Original Article

Association of Lysosome Associated Protein Transmembrane 4 Beta Gene Polymorphism with the Risk of Pancreatic Cancer

Shan Wang, Qing-Yun Zhang*

Key Laboratory of Carcinogenesis and Translational Research(Ministry of Education), Clinical Laboratory, Peking University Cancer Hospital & Institute, Beijing 100142, China

CLC number: R735.9 Document code: A Article ID: 1000-9604(2010)04-0291-05
DOI: 10.1007/s11670-010-0291-5

ABSTRACT

Objective: Lysosome associated protein transmembrane 4 beta (*LAPTM4B*) was originally identified as a gene in human hepatocellular carcinoma (HCC). It was successfully cloned by fluorescence differential display, rapid amplification of cDNA ends (RACE) and reverse transcription polymerase chain reaction (RT-PCR). Previous study showed that the novel gene played an important role in the occurrence, development, migration and prognosis of tumors. Pancreatic cancer is an aggressive malignancy with the majority of patients dying within one year after diagnosis. This study tries to find out the relationship between lysosome associated protein transmembrane 4 beta gene polymorphism and the susceptibility of pancreatic cancer.

Methods: A case-control study was conducted in China, including 58 pancreatic cancer cases and 156 healthy controls. Human genomic DNA was used as the template, polymerase chain reaction (PCR) was used to detect the distribution of *LAPTM4B* genotype. Analyses Odds ratio (OR) and corresponding 95% confidence interval (95%CI) with logistic regression were performed.

Results: Two alleles of *LAPTM4B* generated three kinds of genotypes in population, *1/1, *1/2, and *2/2. The genotype frequency of *1/1, *1/2 and *2/2 in the pancreatic cancer group were 41.4%, 44.8% and 13.8% respectively, which were not significantly different from those of healthy group (47.4%, 42.9%, 9.6%) (P=0.773, P=0.291). Also the *2 allele frequency of *LAPTM4B* among pancreatic cancer had no significantly difference with the controls (P=0.354). When compared to the *1 allele, the people with *2 allele had no increased risk of pancreatic cancer.

Conclusion: The gene polymorphism of *LAPTM4B* may not influence the susceptibility of pancreatic cancer.

Key words: Polymorphism; Lysosome associated protein transmembrane 4 beta; Pancreatic cancer; Susceptibility

INTRODUCTION

The prevalence of pancreatic cancer has increased dramatically over the past decades. Its mortality accounts for one fifth of the gastrointestinal cancers[1]. The causes of pancreatic cancer remain unknown, and risk factors can be classified into three broad categories: demographic, environmental, and genetic predisposition[2]. Despite recent diagnostic and therapeutic advances, pancreatic carcinoma still carries a poor prognosis, the overall 5-year survival rate among patients is <5%[3]. So a tumor marker for early diagnosis is essential.

Recently, *LAPTM4B* (lysosome associated protein transmembrane 4 beta) was successfully cloned by fluorescene differential display, rapid amplication of cDNA ends (RACE) and RT-PCR. According to BLAST program analysis the *LAPTM4B* gene is mapped to chromosome 8q22.1, with seven exons separated by six introns. It encodes two proteins with different molecular weight, 35kDa and 24kDa. There are two alleles in *LAPTM4B* gene, named as *LAPTM4B* *1 and *LAPTM4B* *2 (Genebank

Received 2010−04−25; **Accepted** 2010−09−10
This work was supported by the National Natural Science Foundation of China(No. 81071422).
Corresponding author.
E-mail: zhqy_208@163.com

accession No: the first AY219177 and AY219176, respectively), so three genotypesist[4]. They are differentiated at the 5'UTR in exon 1. Allele *1 contains only one copy of a 19-bp sequence, allele *2 has two tight tandem segments.

Previous study indicated that *LAPTM4B* gene polymorphism was associated with the susceptibility of gastric cancer[5] and colon cancer[6], whereas no statistical difference was found for rectal or esophageal cancer. So, we intend to investigate whether *LAPTM4B* gene polymorphism are associated with the risk of another gastrointestinal carcinomas-pancreatic cancer or not.

PATIENTS AND METHODS

Patient Selection

In the present study, 58 pancreatic cancer patients (37 male, 21 female, mean age 61.7±11.4 years) were enrolled in Beijing Cancer Hospital between September 2009 and April 2010, all pancreatic cancer cases were confirmed by pathological or radiological examination. The 156 control subjects were individuals recruited from physical examination of clinical laboratory in Beijing Cancer Hospital (75 male, 81 female, mean age 66.8±11.5 years).

DNA Preparation

Blood samples were collected with the anticoagulant EDTA K2 and stored at -20°C. Genomic DNA was extracted by the Phenol/Chloroform method. Briefly, the tube was directly thawed in 20°C water bath, then 1 ml blood was transferred to a 10 ml centrifuge tube. After adding phosphate-buffered saline, the tube was centrifuged at 3500g for 15 min. The supernatants were discarded and the tube was centrifuged again until the precipitation turned white. The pellet was resuspend in 0.75ml lysis buffer containing 7.5μl proteinase K (20mg/ml) and digested in 37°C water bath for 3 hours. Then the reaction solution was extracted 3 times using Phenol/ Chloroform method. Finally, the purified DNA was stored at -20°C.

DNA Analysis

LAPTM4B gene polymorphisms were typed by polymerase chain reaction. The primer sequences used are listed in Table 1. The 20 μl final PCR volume was as follows: 1μl DNA (200ng/ml), 10μl 2×EASY Tag mix, 10μmol/L each primer and 7μl ddH_2O. The cycling conditions were 95°C for 5 min, and 38 cycles for 30 s at 94°C, 30 s at 66°C and 30 s at 72°C, followed by a final extension for 5 min at 72°C. The PCR products were visualized on 3% agarose gel stained with ethidium bromide.

Table 1. *The primer sequence of LAPTM4B*

Gene	Primer sequence 5'-3'
LAPTM4B	F: 5'-GCCGACTAGGGGACTGGCGGA-3' (nt72-nt92) R: 5'-CGAGAGCTCCGAGCTTCTGCC-3' (nt255-nt275)

Statistical Analyses

The χ^2 test for Hardy-Weinberg equilibrium was performed among case and control subjects.

The frequencies of the alleles and genotypes between the groups were compared by the χ^2 test. $P<0.05$ was considered statistically significant. Unconditional logistic regression analysis models were used to evaluate the relationships between different genotypes and disease risk [Odds ratio (OR), 95% confidence interval (95% CI)] adjusted by gender and age.

RESULTS

We designed primers on both sides of the 19-bp sequence in the first exon, and genomic DNA was used as template to amplify gene *LAPTM4B* by PCR. Three different genotypes were identified in the PCR products---*LAPTM4B* *1/1, *1/2, *2/2 (Figure 1). A 204-bp band represented the homozygous genotype *1/1, a 223-bp band the homozygous genotype *2/2, while both the 204-bp and 223-bp bands exist in the heterozygous genotype *1/2.

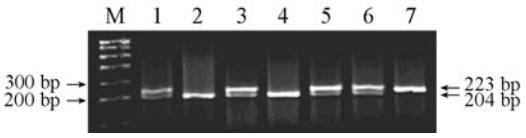

Figure 1. Genotyping of *LAPTM4B*
M: DNA marker (100,200,300,400,500,600,700bp)
Lane 2,4: *LAPTM4B**1/1
Lane 7: *LAPTM4B**2/2
Lane 1,3,5,6: *LAPTM4B**1/2

The distribution of these observed genotypes was not significantly different from the expected distribution according to Hardy-Weinberg test

(*P*>0.05). Analyses of the age and gender in the groups showed a significant difference in the distribution of pancreatic cancers and healthy controls (*P*=0.041, *P*=0.021, respectively) (Table 2).

Table 2. *Distribution of gender and age in case and control groups*

	Controls (n=156)	Cases (n=58)	*P* value
Gender (n%)			0.041
Male	75(48.1%)	37(63.8%)	
Female	81(51.9%)	21(36.2%)	
Age (n %)			0.021
< 60	44(28.2%)	26(44.8%)	
≧60	112(71.8%)	32(55.2%)	

*χ^2 test

According to the analysis of genomic DNA, these genotypes were distributed in pancreatic cancer patients and normal donors with the frequencies of 41.4% and 47.4% for *LAPTM4B**1/1, 44.8% and 42.9% for *LAPTM4B**1/2, 13.8% and 9.6% for *LAPTM4B**2/2, respectively, which are showed in Table 3. Statistical comparisons showed that there was no significant difference in the distribution of *LAPTM4B**1/2 and *LAPTM4B**2/2 between the two groups (*P*=0.773, *P*=0.291). Though different frequency of allele *LAPTM4B**2 was noticed among case and control groups (36.2%, 31.1%), no significant statistical difference was found using crosstabs test (P=0.354). These data indicated that there was no association with *LAPTM4B* gene polymorphism with the risk of pancreatic cancer.

Table 3. *Distribution of genotypes and alleles of LAPTM4B in case and control groups*

	Controls(n%)	Cases(n%)	OR (95%CI)	*P* value
Genotype				
*1/1	74(47.4)	24(41.4)		
*1/2	67(42.9)	26(44.8)	1.103(0.566-2.150)	0.773
*2/2	15(9.6)	8(13.8)	1.712(0.631-4.639)	0.291
Total	156(100)	58(100)		
Allele				
*1	215(68.9)	74(63.8)		
*2	97(31.1)	42(36.2)	1.243(0.785-1.968)	0.354
Total	312(100)	116(100)		

*Dates were calculated by unconditional logistic regression adjusted by gender and age status.

DISCUSSION

Despite of recent diagnostic and therapeutic advances, pancreatic carcinoma still carries a poor prognosis[7]. Its 5-year survival rate is only 5%, the lowest of all malignancies[8]. Similar to other cancerous processes, pancreatic cancer arises from genetic dysregulation and/or environmental factors. Smoking is the only risk factor confirmed of pancreatic carcinoma, diabetes mellitus, high cholesterol diet, chronic pancreatitis may all increased the risk of pancreatic cancer[9]. Hereditary pancreatitis, Peutz-Jeghers syndrome, and hereditary melanoma due to CDKN2A gene mutations have been associated with the highest risk for developing pancreatic cancer (more than 10-fold)[10]. Not many tumor markers are available for pancreatic cancer, and CA19-9 is the most common one, which does not reliably detect early, small pancreatic cancers[11]. With the rapid progression in the research of molecular and gene, the study about genetic susceptibility may make the early diagnosis possible.

LAPTM4B was first cloned in the hepatocellular carcinoma (HCC) as a novel gene, which was up-regulated in most solid tumors and hormone-related tumors, such as lung cancer, colon cancer, breast cancer and cervical cancer[12]. Studies showed that *LAPTM4B* overexpression in HLE cells promoted anchorage independent growth; moreover, the use of antisense oligonucleotides against *LAPTM4B* suppressed the proliferation of hepatocellular carcinoma cells BEL-7402[13]. These results indicated that *LAPTM4B* plays an active role in cell proliferation during tumor development and/or progression rather than being upregulated as a secondary effect during tumourgenesis[14].

In addition, the *LAPTM4B* mRNA expression levels were significantly related to the differentiation status of HCC tissues. They were highest in poorly differentiated HCCs, higher in moderately

differentiated HCCs, and relatively low in well differentiated HCCs[4]. Same regularities were noticed on the protein level[15]. *LAPTM4B* encodes a 35-kDa protein, LAPTM4B-35, which is a type III transmembrane protein with four putative transmembrane regions. This protein is localized mainly on plasma membrane and membranous organelles including endosomes and lysosomes[16]. Recent data showed that LAPTM4B-35 is a risk factor for tumor recurrence and an independent molecular marker of prognosis for HCC[17], extrahepatic cholangiocarcinoma[18], gallbladder carcinoma[19] and ovarian cancer[20]. The facts above indicated that *LAPTM4B* plays an important role in the progression of the tumors[21].

Through of analysis of the genotypes of 58 pancreatic cancer patients and 156 normal controls, we found that the frequencies of *LAPTM4B**1/2, *LAPTM4B**2/2 in case and control groups were 44.8%, 13.8% and 42.9%, 9.6% respectively, and there was no significant difference in the distribution of *LAPTM4B**1/2 and *LAPTM4B**2/2 between the two groups (P=0.773, P=0.291). Similarly, statistical comparisons showed the frequency of allele *LAPTM4B**2 had no significant statistical difference in pancreatic cancers and normals (P=0.354). So there was no association of *LAPTM4B* gene polymorphism with the risk of pancreatic cancer.

Former researches on the relationship between *LAPTM4B* gene polymorphism and susceptibility of gastrointestinal tumors showed that allele *LAPTM4B* *2 was associated with the susceptibility of liver cancer (unpublished), gastric cancer[5], and colon cancer[6]. The risk of suffering these cancers was increased 1.652, 1.710 and 1.523 times in the individuals of allele *2 compared with *1, whereas no statistical difference was found for rectal or esophageal cancer. These results agreed with the findings of Peng[22], who showed through immunohistochemical analysis the expression of LAPTM4B protein in cancer tissues derived from single layer cuboidal and columnar epithelia, such as hepatocellular carcinoma, gastric cancer and colon cancer, and the lack of expression in cancer tissues from stratified epithelia, such as esophageal cancer and rectal cancer[23].

There are many generalities in the process of tumorigensis[24], for example, activation of oncogenes and inactivation of tumor suppressor genes, but different mechanisms of the tumors determined different changes in the molecular genetics[25]. *LAPTM4B* gene polymorphism is not associated with all of the tumor susceptibility, while no correlation between polymorphism and risk of pancreatic cancer was noticed in our study. Due to the lower incidence of pancreatic cancer in all the malignant tumors, we failed to collect large-sample cases. Therefore, more evidences are needed to support the conclusion.

REFERENCES

[1] Zaret KS, Watts J, Xu J, et al. Pioneer factors, genetic competence, and inductive signaling: programming liver and pancreas progenitors from the endoderm[J]. Cold Spring Harb Symp Quant Biol 2008; 73: 119-26.
[2] Chu D, Kohlmann W, Adler DG. Identification and screening of individuals at increased risk for pancreatic cancer with emphasis on known environmental and genetic factors and hereditary syndromes[J]. JOP 2010; 11: 203-12.
[3] Jemal A, Siegel R, Ward E, et al. Cancer Statistics, 2009[J]. CA Cancer J Clin 2009; 59: 225-49.
[4] Shao GZ, Zhou RL, Zhang QY, et al. Molecular cloning and characterization of *LAPTM4B*, a novel gene unregulated in hepatocellular carcinoma[J]. Oncogene 2003; 22: 5060-9.
[5] Liu Y, Zhang QY, Qian N, et al. Relationship between *LAPTM4B* gene polymorphism and susceptibility of gastric cancer[J]. Ann Oncol 2007; 18: 311-6.
[6] Cheng XJ, Xu W, Zhang QY, et al. Relationship between *LAPTM4B* gene polymorphism and susceptibility of colorectal and esophageal cancers[J]. Ann Oncol 2008; 19: 527-32.
[7] Thomson BN, Banfing SW, Gibbs P. Pancreatic cancer-current management[J]. Aust Fam Physician 2006; 35(4): 212-7.
[8] Lowenfels AB, Maisonneuve P. Epidemiology and risk factors for pancreatic cancer. Best Pract Res Clin Gastroenterol 2006; 20: 197-209.
[9] Batty CD, Kivimaki M, Morrison D, et al. risk factors for pancreatic cancer mortality: extended follow-up of the original Whitehall study[J]. Cancer Epidemiol Biomarkers Prev 2009; 18: 673-5.
[10] Pogue-Geile KL, Chen R, Bronner MP, et al. Palladin mutation causes familial pancreatic cancer and suggests a new cancer mechanism. PLoS Med 2006; 3: e516.
[11] Leung TK, Lee CM, Wang FC, et al. Difficulty with diagnosis of malignant pancreatic neoplasms coexisting with chronic pancreatitis. World J Gastroenterol 2005; 11: 5075-8.
[12] Kasper G, Vogel A, Klaman I, et al. The human LAPTM4b transcript is upregulated in various types of solid tumors and seems to play a dual functional role during tumor progression. Cancer Lett, 2005; 224: 93-103.
[13] He J, Shao GZ, Zhou RL. Effects of the novel gene, *LAPTM4B* highly expression in hepatocellular carcinoma on cell proliferation and tumorigenesis of NIH3T3[J]. Beijing Da Xue Xue Bao (in Chinese) 2003; 35: 348-52.
[14] Maeda K, Horikoshi T, Nakashima E, et al. MATN and LAPTM are parts of larger transcription units produced by intergenic splicing: intergenic splicing may be a common phenomenon[J]. DNA Res 2005; 12: 365-72.
[15] Liu XR, Zhou RL, Zhang QY, et al. Identification and characterization of *LAPTM4B* encoded by a human

hepatocellular carcinoma-associated novel gene[J]. Beijing Da Xue Xue Bao (in Chinese) 2003; 35: 340-7.
[16] Liu XR, Zhou RL, Zhang QY, et al. Structure analysis and expressions of a novel tetratransmembrane protein, lysosome-associated protein transmembrane 4B associated with hepatocellular carcinoma[J]. World J Gastroenterol 2004; 10(11):1555-1559.
[17] Yang H, Xiong F X, Lin M, et al. LAPTM4B-35 overexpression is a risk factor for tumor recurrence and poor prognosis in hepatocellular carcinoma[J]. J Cancer Res Clin Oncol 2010; 136: 275-81.
[18] Zhou L, He XD, Cui QC, et al. Expression of LAPTM4B-35: A novel marker of progression, invasiveness and poor prognosis of extrahepatic cholangiocarcinoma[J]. Cancer Lett 2008; 264: 209-17.
[19] Zhou L, He XD, Chen J, et al. Overexpression of LAPTM4B-35 closely correlated with clinico-pathological features and post-resectional survival of gallbladder carcinoma[J]. Eur J Cancer 2007; 43: 809-15.
[20] Yang Y, Yang H, Mcnutt MA, et al. *LAPTM4B* overexpression is an independent prognostic marker in ovarian carcinoma[J]. Oncol Rep 2008; 20: 1077-83.
[21] Yang H, Xiong FX, Qi RZ, et al. APTM4B-35 is a novel prognostic factor of hepatocellular carcinoma [J]. J Surgl Oncol 2010; 101: 363-9.
[22] Peng C, Zhou RL, Shao GZ et al. Expression of lysosome-associated protein transmembrane 4B-35 in cancer and its correlation with the differentiation status of hepatocellular carcinoma[J]. World J Gastroenterol 2005; 11: 2704–2708.
[23] Liu XR, Xiong FX, Wei XH, et al. LAPTM4B-35, a novel tetratransmembrane protein and its PPRP motif play critical roles in proliferation and metastatic potential of hepatocellular carcinoma cells[J]. Cancer Sci 2009; 100: 2335-40.
[24] Xu F, Paquette AJ, Anderson DJ, Charalambous A, Askew DS. Identification of a cell type-specific silencer in the first exon of the His-1 gene[J]. J Cell Biochem 2000; 76(4): 615-24.
[25] Smith G, Stanley LA, Sim E, et al. Metabolic polymorphisms and cancer susceptibility[J]. Cancer Surv 1995, 25: 27-65.