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Original Article

Association of *AXIN2* and *MMP7* polymorphisms with non-small cell lung cancer in Chinese Han population

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Abstract: Objectives: This study aimed to explore the effect of AXIN2 and MMP7 polymorphisms on non-small cell lung cancer (NSCLC) susceptibility; in addition, the interaction between gene polymorphisms and environment was also displayed. Methods: The genotyping was conducted by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) in 102 patients with NSCLC and 120 healthy controls. Odds ratio (OR) and 95% confidence interval (CI) were calculated to assess the relevance strength of AXIN2 and MMP7 polymorphisms with NSCLC. The x^2 test was used to compare to the frequencies difference of genotypes and alleles in cases and controls and Hardy-Weinberg equilibrium (HWE) test. The haplotype and interaction analyses were performed by haploview and MDR software, respectively. Results: The genotype frequencies of all polymorphisms in the control group conformed to HWE. GG genotype frequency of *AXIN2* rs2240307 polymorphism was significantly higher in cases than controls (*P*=0.041). Similarly, rs2240308 in *AXIN2* gene was also increased the susceptibility to NSCLC remarkably (OR=2.412, 95% CI=1.025-5.674). What's more, haplotype A-G-G in *AXIN2* might play a protective role in NSCLC (OR=0.462, 95% CI=0.270-0.790). Genotype GG and allele G in *MMP7* rs11568818 polymorphism were associated with the risk of NSCLC development (*P*=0.024 and *P*=0.038). In addition, the interaction existed in gene polymorphsims and environment (*P*=0.011). Conclusion: *AXIN2* rs2240307 and rs2240308 and *MMP7* rs11568818 polymorphisms might be the independent risk factors for NSCLC and showed the interaction with environmental factor.

Keywords: *AXIN2*, *MMP7*, polymorphism, non-small cell lung cancer

Introduction

Lung cancer a kind of malignant tumor with high mortality and metastasis capacity [1]. The common symptoms are constant coughing, fever, blood-stained sputum, shortness of breath, loss of weight and chest pains and patients show different symptoms with the advancing of stage [2]. Non-small cell lung cancer (NSCLC) accounts for 85% of all lung cancer cases and has a good respond to surgery treatment, but not chemotherapy and radiotherapy [3, 4]. However, lung cancer is a complicated disease regulated by multiple elements, genetic damage to DNA has been proved to have a vital influence on the development of cancers [5-7].

The Wnt signaling pathway regulating cell proliferation, apoptosis and cell cycle, has been recognized that play a vital role in embryonic and organs development [8, 9]. Recently, it is found to participate in the process of carcinogenesis and reported in lung cancer, too [10, 11]. *AXIN2* and *MMP7* are the regulator genes involved in the Wnt signaling pathway.

AXIN2 located in chromosome 17q23-q24, comes in regulating the stability of β-catenin indispensably [12]. The genetic variants of *AXIN2* can lead to the degradation of β-catenin, an important event in the development of multiple malignancies and it has been reported to be associated with a number of cancers [13-15]. Matrix metalloproteinase7 (MMP7) is a crucial member of MMPs family and encoded by *MMP7* gene located in chromosome 11q 22.3 [16]. In physiological condition, MMP7 is in inactivated zymogen mostly and keep stable balance with corresponding inhibitor, however, MMP7 is activated in pathological process, such as inflammation, embryogenesis and angiogenesis [17]. It is a key enzyme in the invasion and metastasis process of tumors and the expression is

Table 1. The detailed characteristics of subjects

Index		Case (n=102, %)	Control (n=120, %)	x^2	*P* value
Age	$\bar{x} \pm s$	51.27±11.64	50.96±11.71	0.026	0.872
Gender	Male	64 (62.75)	71 (59.17)	0.296	0.586
	Female	38 (37.25)	49 (40.83)		
Smoking	Yes	67 (65.69)	59 (49.17)	6.130	0.013
	No	35 (34.31)	61 (50.83)		
Alcohol drinking	Yes	58 (56.86)	62 (51.67)	0.599	0.439
	No	44 (43.14)	58 (48.33)		
Family history	Yes	32 (31.37)	26 (21.67)	2.691	0.101
	No	70 (68.63)	94 (78.33)		
Tumor stage	Early-middle	76 (74.51)		-	-
	Advanced	26 (25.49)			

Table 2. The PCR primer sequences of *AXIN2* and *MMP7* polymorphisms

Polymorphism			Primer sequences	Length
AXIN2	rs2240307	Forward	5'-CTGCCACCAAGACCTACA-3'	102 bp
		Reverse	5'-TTTCCTCCATCACCGACT-3'	
	rs4791169	Forward	5'-GCCTCTGGATTTCCCTTTC-3'	185 bp
		Reverse	5'-TTGGAGAACAAGCCCTTC-3'	
	rs2240308	Forward	5'-CGCTATGTTGGTGACTTGCC-3'	170 bp
		Reverse	5'-CTTCGTTCCGCCTGGTGT-3'	
MMP7	rs11568818	Forward	5'-TGGTACCATAA TGTCCTGAATG-3'	150 bp
		Reverse	5'-TCGTTATTGGCAGGAAGCACACAAGATT-3'	

regulated by the Wnt signaling pathway [18, 19].

Due to both of *AXIN2* and *MMP7* participated in regulating the Wnt signaling pathway, the interaction may exist between the two, which effects the development of disease synergistically. In present case-control study, we selected three polymorphisms of *AXIN2* gene in a block and *MMP7* rs11568818 to conduct our design, in addition, environmental factor is also considered.

Materials and methods

Study subjects

In present study, 102 patients with NSCLC were confirmed by the pathological and clinical diagnoses in oncology department of Chinese PLA general hospital from July in 2012 to November in 2014 as the case group. Their age span was 30-71 with a mean age of 51.27±11.64, including 64 males and 38 females. The control group was frequency-matched with the cases by age and gender and included 71 males and 49 females with an average age of 50.96±11.71. They were enrolled in the health evaluation center of Chinese PLA general hospital with good health and without tumor history. This study design conformed to the rule of the Research Ethics Committee of Chi nese PLA general hospital and written consents should be signed by every subject. In addition, all subjects came from Han population unrelated by blood.

Some clinical characteristics of subjects were obtained to evaluate the difference between the cases and controls and the effect on NSCLC. **Table 1** summarized these information, such as gender, age, smoking, alcohol drinking. The person was as a smoker if he smoked average one or more than one every day and sustained half a year at least. More than one time every week for male or one or more than one times for females was as a drinker.

Sample collection and DNA extraction

5 ml peripheral venous blood was collected from every enrolled subject and then put in EDTA anticoagulative tube. Blood genome DNA

Table 3. The comparison results of genotype frequencies in *AXIN2* and *MMP7* polymorphisms between the case and control groups

Genotype/Allele		Case (n=102, %)	Control (n=120, %)	OR (95% CI)	*P* value
AXIN2					
rs2240307	AA	63 (61.76)	79 (65.83)	1.000 (Ref.)	-
	AG	27 (26.47)	36 (30.00)	0.940 (0.517-1.712)	0.841
	GG	12 (11.77)	5 (4.17)	3.010 (1.007-8.992)	0.041
	A	153 (75.00)	194 (80.83)	1.000 (Ref.)	-
	G	51 (25.00)	46 (19.17)	1.406 (0.895-2.208)	0.138
rs4791169	GG	62 (60.78)	70 (58.33)	1.000 (Ref.)	-
	GA	23 (22.55)	38 (31.67)	0.683 (0.367-1.271)	0.228
	AA	17 (16.67)	12 (10.00)	1.599 (0.709-3.611)	0.256
	G	147 (72.06)	178 (74.17)	1.000 (Ref.)	-
	A	57 (27.94)	62 (25.83)	1.113 (0.731-1.696)	0.617
Rs2240308	GG	50 (49.02)	67 (55.83)	1.000 (Ref.)	-
	GA	34 (33.33)	43 (35.83)	1.060 (0.593-1.893)	0.845
	AA	18 (17.65)	10 (8.34)	2.412 (1.025-5.674)	0.040
	G	134 (65.69)	177 (73.75)	1.000 (Ref.)	-
	A	70 (34.31)	63 (26.25)	1.468 (0.976-2.206)	0.065
MMP7					
rs11568818	AA	42 (41.18)	62 (51.67)	1.000 (Ref.)	-
	AG	47 (46.08)	52 (43.33)	1.334 (0.765-2.326)	0.309
	GG	13 (12.74)	6 (5.00)	3.198 (1.126-9.082)	0.024
	A	131 (64.22)	176 (73.33)	1.000 (Ref.)	-
	G	73 (35.78)	64 (26.67)	1.532 (1.022-2.297)	0.038

Table 4. Haplotypes analysis of *AXIN2* rs2240307, rs4791169, rs2240308 polymorphisms in a block

Haplotype	Case (2n=204, %)	Control (2n=240, %)	OR (95% CI)	*P* value
A-G-A	59 (28.92)	56 (23.33)	1.000 (Ref.)	-
A-G-G	37 (18.14)	76 (31.67)	0.462 (0.270-0.790)	0.005
A-A-G	57 (27.94)	62 (25.83)	0.873 (0.522-1.457)	0.602
G-G-G	40 (19.61)	39 (16.25)	0.973 (0.549-1.726)	0.927
G-G-A	11 (5.39)	7 (2.92)	1.492 (0.540-4.119)	0.438

was extracted using conventional chloroform/isoamyl alcohol extraction and stored at -20°C.

PCR and genotyping

PCR primers were designed by Primer 5.0 software and the sequences were listed in **Table 2**. The PCR system was a volume of 25 μl solution, 1 μl DNA template, 0.5 μl forward and reverse primer, respectively, 12.5 Master Mix and 10.5 μl redistilled water. PCR reaction procedure of *AXIN2* polymorphisms: 95°C, 3 min for initial denaturation, followed 36 cycles of 94°C, 30 s for denaturation, 50.3°C, 54.1°C, 58.5°C (rs2240307, rs4791169, rs2240308), 30s for annealing, 72°C, 30s for extension and finally extension at 72°C for 5 min; *MMP7* rs11568818: 94°C, 3 min for initial denaturation, followed 35 cycles of 94°C, 30 s for denaturation, 55.5°C, 30 s for annealing, 72°C, 30 s for extension and finally extension at 72°C for 7 min.

The PCR products were digested by restriction enzyme *Alu I*, *Bgl I*, *Hinf I*, and *EcoR I*, respectively and separated through 2.5% agarose gel electrophoresis and EB staining.

Statistical methods

The data was represented by $\bar{x}\pm s$ or %. The genotype distribution of *AXIN2* and *MMP7* polymorphisms in control group was tested by Hardy-Weinberg equilibrium (HWE). Odds ratio (OR) with 95% confidence interval (CI) was calculated by the chi-squared and was used to represent the association intensity between

Table 5. The interaction analysis of gene polymorphisms and environment in NSCLC patients and controls

Model	Training BA	Testing BA	Sign test/P	CV-Consistency
Smoking X1	0.626	0.511	5 (0.623)	8/10
Smoking X1 X4	0.657	0.485	4 (0.828)	4/10
Smoking X2 X3 X4	0.718	0.613	9 (0.011)	9/10

Note: X1: rs4791169, X2: rs2240307; X3: rs2240308; X4: rs11568818; BA: balanced accuracy; CV-CV-Consistency: Cross-validation Consistency.

polymorphism and disease. The statistical analysis was conducted through SPSS18.0 software. Linkage disequilibrium and haplotypes analyses was also done based on haploview software, what's more, the interaction between the polymorphisms of two genes was analysis in MDR software. $P>0.05$ was considered the statistical significance.

Result

Characteristics of subjects

A total of 222 subjects were enrolled in this study, there was no significant difference by sex and age in 102 cases and 120 controls ($P>0.05$). Alcohol consumption and family history were also not the influence factors for NSCLC, but the frequency difference of smoking was the statistical significance in the case and control groups ($P=0.013$) (**Table 1**). Among the patients with NSCLC, advanced stage accounted for 25.49%.

HWE test

The genotypes distributions of all polymorphisms in *AXIN2* and *MMP7* genes were consistent with HWE in the control group ($P>0.05$), the results suggested that our study subjects possessed a good representativeness.

Genotype and allele frequencies comparison between the cases and controls

As was shown in **Table 3**, GG genotype of rs2240307 and AA genotype of rs2240308 in *AXIN2* gene were associated with the increased risk of NACLC directly and the genotype frequencies had significant different between the cases and controls (OR=3.010, 95% CI=1.007-8.992, $P=0.041$ and OR=2.412, 95% CI=1.025-5.674, $P=0.040$).

Similarly, genotype GG of MMP7 rs11568818 polymorphism had an obviously higher frequency in cases than controls and might be associated with NSCLC susceptibility (OR=3.198, 95% CI=1.126-9.082). Allele G also increased 1.532 times risk for NSCLC development in the case group (OR=1.532, 95% CI=1.022-2.297).

Linkage disequilibrium and haplotypes analyses in AXIN2 polymorphisms

AXIN2 rs2240307, rs4791169, rs2240308 polymorphisms were linkage disequilibrium and five haplotypes were shown in **Table 4**. Among of them, only haplotype A-G-G frequency had remarkable different in the case group compared with the control group and it might be associated with the decreased risk of NSCLC (OR=0.462, 95% CI=0.270-0.790).

Interaction between AXIN2 and MMP7 polymorphisms and environment

In present study, we also explored the interactions of gene-gene and gene-environment. We obtained the results in **Table 5** that *AXIN2* and *MMP7* polymorphisms participated in the Wnt signaling pathway and environmental factor had the interaction and they effected the development and progression of NSCLC synergistically. The superior MDR model consisted of rs2240307, rs2240308 in *AXIN2*, rs11568818 in *MMP7* and smoking ($P=0.011$).

Discussion

The Wnt/β-catenin signaling pathway is in inactivated in normal cells and β-catenin, glycogen synthase kinase 3 (GSK3), adenomatosis polyposis coli (APC) and Axin form a polyprotein complex. But when Wnt lack, β-catenin is the phosphorylation of serine/threonine residues catalyzed by GSK-3α/β and casein kinase1 (CK1), which leads to the degradation of β-catenin [20]. Wnt protein combines with low-density-lipoprotein-receptor-related protein (LRP-5, LRP-6) in the surface of membrane, which catalyzes the phosphorylation of dishevelled (Dah) and combines with Axin, and then inhibits the activation of GSK/3β and the synthesis of β-catenin polyprotein complex [21]. So β-catenin is accumulated in cytoplasm and

then transfers to cell nucleus, followed by synthesizing the complex with transcription factor TCF/LEF, which stimulates the transcription of Wnt target genes, such as Cyclin D1, *MMP7*, c-myc, and regulates cell growth [22].

In the study of Zhang et al., they explore the role of Wnt/β-catenin signaling in maintaining the cancer stem cells (CSCs) in lung cancer, the results show that Wnt signal pathway plays an vital role in maintaining highly resistant CSCs and regulating cell cycle, apoptosis and metastasis in the development and progression of lung cancer [23]. What's more, several genes related with the Wnt signaling pathway have been explored the relationship with lung cancer. Li et al. have found that Wnt3a, a key gene in Wnt signaling pathway, can accelerate cell invasion and the growth of anchorage-independent and promote the metastasis ability of NSCLC through regulating the expression of *Notch3* in Notch signaling pathway [24]. Coscio et al. indicate that the Wnt signaling pathway may play a crucial role in tumorigenesis and the progression of cancer and study 441 single nucleotide polymorphisms (SNPs) from 54 genes in the Wnt signaling pathway in NSCLC patients, the final results show that genetic variants contribute to the clinical outcomes for patients with the early-stage NSCLC [25].

However, in previous studies, they mostly focus on the function of single gene. As we all known, the occurrence of diseases are influenced by many factors in complex human body. Therefore, in our study, we explored the relevance of 4 polymorphisms from two genes in the Wnt signaling pathway with NSCLC susceptibility and the environment factor was also considered. The homozygous mutant genotypes of rs2240307, rs2240308 in *AXIN2* gene significantly increased the risk of NSCLC and *MMP7* rs11568818 polymorphism was also considered to be associated with the increased susceptibility to NSCLC. What's more, the haplotype A-G-G of rs2240307, rs4791169, rs2240308 in a block of *AXIN2* might be a protective factor for against the occurrence of NSCLC in Chinese Han population. In addition, we found that there were the interaction between *AXIN2*, *MMP7* polymorphisms and environment factor.

Axin is an essential component of β-catenin polyprotein complex which works as the key element to maintain the Wnt signaling pathway in stably inactivated. *MMP7* can mediate cell apoptosis signal through digesting extracellular matrix proteins and prohibiting fas/fasl, and affecting the tumorigenesis microenvironment. The report has proved that Wnt/β-catenin pathway influences the development of cancer through activating the downstream target genes mainly [26]. *MMP7* as the target gene of the Wnt signaling pathway may account for the interaction with *AXIN2*.

In present study, we studied the effects of rs2240307, rs4791169, rs2240308 polymorphisms in *AXIN2* and rs11568818 in *MMP7* on NSCLC systematically, in the meanwhile, environment factor was also considered. However, due to the small sample size, few genes and gene polymorphisms and only one environmental factor, the results need to be verified further. In the future, well-design with large sample size and considering more influence factors should be conducted.

Disclosure of conflict of interest

None.

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