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Original Article

Correlation between *survivin* genetic polymorphisms and lung cancer susceptibility

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Abstract: Aims: The purpose of the study was to analyze the relationship of survivin polymorphisms including -31G/C, -625G/C, 9194A/G and 9809T/C with the susceptibility to lung cancer. Methods: Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to test the polymorphisms of -31G/C, -625G/C, 9194A/G and 9809T/C in 104 patients with lung cancer and 104 healthy controls. Then, linkage disequilibrium and haplotypes were analyzed by HaploView software. The differences of genotype, allele and haplotype frequencies in case and control group were assessed via chi-square test. Odds ratio (OR) with 95% CI were used to evaluate the correlation of *survivin* polymorphisms with lung cancer. Results: Genotype distribution of each polymorphism site in control group was in agreement with Hardy-Weinberg equilibrium (HWE) (P>0.05). The frequency of -31G/C CC genotype and C allele in case group were much higher than that of controls, respectively (CC: 33.6% vs. 22.1%; C: 57.2% vs. 46.6%) and CC genotype as well as C allele were appeared to be risk factors for lung cancer. Meanwhile, 9194A/G GG genotype could increase the risk for lung cancer (OR=2.86, 95% CI=1.14-7.20). The risk of G allele carriers for lung caner was higher than that of A allele (OR=1.63, 95% CI=1.08-2.47). The haplotypes analysis indicated that CGGC and GCAT were associated with the susceptibility to lung cancer (OR=2.79, 95% CI=1.58-4.92; OR=2.36, 95% CI=1.29-4.30). Conclusions: *Survivin* -31G/C and 9194A/G polymorphisms were associated with the risk of lung cancer. The CGGC and GCAT haplotypes carriers were more likely to develop lung cancer.

Keywords: *Survivin*, polymorphisms, lung cancer

Introduction

Lung cancer is one of the most common malignant tumors which jeopardizes people's health seriously. There are approximately 1.18 million deaths caused by lung cancer every year all over the world. The 5-year survival rate of lung cancer is less than 15% [1]. Early detection and treatment can effectively improve the survival rate. However, due to limited diagnosis methods, over 70% patients are at late or advanced stage when initial diagnosis. Besides, the pathogen of lung cancer are multiple and its progress is complicated. Against the background, genes were extensively studied to explore the pathogenesis of lung cancer and *survivin* was one of them.

survivin gene locates on chromosome 17q25 with a length of 15 kb. It has been reported that *survivin* is related to cell apoptosis and cell proliferation [2-4]. Moreover, it plays crucial role in the pathogenesis of hepatocellular carcinoma, gastric cancer, cervical cancer and gallbladder cancer [5-8]. Besides, downregulation of *survivin* could suppress proliferation and colony formation of non-small-cell-lung cancer cells [9]. The studies also found that *survivin* could significantly affect the survival rate of lung cancer patients [10]. As we all know, single nucleotide polymorphism (SNPs) are the third generation of genetic markers, which are helpful for the diagnosis, prognosis and treatment of diseases. Although many studies have investigated the effects of *survivin* polymorphisms on the occurrence of cancers [11-14], there were few studies exploring the association between *survivin* polymorphisms and lung cancer.

In current study, we selected four polymorphisms sites (-31G/C, -625G/C, 9194A/G and 9809T/C) in *survivin* gene and analyzed the relationship of them with the risk of lung cancer.

Table 1. Sequences of PCR primers for *survivin* (-31G/C, -625G/C, 9194A/G and 9809T/C) polymorphisms

SNP	SNP locus	Primer (5'→3')
Rs 9904341	-31G/C	F: 5'-AAGAGGGCGTGCGCTCCCGACA-3'
		R: 5'-AAGAGGGCGTGCGCTCCCGACA-3'
rs 8073069	-625G/C	F: 5'-GTTCATTTGTCCTTCATGCGC-3'
		R: 5'-GGCAGAGGGTGCAGTGAGC-3'
rs 2071214	9194A/G	F: 5'-GAAGAAAGAATTTGAGGAAACCGC-3'
		R: 5'-AAACCCTGGAAGTGGTGCAG-3'
rs 1042489	9809T/C	F: 5'-GCTTA CCAGGTGAGAAGTGAGG-3'
		R: 5'-GTATCTGCCAGACGCTTCCTATC-3'

F: forward; R: reverse.

Material and methods

Objects of study

104 patients diagnosed with lung cancer via histopathology were collected from Linyi People's Hospital, aging from 29 to 67 with an average age of 45. Meanwhile, 104 healthy people without history of genetic disease or cancers whose age were from 31 to 70 with an average age of 52 were taken as controls. All patients did not receive any radiotherapy or chemotherapy treatment before sampling. The controls and cases were matched on the age and gender and they had no blood relationship. The process of sample collection was proceeded in accordance with the national ethics criterion for human genome research. Written informed consents were signed in advance. The study was approved by the Research Ethics Committee of Linyi People's Hospital.

DNA extraction and genotyping

5 mL venous blood was extracted from each subject and was put into EDTA blood collection tube immediately. Then the samples were stored at -80°C for DNA extraction. DNA was isolated using the improved method of sodium iodide. PCR primers were designed by Primer Premier 5.0 software and synthesized in Shanghai SANGON biological company. The sequences of primers were displayed in **Table 1**. PCR reaction solution included 1 μl (1×10^8 ng/L) DNA, 1 μL forward primer, 1 μl reverse-primer, 25 μL Master Mix and 22 μL of ddH_2O. The reaction procedure was as follows: initial denaturation at 94°C for 10 min, 35 cycles of denaturation for 30 s at 94°C, annealing at 62°C for 30 s, extension at 72°C for 1 min, and finally extension at 72°C for 5 min. The PCR products were handled with MspI enzyme digestion, and then examined by agarose gel electrophoresis to ascertain the genotypes of each genetic variation.

Statistical analysis

SPSS18.0 software was used to conductstatistical analysis. Hardy-Weinberg equilibrium (HWE) was taken to test whether the subjects in control group was representative. The differences on genotypes, alleles and haplotypes distribution between cases and controls were detected by chi-square method. The difference was considered to be significant if $P<0.05$. Odds ratio (OR) and 95% confidence interval (CI) were used to estimate the relationship of *survivin* polymorphisms with lung cancer susceptibility.

Results

HWE test

The genotypes distribution of -31G/C, -625G/C, 9194A/G and 9809T/C polymorphisms were consistent with HWE ($P>0.05$ for all), which indicated that the people in control group was representative.

Correlation between survivin polymorphisms and risk of lung cancer

In the analyses, the genotype frequencies of -31G/C CC and 9194A/G GG were significantly higher in cases than controls, which manifested that they were closely related to the risk of lung cancer (OR=2.28, 95% CI=1.05-4.94; OR=2.86, 95% CI=1.14-7.20). Meanwhile, similar results were observed on -31G/C C and 9194A/G G alleles (OR=1.53, 95% CI=1.04-2.25; OR=1.63, 95% CI=1.08-2.47). However, -625G/C and 9809T/C polymorphisms showed no effects on the occurrence of lung cancer (**Table 2**).

Association of haplotypes and lung cancer risk

14 haplotypes combinations were established according to the linkage disequilibrium analysis of -31G/C, -625G/C, 9194A/G and 9809T/C with HaploView software. For further analysis, 9 haplotypes with frequency of less than 1% were excluded. The remaining 5 haplotypes account-

Table 2. Genotypes and alleles distribution of -31G/C, -625G/C, 9194A/G and 9809T/C

Genotype/ allelotype	Cases n=104 (%)	Controls n=104 (%)	χ^2	*P* value	OR (95% CI)
-31G/C					
GG	20 (19.2)	30 (28.8)	-	-	1.00
GC	49 (47.1)	51 (49.0)	1.087	0.297	1.44 (0.72-2.87)
CC	35 (33.6)	23 (22.1)	4.447	0.035	2.28 (1.05-4.94)
G	89 (42.8)	111 (53.4)	-	-	1.00
C	119 (57.2)	97 (46.6)	4.661	0.031	1.53 (1.04-2.25)
-625G/C					
GG	54 (51.9)	57 (54.8)	-	-	1.00
GC	39 (37.5)	39 (37.5)	0.033	0.855	1.06 (0.59-1.88)
CC	11 (10.6)	8 (7.7)	0.555	0.456	1.45 (0.54-3.88)
G	147 (70.7)	153 (73.6)	-	-	1.00
C	61 (29.3)	55 (26.4)	0.430	0.512	1.15 (0.75-1.77)
9194A/G					
AA	44 (42.3)	56 (53.8)	-	-	1.00
AG	42 (40.4)	40 (38.5)	0.942	0.332	1.34 (0.74-2.40)
GG	18 (17.3)	8 (7.7)	5.256	0.022	2.86 (1.14-7.20)
A	130 (62.5)	152 (73.1)	-	-	1.00
G	78 (37.5)	56 (26.9)	5.328	0.021	1.63 (1.08-2.47)
9809T/C					
CC	32 (30.8)	33 (31.7)	-	-	1.00
CT	52 (50.0)	54 (51.9)	0.000	0.982	0.99 (0.54-1.84)
TT	20 (19.2)	17 (16.3)	0.219	0.639	1.21 (0.54-2.72)
C	116 (55.8)	120 (57.7)	-	-	1.00
T	92 (44.2)	82 (39.4)	0.555	0.456	1.16 (0.78-1.72)

Table 3. Linkage disequilibrium and haplotype analysis of alleles in -31G/C, -625G/C, 9194A/G and 9809T/C

Haplotype site1-site2-site3-site4	Patients[a] 2n=200 (%)	Controls[a] 2n=198 (%)	χ^2	P	OR (95% CI)
G-G-A-C	28 (14.0)	56 (28.3)	-	-	1.00
C-G-A-C	10 (5.0)	8 (4.0)	3.132	0.077	2.50 (0.89-7.03)
C-G-A-T	31 (15.5)	33 (16.7)	3.457	0.063	1.88 (0.96-3.66)
C-G-G-C	78 (39.0)	56 (28.3)	12.790	0.000	2.79 (1.58-4.92)
G-C-A-T	53 (26.5)	45 (22.7)	7.884	0.005	2.36 (1.29-4.30)

[a]Nine haplotypes that had a frequency of less than 1% were excluded from analysis; controls 30 and cases 20, respectively.

ed for 97.6 of the cases and 96.4% of the controls. The association analyses indicated that CGGC and GCAT haplotypes could increase the risk of lung cancer (OR=2.79, 95% CI=1.58-4.92; OR=2.36, 95% CI=1.29-4.30) (**Table 3**).

Discussion

Lung cancer is one most common malignancy with a high fatality rate. It is the most fatal cancer in males, while the morbidity and mortality were also high in females [15]. The development of lung cancer is influenced by multiple factors and involves complicated progress. In recent years, it has been demonstrated that genetic polymorphisms show strong effects on the onset of lung cancer [16-18].

Survivin, as a defender against cell apoptosis, can reduce the sensitivity of tumor cells to apoptosis stimulation, increase the survival capability of tumor cells and then affect individuals susceptibility to tumors [12, 19, 20]. *Survivin* polymorphisms were widely studied in previous studies and were confirmed to be associated with the risk of many cancers.

George et al. and Marques et al. found that *caspase-9* polymorphism combined with *survivin* -31G/C could increase the risk of pancreatic cancer and renal cell carcinoma [21, 22]. Besides, *survivin* -31G/C polymorphism was a promoter to esophageal cancer as well as prostate cancer and papillary thyroid carcinoma [23-25]. The similar role was found by Li et al. in colorectal cancer for *survivin* -31G/C based on Chinese population [14]. Besides, Rosato et al. revealed that the -31G/C SNP was related to the metastasis of non small cell lung cancer (NSCLC) [26].

In this study, we analyzed the correlation between four polymorphic locus (-31G/C, -625G/C, 9194A/G and 9809T/C) of survivin

gene and the risk of lung cancer through a case-control design. The genotype distribution of each polymorphism locus in control group was conformed to HWE test. The results suggested survivin -31G/C and 9194A/G was involved in pathogenesis of lung cancer among Chinese population, however, survivin -625G/C and 9809T/C were not associated with lung cancer risk. The haplotype analyses indicated that CGGC and GCAT were both risk factors for lung cancer.

In conclusion, *survivin* polymorphism, especially -31G/C and 9194A/G, could increase the risk of lung cancer. However, there are still some defects in the study. First, the sample size was relatively small. Moreover, the study only investigated the role of survivin gene in the pathogenesis of lung caner, while not considered the effects of environmental factors. A larger scale study with comprehensive design is needed to provide more reliable evidences on the issue.

Disclosure of conflict of interest

None.

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References

[1] Strieter RM, Polverini PJ, Arenberg DA, Walz A, Opdenakker G, Van Damme J and Kunkel SL. Role of C-X-C chemokines as regulators of angiogenesis in lung cancer. J Leukoc Biol 1995; 57: 752-762.

[2] Ambrosini G, Adida C, Sirugo G and Altieri DC. Induction of apoptosis and inhibition of cell proliferation by survivin gene targeting. J Biol Chem 1998; 273: 11177-11182.

[3] Li F and Brattain MG. Role of the Survivin gene in pathophysiology. Am J Pathol 2006; 169: 1-11.

[4] Dang SC, Feng S, Wang PJ, Cui L, Qu JG and Zhang JX. Overexpression of Survivin mutant Thr34Ala induces apoptosis and inhibits gastric cancer growth. Neoplasma 2015; 62: 81-87.

[5] Li Y, Wang J, Jiang F, Lin W and Meng W. Association of polymorphisms in survivin gene with the risk of hepatocellular carcinoma in Chinese han population: a case control study. BMC Med Genet 2012; 13: 1.

[6] Han G, Gong H, Wang Y, Guo S and Liu K. AMPK/mTOR-Mediated Inhibition of Survivin Partly Contributes to Metformin-Induced Apoptosis in Human Gastric Cancer Cell. Cancer Biol Ther 2015; 16: 77-87.

[7] Kogo R, How C, Chaudary N, Bruce J, Shi W, Hill RP, Zahedi P, Yip KW and Liu FF. The microRNA-218~Survivin axis regulates migration, invasion, and lymph node metastasis in cervical cancer. Oncotarget 2015; 6: 1090-100.

[8] Nigam J, Chandra A, Kazmi HR, Parmar D, Singh D and Gupta V. Prognostic significance of survivin in resected gallbladder cancer. J Surg Res 2015; 194: 57-62.

[9] Zhang K, Li Y, Liu W and Gao X. Silencing survivin expression inhibits the tumor growth of non-small-cell lung cancer cells in vitro and in vivo. Mol Med Rep 2015; 11: 639-644.

[10] Dai J, Jin G, Dong J, Chen Y, Xu L, Hu Z and Shen H. Prognostic significance of survivin polymorphisms on non-small cell lung cancer survival. J Thorac Oncol 2010; 5: 1748-1754.

[11] Yamak N, Yaykasli KO, Yilmaz U, Eroz R, Uzunlar AK, Ankarali H, Sahiner C and Baltaci D. Association between survivin gene polymorphisms and the susceptibility to colon cancer development in the Turkish population. Asian Pac J Cancer Prev 2014; 15: 8963-8967.

[12] Zhu Y, Li Y, Zhu S, Tang R, Liu Y and Li J. Association of survivin polymorphisms with tumor susceptibility: a meta-analysis. PLoS One 2013; 8: e74778.

[13] Kostic M, Nikolic N, Ilic B, Carkic J, Milenkovic S and Vukadinovic M. Analysis of polymorphism in the survivin gene promoter as a potential risk factor for head and neck cancers development. Srp Arh Celok Lek 2013; 141: 304-307.

[14] Li XB, Li SN, Yang ZH, Cao L, Duan FL and Sun XW. Polymorphisms of survivin and its protein expression are associated with colorectal cancer susceptibility in Chinese population. DNA Cell Biol 2013; 32: 236-242.

[15] Jemal A, Bray F, Center MM, Ferlay J, Ward E and Forman D. Global cancer statistics. CA Cancer J Clin 2011; 61: 69-90.

[16] Uppal V, Mehndiratta M, Mohapatra D, Grover RK and Puri D. XRCC-1 Gene Polymorphism (Arg399Gln) and Susceptibility to Development of Lung Cancer in Cohort of North Indian Population: A Pilot Study. J Clin Diagn Res 2014; 8: CC17-20.

[17] Wang SS, Guo HY, Dong LL, Zhu XQ, Ma L, Li W and Tang JX. Association between a p73 Gene Polymorphism and Genetic Susceptibility to Non-small Cell Lung Cancer in the South of China. Asian Pac J Cancer Prev 2014; 15: 10387-10391.

[18] Kong J, Xu F, Qu J, Wang Y, Gao M, Yu H and Qian B. Genetic polymorphisms in the vitamin D pathway in relation to lung cancer risk and survival. Oncotarget 2015; 6: 2573-82.

[19] Tamm I, Wang Y, Sausville E, Scudiero DA, Vigna N, Oltersdorf T and Reed JC. IAP-family protein survivin inhibits caspase activity and apoptosis induced by Fas (CD95), Bax, caspases, and anticancer drugs. Cancer Res 1998; 58: 5315-5320.

[20] Altieri DC. Survivin, cancer networks and pathway-directed drug discovery. Nat Rev Cancer 2008; 8: 61-70.

[21] Theodoropoulos GE, Michalopoulos NV, Panoussopoulos SG, Taka S and Gazouli M. Effects of caspase-9 and survivin gene polymorphisms in pancreatic cancer risk and tumor characteristics. Pancreas 2010; 39: 976-980.

[22] Marques I, Teixeira AL, Ferreira M, Assis J, Lobo F, Mauricio J and Medeiros R. Influence of survivin (BIRC5) and caspase-9 (CASP9) functional polymorphisms in renal cell carcinoma development: a study in a southern European population. Mol Biol Rep 2013; 40: 4819-4826.

[23] Upadhyay R, Khurana R, Kumar S, Ghoshal UC and Mittal B. Role of survivin gene promoter polymorphism (-31G>C) in susceptibility and survival of esophageal cancer in northern India. Ann Surg Oncol 2011; 18: 880-887.

[24] Chen J, Cui X, Zhou H, Qin C, Cao Q, Ju X, Li P, Cai H, Zhu J, Meng X, Wang M, Zhang Z, Shao P, Li J and Yin C. Functional promoter -31G/C variant of Survivin gene predict prostate cancer susceptibility among Chinese: a case control study. BMC Cancer 2013; 13: 356.

[25] Yazdani N, Sayahpour FA, Haghpanah V, Amiri P, Shahrabi-Farahani M, Moradi M, Mirmiran A, Khorsandi MT, Larijani B, Mostaan LV and Amoli MM. Survivin gene polymorphism association with papillary thyroid carcinoma. Pathol Res Pract 2012; 208: 100-103.

[26] Rosato A, Menin C, Boldrin D, Dalla Santa S, Bonaldi L, Scaini MC, Del Bianco P, Zardo D, Fassan M, Cappellesso R and Fassina A. Survivin expression impacts prognostically on NSCLC but not SCLC. Lung Cancer 2013; 79: 180-186.