RESEARCH ARTICLE

Rs895819 within miR-27a Might be Involved in Development of Non Small Cell Lung Cancer in the Chinese Han Population

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Abstract

MicroRNA-27a (miR-27a) is deemed to be an oncogene that plays an important role in development of various cancers, and single nucleotide polymorphism (SNP) of miR-27a can influence the maturation or aberrant expression of hsa-miR27a, resulting in increased risk of cancer and poor prognosis for non-small cell lung cancer (NSCLC). This study aimed to assess the effects of rs895819 within miR-27a on susceptibility and prognosis of NSCLC patients in 560 clinical confirmed cases and 568 healthy check-up individuals. Adjusted odds/hazard ratios (ORs/HRs) and 95% confidential intervals (CIs) were calculated to evaluate the association between rs895819 and the risk and prognosis of NSCLC. The results showed that allele A and genotype GG of rs895819 were significantly associated with an increased risk of NSCLC (38.9% *vs* 30.8%, adjusted OR=1.26, 95%CI=1.23-1.29 for allele G *vs* A; 18.1% *vs* 11.7%, adjusted OR=1.67, 95%CI=1.59-1.75 for genotype GG *vs* AA). Moreover, positive associations were also observed in dominant and recessive models (53.7% *vs* 49.9%, adjusted OR=1.17, 95%CI=1.13-1.20 for GG/AG *vs* AA; 18.1% *vs* 11.7%, adjusted=1.65, 95%CI=1.58-1.73). However, no significant association was found between rs895819 and the prognosis of NSCLC in genotype, dominant and recessive models. These results suggested that miR-27a might be involved in NSCLC carcinogenesis, but not in progression of NSCLC. The allele G, genotype GG and allele G carrier (GG/AG *vs* AA) of rs895819 might be genetic susceptible factors for NSCLC. Further multi-central, large sample size and well-designed prospective studies as well as functional studies are warranted to verify our findings.

Keywords: miR-27a - single nucleotide polymorphism - NSCLC - Chinese Han

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Introduction

Due to high morbidity and mortality, malignant behaviors and lack of effective treatment, lung cancer is deemed to the leading cause of cancer-related deaths, worldwide. According to cancer statistics in 2014, approximately 116,000 men and 108,210 women will be estimated with new NSCLC cases and 86,930 men and 72,330 women will die of the disease in USA (Siegel et al., 2014). In China, a total of 605,946 persons were diagnosed as new NSCLC patients, and 486,555 cases were died in 2010 (Chen et al., 2014). Non-small cell lung cancer (NSCLC) accounts for 80% of all lung cancer patients (Lee et al., 2006). Although the rapid advantage in understanding the mechanism of carcinogenesis and progression of NSCLC recently, but the precise mechanism remains puzzle. It is well known that NSCLC is a complicated malignant with an intense crosstalk of genetic and environmental factors. Accumulating evidences suggested that single nucleotide polymorphism (SNP) of related genes were significantly associated with the risk and prognosis of NSCLC.

MicroRNA-27a gene (miR-27a) is supposed to be a candidate NSCLC-related gene that transcripts a 22 bp small non-coding RNA. It has been identified as an oncogene in various cancers, including NSCLC (Tian et al., 2014; Wang et al., 2011). It is involved in cell proliferation and apoptosis, metastasis and drug resistance in cancer cell lines by specially binding to 3'-UTR of its targeted genes (Li et al., 2014; Tian et al., 2014; Noratto et al., 2013). High expression of hsa-miR-27a was observed in gastric cancer, pancreatic carcinoma and lung adenocarcinoma tissues as well as cisplatin-resistant lung adenocarcinoma A549/CDDP cell line (Liu et al., 2009; Ma et al., 2010; Li et al., 2014). It regulated cell proliferation and division, colony formation and migration in pancreatic cell line (Ma et al., 2010). Also, it induced the drug resistance of esophageal, gastric and lung cancer cell line as well as leukemia (Zhang et al., 2010; Feng et al., 2011; Noratto et al., 2013; Li et al., 2014). Furthermore, it reported that miR-27a could regulate epithelial-mesenchymal transition (EMT) in A549 cell line, and it was significantly down-regulated when MET tyrosine kinase (MET) was stably silenced in A549 lung cancer cell (Li et al., 2014). Since MET is basally overexpressed in NSCLC and activated in A549 cell line, and activated MET signaling plays an

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important role in human cancer tumorigenesis, metastasis, and drug resistance (Feng et al., 2014). So we speculate that miR-27a may be involved in carcinogenesis and progression of NSCLC.

Rs895819, a SNP locus located in the terminal loop of pre-miR-27a, was reported to be significantly associated with the risk of gastric and cervical cancers (Sun et al., 2010; Xiong et al., 2014). However, there is no study reported the association between the locus and the risk of NSCLC, and prognostic results between them remain elusive (Xu et al., 2013; Yoon et al., 2012). Therefore, in order to address the role of rs895819 in the risk and prognosis of NSCLC, we genotyped the locus in 560 NSCLC patients and 568 healthy check-up individuals and followed-up the cases to examined the possible association between them.

Materials and Methods

560 NSCLC patients who were diagnosed between January 2007 to October 2009 and 568 healthy check-up individuals matched with sex and age from May 2014 to August at Nanjing First Hospital were enrolled in the study. All cases were confirmed with histological evidence and all subjects in the control group were free clinical symptom, CT detection and serum tumor protein biomarkers (CEA, CA199, and CyfRA211) were normal. Follow-up was conducted every three months from the time of recruitment until the last 5-year's scheduled follow-up or death. The last follow-up time was October 2014 and the medial follow-up time was 23.0 months. All subjects of the study are Han nationality, which is consisted of 95% Chinese population. This study was approved by the ethical committee of Nanjing First Hospital and written informed consents were obtained from all individuals enrolled in the present study.

The genomic DNA of each subject of the cases and controls were extracted from 200ul EDTA-anticoagulated peripheral blood samples using Tiangen genomic DNA isolation kit (Tiangen, Beijing, China) according to the manufacturer's protocol. The DNA concentration and purity of each sample were measured by an ultraviolet spectrophotometer (GE Healthcare, USA) and samples with A260/A280 ratio ranged from 1.8 to 2.0 were selected as eligible samples and stored at -20°C until detection. TaqMan allelic discrimination assay was selected to detect genotype of each sample using ABI7500 fluorescence quantitative PCR system (Applied Biosystems, Foster City, USA). The detections were performed in a total volume of 20ul which contained 100ng genomic DNA temple, 10pM of each primer, 0.2mM dNTPs, 2.5ul 10*PCR buffer, 1.5mmol/l $MgCl_2$, and 0.5 U of Taq polymerase (Tiangen, Being, China). Primer and probe sequences, reaction conditions used were described previously by Wang et al (Wang et al., 2014). In order to confirm the accuracy of the detected results, 5% PCR products were randomly selected to DNA sequencing and the results were 100% consistent.

Allele and genotype frequencies of the locus were obtained by directed counting. Hardy-Weinberg equilibrium (HWE) was tested using HWE software and $p<0.05$ was considered as a significant departure from HWE (Rodriguez et al., 2009). Odds ratio (OR) and 95% confidential interval (CI) were calculated to estimate the strength of the locus and cancer risk. Recurrence-free survival (RFS) was defined as the time from diagnosis to the first data of recurrence, and the time from diagnosis to death was considered as overall survival (OS). Kaplan-Meier curve and long-rank test were used to assess the association between the locus and survival of the cases. Cox proportional hazard model was selected to estimate the hazard ratio (HR) and 95%CI for RFS and OS. All the data analyses were performed using SPSS 17.0 statistical software (SPSS Company, Chicago, IL).

Results

Participant characteristics

Due to insufficient volume sample ad genomic DNA purity, 18 cases and 11 controls samples were excluded for detecting the genotype. Finally, a total of 542 cases and 557 controls were enrolled in our study. The baseline characteristics of the cases and controls were listed in Table 1. No significant difference was observed with regard to gender, age, smoking and drinking in the case and control groups. The cases comprised of 542 NSCLC cases receiving surgical operation. Among these patients, there were 268 (49.4%) adenocarcinomas, 230 (42.4%) squamous cell carcinomas, and 44(8.2%) cases mucoepidermoid, adenosquamous or large cell carcinoma. There were 315 males (58.1%) and 284 (52.4%) smokers. The distributions of TNM I/II and III stage in cases were 332(61.3%) and 210(38.7%). And most of the cases (301 (55.5%)) had well or moderate differentiation. All of the cases underwent radical surgery, and 287(53.0%) patients received adjuvant chemotherapy with platinum-based regimen or radiotherapy (Table 1). In the period of follow-up time, 427 patients were detected as recurrence, and 361 patients died of NSCLC. No significant difference of NSCLC recurrence or death risk was observed in cases in comparison of male *vs* female, smoking *vs* no smoking, drinking *vs* no drinking, adnocarcinoma *vs* squamous cell carcinoma and others as well as well/moderate *vs* poor/undifferentiated. However, the risk of death recurrence of NSCLC was significantly increased in patients with advanced TNM III stage diseases (HR=1.32 for RFS; HR=1.30 for OS). In addition, patients receiving adjuvant chemotherapy or radiotherapy had better both OS and RFS compared with those without any adjuvant therapy (HR=0.41 and 0.38, respectively) (Table 4).

Rs895819 and clinical characteristics

In order to explore the association of genotype with phenotype, we conducted the analysis between allele and genotype of rs895819 and the case clinical pathological characteristics. No significant association was found between cancer histology, cell differentiation, TNM stage, node metastasis and genotypes and alleles of rs895819 (Table 2).

Rs895819 and the risk of NSCLC

The distributions of rs895819 allele and genotype

Table 1. The Baseline Characteristics in Case and Control Groups

Parameters	Cases (542)	Controls (557)
Age (years, M±SD) (p=0.605)	60.2±10.7	59.9±10.7
Gender (p=0.89)		
Male	315(58.1%)	326(58.5%)
Female	227(41.9%)	231(41.5%)
Smoking (p=0.586)		
Yes	284(52.4%)	301(46.0%)
No	258(47.6%)	256(54.0%)
Drinking (p=0.415)		
Yes	264(48.7%)	285(51.2%)
No	278(51.3%)	272(48.8%)
Histology		
Adenocarcinoma	268(49.4%)	
Squamous cell carcinoma	230(42.4%)	
Others*	44(8.2%)	
Differentiation		
Well/moderate	301(55.5%)	
Poor/Undifferentiated	241(44.5%)	
TNM stage		
I/II	332(61.3%)	
III	210(38.7%)	
Node metastasis		
N0/N1	95(17.5%)	
N2/N3	447(82.5%)	
Adjuvant treatment		
None	255(47.0%)	
Chemotherapy or radiotherapy	287(53.0%)	

Other*: mucoepidermoid, adenosquamous or large cell carcinoma. Squamous cell carcinoma

in two groups were listed in Table 3. The genotype distributions in control group were consistent with the HWE model (p>0.05). Allele G frequency in cases was significant higher than it in controls (38.9% *vs* 30.8%, p<0.001), indicating that allele G is positive associated with the risk of NSCLC (adjusted OR=1.26, 95%CI=1.23-1.29); genotype AG distribution in two groups did not show the significant difference (35.6% *vs* 38.2%, p=0.240), however, genotype GG frequency in cases was significant higher comparing to it in controls (18.1% *vs* 11.7%, p<0.001), suggesting that only genotype GG was significantly associated with NSCLC risk (adjusted OR=1.67, 95%CI=1.59-1.75). In addition, significant differences in genotype distributions were examined in dominant model (GG/AG *vs* AA, p<0.001, adjusted OR=1.17, 95%CI=1.13-1.20) and recessive model (GG *vs* AA/AG, p<0.001, adjusted OR=1.65, 95%CI=1.58-1.73).

Rs895819 and the survival of NSCLC

Cox regression analysis was used to assess the associations between rs895819 genotype and the RFS and OS of NSCLC patients. No significant association was found between rs895819 and NSCLC RFS in genotype, dominant and recessive models (HR=1.11, 95%CI=0.87-1.42 for AG *vs* AA; HR=0.95, 95%CI=0.73-1.25 for GG *vs* AA; HR=1.08, 95%CI=0.88-1.33 for AG/GG *vs* AA; HR=0.90, 95%CI=0.70-1.16 for GG *vs* AA/AG) (Table 4 and Figure 1A). In addition, there is no significant association between rs895819 and OS of NSCLC

Table 2. Rs895819 Polymorphism and Clinical Pathological Characteristics in the Cases

Variables	Genotype and allele					p-value		
	AA	AG	GG	A	G	[1]	[2]	[3]
Histology								
Adenocarcinoma	130	93	45	353	183	0.492	0.402	0.579
Others*	121	100	53	342	206			
Differentiation								
Well/moderate	141	109	51	391	211	0.949	0.485	0.521
Poor/Undifferentiated	110	84	47	304	178			
TNM								
I/II	159	110	65	428	240	0.175	0.771	0.867
III	92	83	35	267	153			
Node metastasis								
N0/N1	41	43	11	125	65	0.113	0.228	0.596
N2/N3	210	150	87	570	324			

Other*: Squamous cell carcinoma, mucoepidermoid, adenosquamous or large cell carcinoma; [1]: genotype AG *vs* AA; [2]: genotype GG *vs* AA; [3]: allele G *vs* allele A

Table 3. Rs895819 Genotype and Allele Distributions in Two Groups

Genotype and allele	Cases	Controls	p-value	Crude OR and 95%CI	Adjusted OR and 95%CI*
Allele A	695(61.1%)	771(69.2%)			
Allele G	389(38.9%)	343(30.8%)	<0.001	1.27(1.24-1.30)	1.26(1.23-1.29)
AA	251(46.3%)	279(50.1%)			
AG	193(35.6%)	213(38.2%)	0.24	1.03(0.99-1.06)	0.98(0.95-1.01)
GG	98(18.1%)	65(11.7%)	<0.001	1.79(1.70-1.87)	1.67(1.59-1.75)
AA	251(46.3%)	279(50.1%)			
GG/AG	291(53.7%)	278(49.9%)	<0.001	1.18(1.14-1.22)	1.17(1.13-1.20)
AA/AG	444(81.9%)	492(88.3%)			
GG	98(18.1%)	65(11.7%)	<0.001	1.65(1.58-1.72)	1.65(1.58-1.73)

*adjusted by gender, age, smoking and drinking

Table 4. Associations of Baseline Characteristics and rs895819 Polymorphism with Clinical Outcomes in NSCLC Patients

Variables	Cases	Recurrence free survival (RFS)			Overall survival (OS)		
	-542	Recurrence -427	Adjusted HR (95%CI)	*p*-value	Death -361	Adjusted HR (95%CI)	*p*-value
Gender(male)	315	251	0.99(0.78-1.27)	0.964	216	0.95(0.73-1.22)	0.667
Smoking(Yes)	284	225	1.06(0.94-1.34)	0.62	193	1.04(0.81-1.35)	0.74
Drinking(Yes)	264	204	1.18(0.97-1.43)	0.103	174	0.97(0.78-1.19)	0.74
Histology(others*)	254	197	0.94(0.77-1.13)	0.492	166	0.95(0.771-1.72)	0.633
Differentiation (poor/ undifferentiated)	301	238	1.08(0.89-1.31)	0.437	197	0.71(0.79-1.20)	0.785
TNM(III)	210	159	1.32(1.05-1.67)	0.019	130	1.30(1.12-1.42)	0.049
Adjuvant treatment (Yes)	453	341	0.41(0.32-0.53)	<0.001	283	0.38(0.29-0.49)	<0.001
AG vs. AA	193/251	15/193	1.11(0.87-1.42)	0.4	132/160	1.24(0.95-1.61)	0.111
GG vs. AA	98/251	76/193	0.95(0.73-1.25)	0.723	69/160	1.22(0.92-1.63)	0.169
AG/GG vs. AA	291/251	234/193	1.08(0.88-1.33)	0.474	201/160	1.25(0.73-1.56)	0.54
GG vs. AG/AA	98/444	76/351	0.90(0.70-1.16)	0.414	69/160	1.13(0.86-1.47)	0.377

Other*: Squamous cell carcinoma, mucoepidermoid, adenosquamous or large cell carcinoma

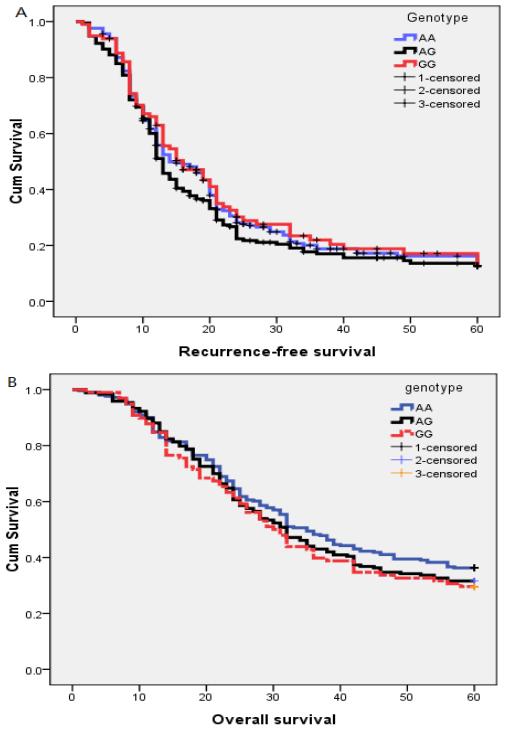

Figure 1. Kaplan-Meier Curves of rs895819 Genotypes for Survival of NSCLC Patients. A: RFS; B: OS

(HR=1.24, 95%CI=0.95-1.61 for AG *vs* AA; HR=1.22, 95%CI=0.92-1.63 for GG *vs* AA; HR=1.25, 95%CI=0.73-1.56 for AG/GG *vs* AA; HR=1.30, 95%CI=0.86-1.47 for GG *vs* AA/AG) (Table 4 and Figure 1B).

Discussion

MicroRNAs (miRNAs) are a class of evolutionarily conserved small noncoding RNAs that contain only 17-25 nucleotides. They can negatively regulate gene expression at the post-transcriptional level predominantly by Watson-Crick base pairing to the 3'-UTR of target messenger RNA (Lambert et al., 2011). They represent only a small proportion of the genome, but can regulate almost one-third of human genes, and are involved in most biological and pathological processes, such as organ growth and development (Aparicio et al., 2014), cell proliferation and differentiation as well as tumorigenesis and cancer progression (Ni et al., 2014; Rupaimoole et al., 2014; Zhao et al., 2014). Polymorphisms in the miRNA are emerging as powerful tools to study the biology of a disease and have the potential to be used as diagnostic and prognostic biomarkers in diseases (Joshi et al., 2014). Rs712 within let-7 binding site of KRAS gene were reported to be significantly associated with cancer risk in Chinese population (Ying et al., 2014). Report from a meta-analysis indicated that miR-196a2 gene rs11614913 polymorphism and the miR-146a gene rs2910164 polymorphism might increase susceptibility to the risk of lung cancer (Fan and Wu, 2014). Furthermore, Hong et al reported that miR-196a rs11614913 and miR-149 rs2292832 can be used as prognostic markers for patients with surgically resected early-stage NSCLC (Hong et al., 2013).

In our study, we evaluated the association between rs895819 within miR-27a and the risk and survival of NSCLC in 542 cases undergoing radical surgery and 557 healthy check-up individuals. In our study, we found that allele G, genotype GG and allele G carrier (genotype AG/GG) of rs895819 were significantly associated with an increased risk of NSCLC, suggesting that miR-27a was involved in developing of NSCLC and allele G, genotype GG and allele G carrier (genotype AG/GG) of rs895819 might be genetic susceptible factors for NSCLC carcinogenesis. However, no significant association was found between rs895819 and clinical pathological characteristics as well as RFS or OS of NSCLC, indicating that miR-27a was not associated with NSCLC progression and rs895819 could not be used as a prognostic molecular biomarker of NSCLC in Chinese Han population. These findings illustrated that miR-27a was only involved in carcinogenesis of NSCLC and rs895819 might be only used as molecular susceptible factor to evaluate the risk of NSCLC. The following reasons might account for our results. Rs895819 was located in the terminal loop of pre-miRNA region of miR-27a (Yang et al., 2010). It may cause spatial structure change in a critical region of hsa-miR-27a, affecting the process and the maturation of hsa-miR-27a. A relative higher expression of miR-27a and a significantly reduced expression of ZBTB10 were

examined in patients with the genotype GG or allele G carrier (AG/GG) compared to genotype AA and up-regulation of miR-27a might contribute to the malignant transformation of human bronchial epithelial cells induced by SV40 small T antigen (Sun et al., 2010; Wang et al., 2011). Since miR-27a functions as an oncogene in human carcinogenesis (Pan et al., 2014; Wang et al., 2011), a higher expression of miR-27a might promote cancer cell growth, increase cell viability and colony formation and inhibition of the late apoptosis arising from the suppression of PLK2, ZBTB10 and FBXW7 (Sun et al., 2010; Tian et al., 2014; Wang et al., 2011), leading to responsible for the viral oncoprotein small T antigen-induced malignant transformation.

To the best of our knowledge, this is the first study to evaluate the effects of rs895819 within miR-27a on the risk of NSCLC and the largest sample size to explore the prognostic value of rs895819 in NSCLC. Recently, Yoon et al reported that rs895819 of miR-27a was not associated with prognosis in patients with completely resected NSCLC in Korean population (Yoon et al., 2012). A study conducted by Xu et al suggested that miR-27a rs895819 polymorphism might influence NSCLC patients' clinical outcome (Xu et al., 2013). However, our result indicated rs895819 wasn't associated with NSCLC progression, and it could not be used to evaluate the survival of NSCLC in Chinese Han population. The inconsistent results may cause by selection of the cases and small sample size. In our study, the cases with TNM stage I-III were selected and all of them performed surgical operation, which was the same to the study by Yoon et al. However, the study by Xu et al included the TNM stage 1-IV NSCLC patients (Xu et al., 2013). In addition, the cases in two studies were from one single hospital with small size, but a small size was not large enough to reach a more precise conclusion.

In summary, our study found that rs895819 within miR-27a was significantly associated with NSCLC carcinogenesis, but not with NSCLC progression in Chinese Han population. These findings suggested that miR-27a was involved in carcinogenesis of NSCLC, allele G, allele G carrier (AG/GG) and genotype GG of rs895819 only could be used as susceptible factors for evaluating the risk of NSCLC. Further, multiple central, large-sample size and well-designed prospective study are recommended to further verify our finding.

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