with advanced T classification ($p=0.003$) and TNM stage ($p=0.005$). However, there was no association between miR-610 expression and age, smoking status, histology/differentiation, distant organ metastasis or local recurrence ($p>0.05$). Subsequently, the effect of miR-610 on long-term survival of OSCC patients was also analyzed. As shown in Figure 1C, our results indicated that the overall survival of patients with low miR-610 expression was significantly shorter than those with high miR-610 expression ($p=0.0167$). More importantly, univariate analysis further confirmed that T classification, TNM stage and miR-610 expression were associated with the overall survival of OSCC patients (Table III). Finally, multivariate analysis demonstrated that miR-610 expression was an independent prognostic marker for the overall survival of OSCC patients (HR=2.956, 95% CI: 1.211-4.032, $p=0.008$, Table III). Our findings revealed that miR-610 may act as a clinical biomarker for OSCC patients.

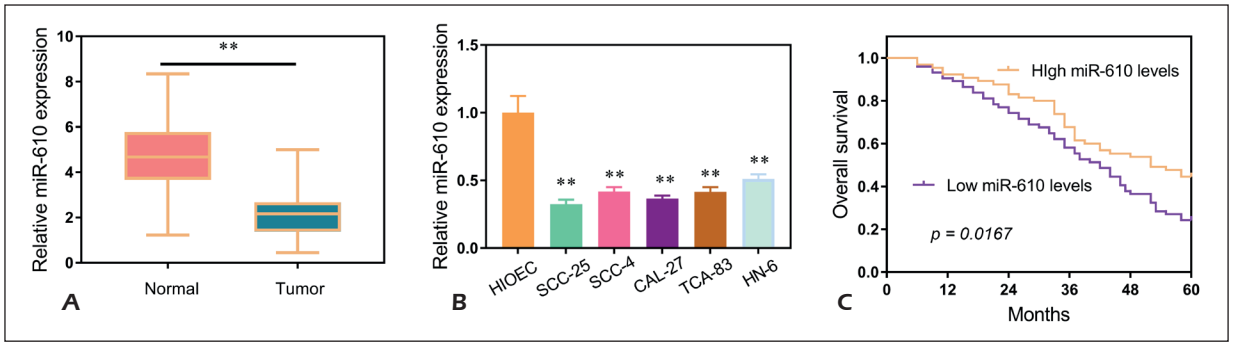

Figure 1. Expression levels of miR-610 in OSCC and its prognostic value. **A**, Real Time-PCR analysis of miR-610 expression levels in 137 paired OSCC tissues and corresponding adjacent normal tissues. **B**, Real Time-PCR analysis of miR-610 in SCC-25, SCC-4, CAL-27, TCA-83, and HN-6 OSCC cells and the human immortalized oral epithelial cell line HIOEC. **C**, Kaplan-Meier survival curves for cervical cancer patients according to the expression of miR-610. *$p<0.05$, **$p<0.01$.

Table III. Summary of univariate and multivariate Cox regression analyses of overall survival duration.

Parameter	Univariate analysis			Multivariate analysis		
	HR	95% CI	*p*	HR	95% CI	*p*
Age	1.644	0.732-2.231	0.216	-	-	-
Gender	1.324	0.842-2.445	0.155	-	-	-
Smoking status	1.743	0.524-2.421	0.387	-	-	-
Histology/differentiation	1.213	0.864-1.658	0.133	-	-	-
Distant organ metastasis	0.984	0.572-1.874	0.119	-	-	-
Local recurrence	1.478	0.784-2.322	0.147	-	-	-
T classification	3.324	1.428-4.988	0.005	2.876	1.216-4.321	0.009
TNM stage	3.895	1.655-5.325	0.001	3.123	1.166-4.326	0.006
MiR-610 expression	3.437	1.545-4.763	0.003	2.956	1.211-4.032	0.008

Ectopic Expression of MiR-610 Inhibited the Proliferation of OSCC Cells

Next, we performed a gain of function experiment to evaluate the role miR-610 played in the proliferation of OSCC cell lines, SCC-25 and CAL-27. We first validated that transfection of miR-610 mimic enhanced miR-610 expression remarkably in SCC-25 and CAL-27 cells using qRT-PCR assays (Figure 2A). Afterward, we conducted CCK-8 assays to assess the proliferation of SCC-25 and CAL-27 cells transfected with miR-610 mimics. As the data shown in Figure 2B and C, forced expression of miR-610 resulted in a notable decrease of proliferative rates in SCC-25 and CAL-27 cells. In addition, the cell colony formation assays were also applied to evaluate the alteration of clonogenic abilities in OSCC cells after transfecting with miR-610 mimics. Consistent with the results of the

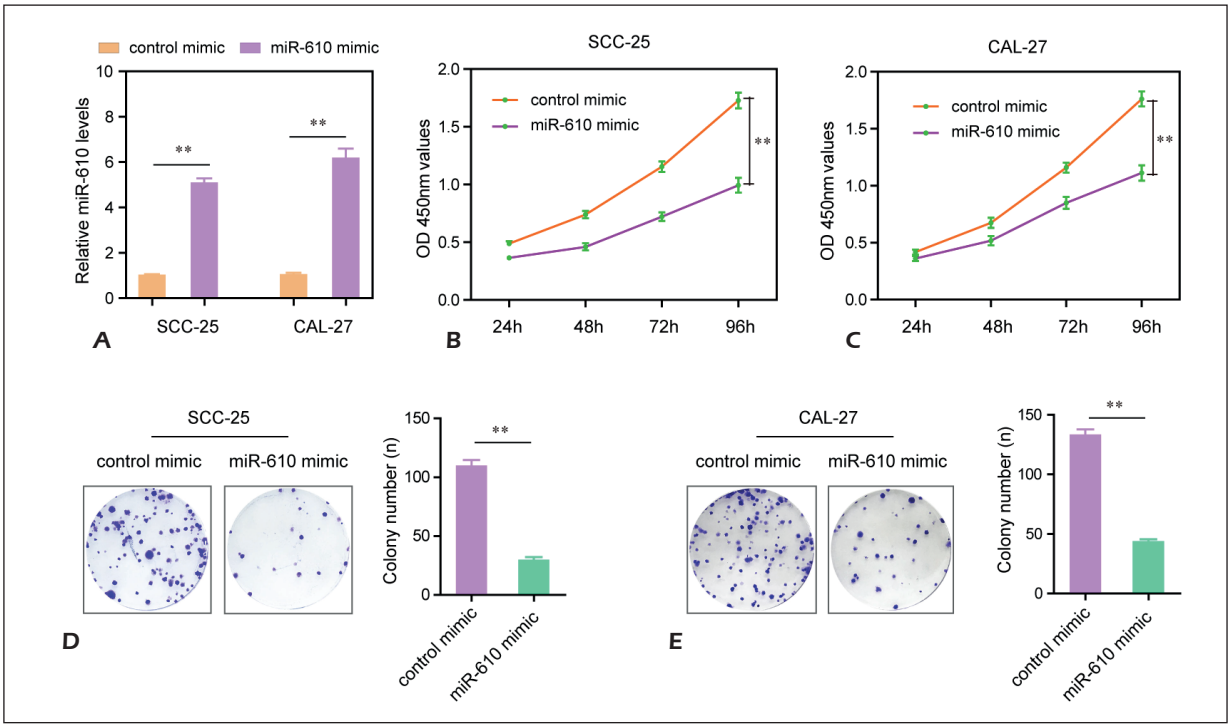

Figure 2. The effects of miR-610 on the proliferation and apoptosis of SCC-25 and CAL-27 cells. **A**, Expression levels of miR-610 in SCC-25 and CAL-27 cells transfected with miR-610 mimics. **B**-**C**, The cell viabilities of SCC-25 and CAL-27 cells were evaluated by CCK-8 assays. **D**-**E**, Transfection of miR-610 mimics decreased the cell colony number of SCC-25 and CAL-27 cells. **p*<0.05, ***p*<0.01.

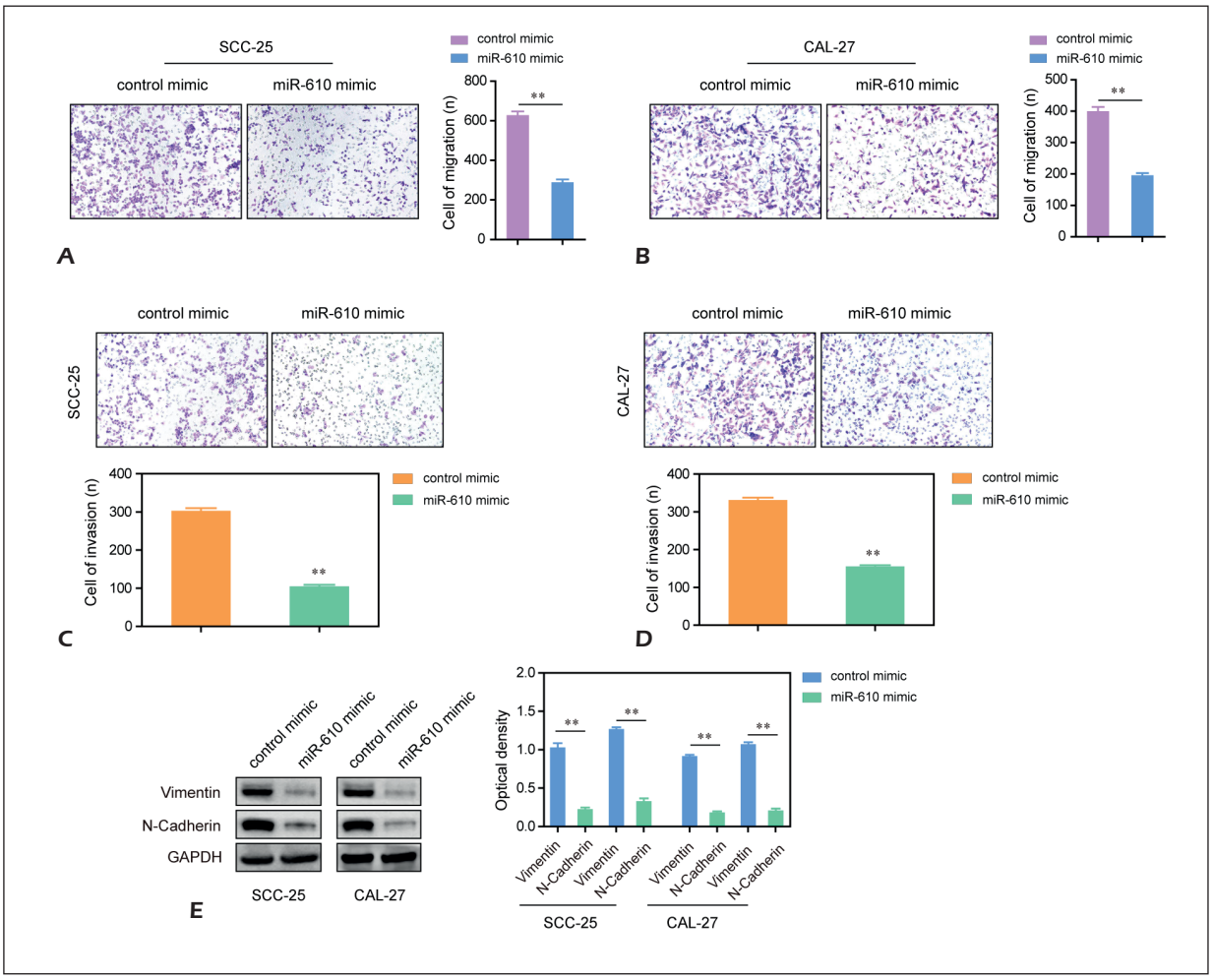

Figure 3. The effects of miR-610 on the migration and invasion of SCC-25 and CAL-27 cells. **A-B**, Transfection of miR-610 mimics inhibited the migratory capabilities of SCC-25 and CAL-27 cells. **C-D**, The transwell invasion assays were conducted using SCC-25 and CAL-27 cells transfected with control mimics or miR-610 mimics, respectively. E, The protein levels of N-cadherin and vimentin examined by Western blot assays. *p<0.05, **p<0.01.

CCK-8 assays, the number of SCC-25 and CAL-27 cell colonies was significantly reduced after transfection of miR-610 mimics (Figure 2D and E). Overall, these data indicated that enhancing expression of miR-610 significantly suppressed the proliferation of OSCC cells.

Overexpression of MiR-610 Suppressed the Migration and Invasion of OSCC Cells

To investigate whether miR-610 affected the metastasis potentials of OSCC, we next evaluated the migratory and invasive abilities of SCC-25 and CAL-27 cells after transfection with miR-610 mimics or negative control mimics (control mimic) using transwell assays. The results of transwell migration assays indicated that enhancing expression of miR-610 in SCC-25 and CAL-27 cells exhibited a remarkable suppression on cell migration (Figure 3A and B). Furthermore, the results of transwell invasion assays revealed that miR-610 mimic transfection caused significant suppression of the invasive capabilities of SCC-25 and CAL-27 cells (Figure 3C and D). We next aimed to explore whether miR-610 affected the epithelial to mesenchymal (EMT) of OSCC cells. Thus, the expression levels of EMT markers in miR-610 overexpressing SCC-25 and CAL-27 cells were examined by Western blot. The results suggested that the protein levels of both vimentin and N-cadherin were dramatically decreased in miR-610 mimics transfected SCC-25 and CAL-27 cells (Figure 3E). Collectively, these results indicated that miR-610 might be involved in the progression of OSCC via modulating EMT and cell metastasis potentials.

MiR-610 Modulated the Expression of AGK by Directly Targeting its 3'-UTR

To elucidate the molecular mechanism by which miR-610 impacted the proliferation, migration and invasiveness of OSCC cells, we next searched "miRDB" (http://www.mirdb.org/) and found that AGK might be a potential target of miR-610 (Figure 4A). To verify that, we first constructed Luciferase reporter plasmids containing the wild-type 3'UTR of AGK (AGK WT) and its corresponding mutant sequence (AGK MUT). Subsequently, we carried out Dual-Luciferase reporter assays to examine the alteration of the Luciferase activities in SCC-25 and CAL-27 cells under various experimental conditions. As the data presented in Figure 4B, co-transfection of miR-610 and AGK WT notably decreased the Luciferase activities in SCC-25 and CAL-27 cells, while co-transfection with miR-610 and AGK MUT did not change the Luciferase activities. Moreover, qRT-PCR assays suggested that the overexpression of miR-610 significantly reduced the mRNA levels of AGK in SCC-25 and CAL-27 cells (Figure 4C). Similarly, the results of Western blot assays confirmed that transfection of miR-610 mimics led to a remarkable decline of the protein levels of AGK in SCC-25 and CAL-27 cells (Figure 4D). Our data demonstrated that AGK was a direct target of miR-610 in OSCC cells.

Forced Expression of AGK Abrogated the Suppressive Effects of MiR-610 on OSCC Cell Proliferation and Invasion

Based on the finding that miR-610 could target and modulate the expression of AGK, we next aimed to explore the ability of AGK to alter the inhibitory effects of miR-610 on cell prolif-

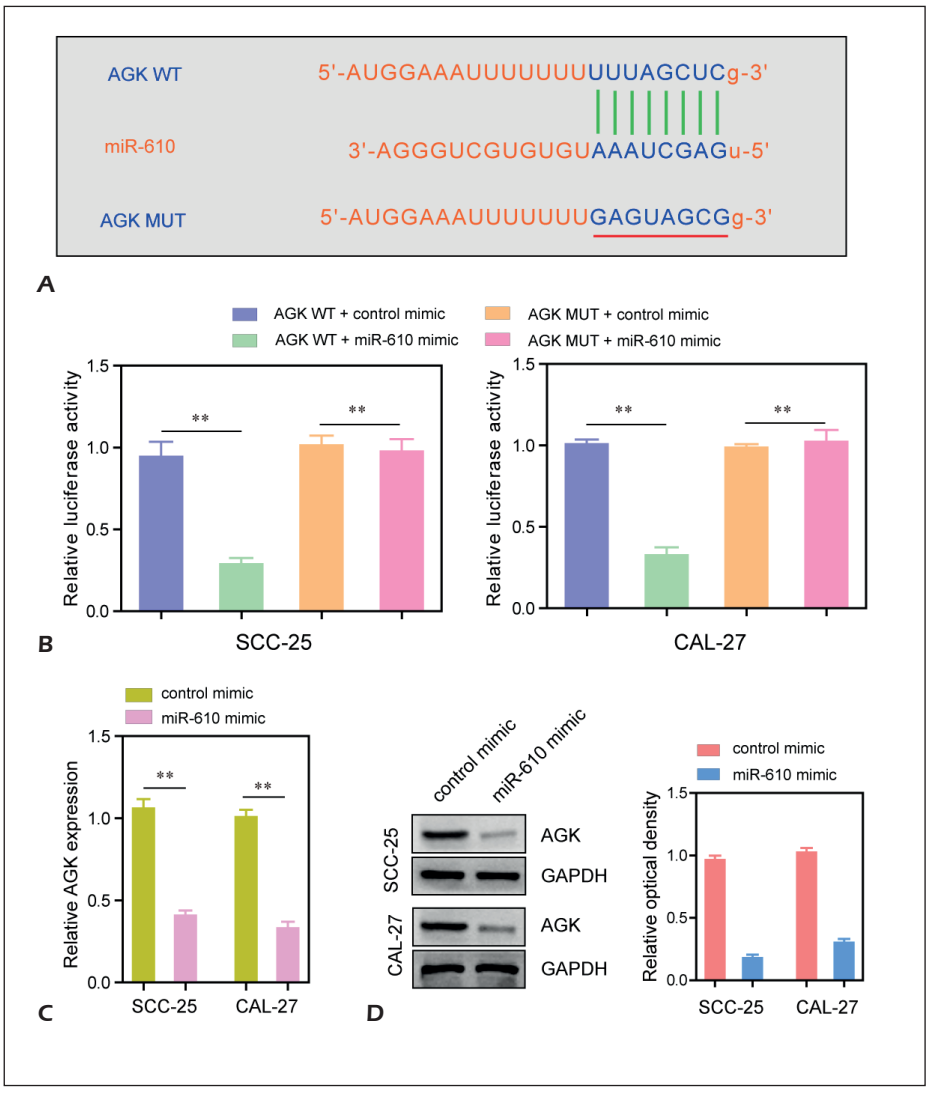

Figure 4. MiR-610 was directedly interacted with AGK. **A**, The predicted binding site by bioinformatics website "starBase". **B**, The Luciferase activities of SCC-25 and CAL-27 cells were determined by Dual-Luciferase reporter assays. **C**, QRT-PCR assays indicated that transfection of miR-610 mimics reduced the mRNA levels of AGK in SCC-25 and CAL-27 cells. **D**, The protein levels of AGK were decreased in SCC-25 and CAL-27 cells transfected with miR-610 mimics. *p<0.05, **p<0.01.

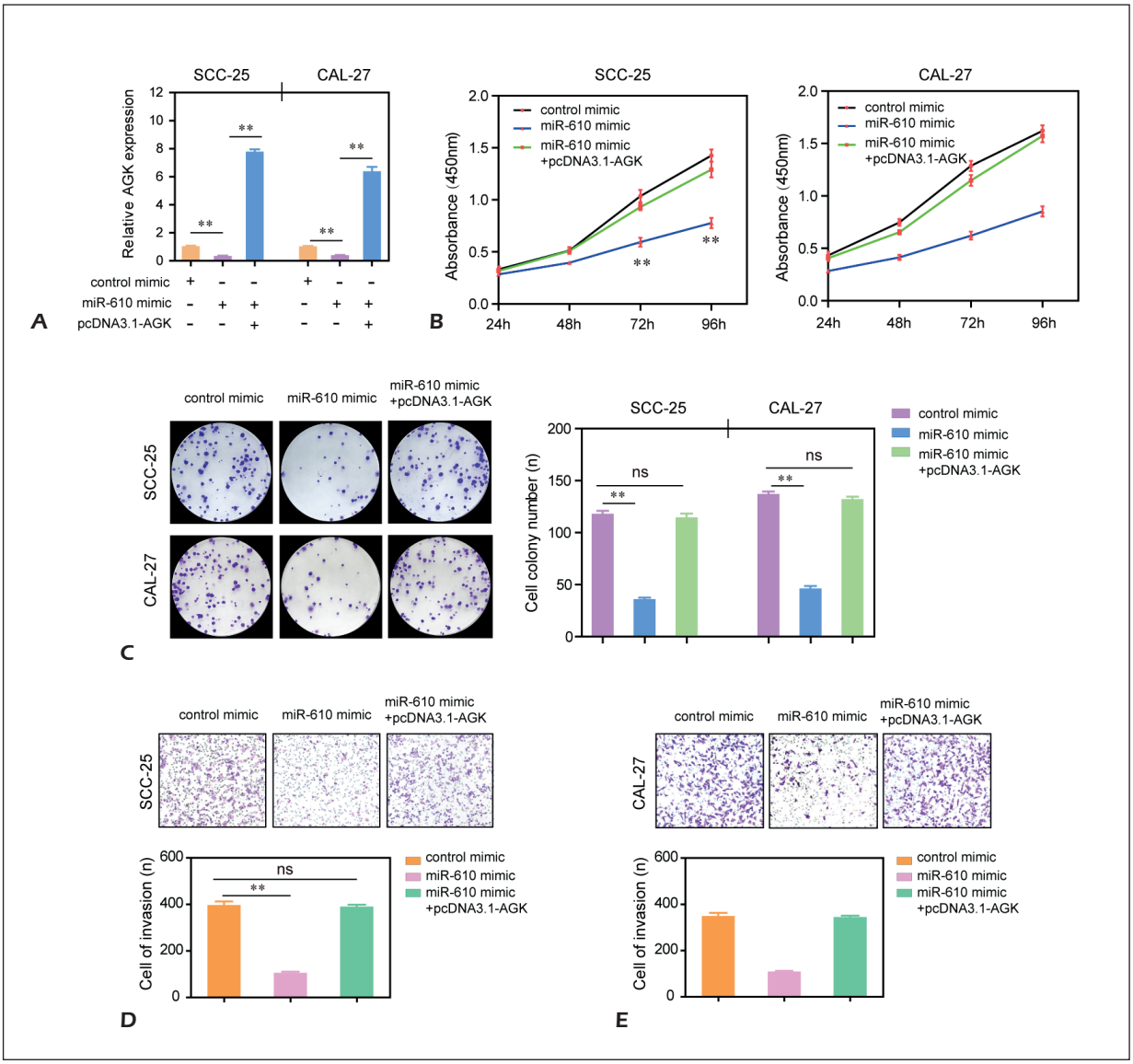

Figure 5. Overexpression of AGK abrogated the tumor suppressive roles of miR-610. **A**, Relative mRNA expression levels of AGK in SCC-25 and CAL-27 cells under various experimental conditions. **B**, CCK-8 assays detected the proliferative rates of SCC-25 and CAL-27 cells in different groups. **C**, The congenic capabilities of SCC-25 and CAL-27 cells in different groups examined by colony formation assays. **D-E**, The transwell invasion assays were performed to evaluate the invasive abilities of SCC-25 and CAL-27 cells transfected with control mimic, miR-610 mimic or miR-610 mimic as well as pcDNA3.1-AGK plasmids. *p<0.05, **p<0.01.

eration and invasion. Although transfection of miR-610 mimic reduced the expression of AGK, the mRNA levels of AGK were significantly increased when SCC-25 and CAL-27 cells were transfected with pcDNA3.1-AGK plasmids (Figure 5A). Moreover, the CCK-8 assays suggested that restoring expression of AGK in SCC-25 and CAL-27 cells rescued the suppression of the proliferation which was mediated by miR-610 (Figure 5B). Similarly, colony formation assays confirmed that pcDNA3.1-AGK plasmids abrogated the suppressive effects of miR-610 on the cell colony formation of SCC-25 and CAL-27 cells (Figure 5C). Besides, transwell invasion assays demonstrated that the number of invasive SCC-25 and CAL-27 cells was significantly increased when they were co-transfected with miR-610 mimics as well as pcDNA3.1-AGK plasmids compared with the cells transfected with miR-610 mimics alone (Figure 5D and E). In summary, these data suggested that miR-610 modulated the development and progression of OSCC via AGK.

Discussion

OSCC is a serious health problem and accounts for approximately 3% of all newly diagnosed clinical cancer cases in China[19]. Smoking, alcohol abuse and betel quid chewing are the major risk factors of OSCC[20]. Despite significant advancement in understanding the potential molecular of OSCC, the use of genetic alterations for diagnostic and prognostic purposes remains limited[21,22]. Recently, miRNAs have emerged as novel biological regulators which could be used as novel diagnostic and prognostic biomarkers[23,24]. In this work, we found a newly identified miRNA miR-610, which has been reported to be down-regulated in several other tumors. Similarly, our results confirmed miR-610 as a down-regulated miRNA in OSCC, which implied that miR-610 may act as a functional regulator in this disease. Furthermore, clinical data by analyzing the association between miR-610 and clinicopathological characteristics indicated that low expression of miR-610 was significantly associated with advanced clinical stages, including T classification and TNM stage. Importantly, Kaplan-Meier analysis by analyzing five-year follow-up data revealed that OSCC patients with low miR-610 expression tended to have worse overall survival. Subsequent univariate and multivariate analysis further confirmed that miR-610 expression was an independent prognostic factor for overall survival. Overall, these above results provided important clinical evidence that miR-610 had an important clinical value for OSCC prognosis. Abnormal expression of various miRNAs has been reported in OSCC, acting as oncogenes or tumor suppressors[25]. miR-610 has been reported to be lowly expressed in several tumors and play a functional role. For instance, Sun et al[26] reported that miR-610 acted as a down-regulated miRNA in colorectal cancer and its overexpression suppressed colorectal cancer cell growth and invasion *in vitro* via modulating hepatoma-derived growth factor. Zhang et al[16] showed that miR-610 was lowly expressed in melanoma and associated with adverse prognostic characteristics. In their functional investigation, it was observed that miR-610 suppressed tumor growth of melanoma via modulating LRP6. The down-regulation of miR-610 was also observed in gastric cancer, osteosarcoma and hepatocellular carcinoma, and its anti-oncogenic effect was mediated by targeting tumor genes and Wnt/β-catenin signaling[17,18,27]. These results highlighted miR-610 as an important negative regulator in the above tumors. However, the biological function of miR-610 in OSCC remains unknown. In this study, we designed several experiments to explore the function of miR-610. First, we up-regulated the levels of miR-610 in OSCC by transfection of miR-610 mimics. Secondly, we performed CCK-8 and colony-formation assay to explore the roles of miR-610 on the proliferation of OSCC, finding that the overexpression of miR-610 significantly suppressed the proliferation of OSCC. Thirdly, transwell assay was used to analyze the influence of miR-610 on the metastasis of OSCC and the results indicated that up-regulation of miR-610 significantly suppressed the migration and invasion of OSCC cells. On the other hand, the results of Western bot revealed that the overexpression of miR-610 could suppress EMT progression. Overall, the above data indicated that miR-610 may act as a tumor suppressor in OSCC because its overexpression may suppress the proliferation, migration, invasion and EMT. The Acylglycerol kinase (AGK) gene, located on the 7th chromosome, encodes the enzyme mitochondrial acylglycerol kinase[28]. It is found that AGK is abundantly expressed in the heart, kidney and brain[29]. In clinical research, AGK plays a critical role in several diseases, such as diabetic retinopathy and tumors[30,31]. Wang et al[32] reported that AGK promoted cell proliferation in breast cancer by inhibiting the FOXO1 transcription factor. Cui et al[33] suggested that AGK promoted the tumorigenicity of hepatocellular carcinoma cells by modulating the NF-κB signaling pathway. Importantly, a recent study by Liu et al[34] revealed that AGK was highly expressed in OSCC and promoted the proliferation and cell cycle progression of OSCC. On the other hand, Chi et al[35] reported that AGK, targeted by miR-194, could reverse the anti-oncogenic role mediated by miR-194. Interestingly, we found that AGK contained a binding sequence for miR-610 by searching Database TargetScan. Using a Dual-Luciferase reporter assay, we confirmed that miR-610 directly bound to the 3'-UTR of AGK. In addition, the overexpression of miR-610 could suppress the levels of AGK at both mRNAs and proteins levels. Moreover, functional experiments showed that the overexpression of miR-610 significantly inhibited OSCC cell proliferation, migration and invasion, whereas AGK overexpression abrogated these effects. Our results indicated that miR-610 displayed its tumor suppressor role by targeting AGK.

Conclusions

We demonstrated for the first time that miR-610 expression was reduced in OSCC and associated with poor prognosis. The overexpression of miR-610 promotes OSCC cell growth, migration and invasion by targeting AGK. In the future, miR-610 may serve as a potential prognostic biomarker and treatment targeting in OSCC.

Conflict of Interests

The authors declare that they have no conflict of interest.

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