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Genetic variants of *p21* and *p27* and pancreatic cancer risk in non-Hispanic whites: a case-control study

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Abstract

Objectives—*p21* (WAF1/Cip1/CDKN1A) and *p27* (Kip1/CDKN1B) are members of the Cip/Kip family of cyclin-dependent kinase inhibitors, which can induce cell cycle arrest and serve as tumor suppressors. We hypothesized that genetic variants in *p21* and *p27* may modify individual susceptibility to pancreatic cancer.

Methods—To test this hypothesis, we evaluated the associations of the Ser31Arg polymorphism in *p21* and the Gly109Val polymorphism in *p27*, as well as their combinations, with pancreatic cancer risk in a case-control study of 509 pathologically confirmed pancreatic adenocarcinoma patients and 462 age- and sex-matched cancer-free controls in non-Hispanic whites.

Results—We found that the heterozygous and homozygous variant genotypes combined in a dominant model of the *p21* polymorphism were associated with increased risk of pancreatic cancer compared with the homozygous wild-type (odds ratio [OR_{adjusted}] = 1.70; 95% confidence interval [CI], 1.13–2.55). This increased risk was more pronounced in carriers with the *p27* homozygous wild-type (OR_{adjusted}, 2.20; 95% CI, 1.32–3.68) as well as in nonsmokers (OR_{adjusted}, 2.16; 95% CI, 1.14–4.10), although the *p27* polymorphism alone was not associated with pancreatic cancer risk.

Conclusions—These results indicate that the *p21* polymorphism may contribute to susceptibility to pancreatic cancer, particularly among *p27* homozygous wild-type carriers and nonsmokers.

Keywords

p21; *p27*; polymorphisms; pancreatic cancer; case control

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Introduction

Pancreatic cancer is the fourth leading cause of cancer-related death in both men and women in the United States, with an estimated 34,290 deaths in 2008 ¹. The median survival time for all patients diagnosed is less than six months with a 5-year survival rate of <5% ². Because pancreatic cancer lacks early disease-specific signs and symptoms and progresses rapidly, it is usually diagnosed at a late stage of the disease, resulting in the worst prognosis of all solid tumors. Thus, it is of critical importance to understand the etiology and identify risk factors for the primary prevention of this deadly disease.

Cell cycle control proteins p21 and p27, members of the Cip/Kip family of CDK inhibitors, bind to cyclin-CDK complexes to inhibit their catalytic activity and induce cell-cycle arrest ³. p27 inhibits activation of cyclin E-CDK2 complex and plays a pivotal role in the progression from the G1 to the S phase of the cell cycle. When p27 is absent, cells do not follow cell cycle control signs and thus proliferate in an exaggerated way ⁴. p21 acts as a universal inhibitor of CDK's with inhibition of a variety of CDK-cyclin complexes and has been demonstrated to be a critical mediator of G1-phase cell cycle arrest in order to prevent G1/S transition ⁵. A previous study demonstrates that p21 not only inhibits CDK activity directly, but also indirectly through stabilization of p27 protein that increases cellular levels of this inhibitor ⁶.

Recent studies have provided evidence showing that single nucleotide polymorphisms (SNPs) in genes with roles in cell cycle control play an important role in carcinogenesis and may lead to altered susceptibility to different cancers ⁷⁻¹⁵. A C-to-A SNP in *p21* results in an amino acid change in codon 31 from Serine to Arginine. This SNP is located in a highly conserved region of *p21* and is predicted to affect its molecular function ¹⁶. Another SNP (T-to-G) in *p27* is located at codon 109 and results in an amino acid change from valine to glycine. It has been suggested that this V109G polymorphism may have an effect on p27 degradation. This polymorphism falls within a region (amino acids 97-151) that physically interacts with the Jun activation domain binding protein 1 (JAB1). JAB1 triggers the proteolytic degradation of p27 ¹⁷. It has been speculated that the V109G polymorphism enhances this degradation ¹⁸. Given the major roles that p21 and p27 play in cell cycle checkpoint regulation, we hypothesized that polymorphisms in the two genes may modify individual susceptibility to pancreatic cancer. Therefore, we evaluated the association between the two SNPs of *p21* and *p27* and pancreatic cancer risk in a hospital-based case-control study.

Materials and Methods

Study subjects

The study included 509 pancreatic cancer patients and 426 age- and sex-matched cancer-free controls. All subjects were genetically unrelated non-Hispanic whites. The cases were patients with pathologically confirmed primary pancreatic adenocarcinoma and were consecutively recruited from the Gastrointestinal Center at The University of Texas M. D. Anderson Cancer Center in Houston, Texas between February 1999 and May 2007. Cancer-free control subjects were recruited during the same period from among self-reported cancer-free visitors to M.D. Anderson Cancer Center who were not seeking medical care but were instead accompanying patients on outpatient clinic visits. The controls were not blood relatives of the patients or each other and were frequency-matched with the cases by age at enrollment (5-year interval) and sex. At recruitment, each participant gave written informed consent and contributed a blood sample. The study was approved by the Institutional Review Board of M. D. Anderson Cancer Center.

Polymorphism analysis

Genotypes of *p21* C-to-A (dbSNP: rs1801270) and *p27* G-to-T (dbSNP: rs2066827) were analyzed using two different methods. The first method was by pyrosequencing using a PSQTMHS96A system. The pyrosequencing was carried out according to the manufacturer's instructions (Biotage, Inc., Foxboro, MA). A polymerase chain reaction (PCR) was performed on 5 ng of DNA in a 50 μ L reaction mixture containing 50 mM KCl; 10 mM Tris-HCl (pH 8.3); 2.0 mM MgCl₂; 0.125 mM dATP, dCTP, dGTP, and dTTP; 1.5 U AmpliTaq Gold DNA polymerase (Applied Biosystems, Branchburg, NJ); and 10 pmol of each primer (Sigma/Genosys, The Woodlands, TX). The PCR reaction mixture was initially incubated at 95°C for 6 min, followed by 45 cycles at 95°C for 15 s, 63°C for 30 s for *p21* and 65°C for 30 s for *p27* followed by 72°C for 15 s, and then an extension of 72°C for 5 min. The PCR primers used were 5'-CTCTTCGGCCAGTGGACA-3' (forward) and 5'-CTCACGGGCTCCTGGAT-3' (reverse) for *p21*, and 5'-CGAGTGGCAAGAGGTGGAGA-3' (forward) and 5'-GGAGCCCCAATTAAAGGCG-3' (reverse) for *p27*. The forward primer was biotinylated for *p21* and the reverse primer was biotinylated for *p27* to allow subsequent immobilization on magnetic bead. The sequencing primers were 5'-AGCGCATCACAGTCG-3' (Sigma Genosys) for *p21* and 5'-CAGGAGAGCCAGGAT-3' for *p27*. The second genotyping method used was a PCR-Restriction Fragment Length Polymorphism (PCR-RFLP) assay as described previously^{19, 20}. For each type of assay, the genotypes were read independently by two different persons. We analyzed 10% of the samples using both genotyping methods, and the same results were obtained.

Statistical analysis

Differences in demographic characteristics, selected variables and genotype frequencies in the cases and controls were evaluated using the χ^2 test. The associations between the two SNPs and pancreatic cancer risk were estimated by computing the ORs and their 95% CIs from both univariate and multivariate logistic regression analyses with adjustment for age, sex, and smoking status. We tested for Hardy-Weinberg equilibrium by using an exact test based on genotypic frequencies. All statistical analyses were two-sided and performed using the Stata 8.0 (Stata Corporation, College Station, TX).

Results

Subject characteristics

The demographic data for the 509 cases and 462 controls are described in Table 1. Because of frequency matching, there was no statistically significant difference in the distributions of age and sex between cases and controls. There was no significant difference between cases and controls in the distribution of smoking status. The distribution of genotypes of the two polymorphisms between cases and controls is also shown in Table 1. The genotypic frequencies for *p21* and *p27* were consistent with the Hardy-Weinberg equilibrium in cases as well as in controls (all $P > 0.1$). The minor allelic frequency for *p21* was 0.08 in cases and 0.05 in control; and for *p27* was 0.23 in cases and 0.22 in controls.

Genotypes and cancer risk

Because the number of subjects with the *p21* as well as *p27* homozygous variant genotype was too low to provide meaningful results, we combined the heterozygous and homozygous variant genotypes for the analysis. We found that the *p21* variant genotypes (CA/AA) exhibited a significantly increased pancreatic cancer risk (OR_{adjusted}, 1.70; 95% CI, 1.13–2.55), compared with the *p21* wild-type genotype (CC). There was no significant difference in pancreatic cancer

risk between the variant genotypes (TG/GG) and wild-type genotype (TT) for the *p27* polymorphism (OR_{adjusted}, 1.05; 95% CI, 0.81–1.36).

Because both *p21* and *p27* are involved in the same cell cycle regulation pathway and bind to cyclin D1-CDK complexes to inhibit their catalytic activity and induce cell-cycle arrest, we evaluated whether the *p21* and *p27* polymorphisms had a joint effect on the risk of pancreatic cancer. We did not find a significant joint effect between these two polymorphisms (data not shown). However, when we analyzed the effect of *p21* polymorphism on cancer risk stratified by the *p27* genotypes, we found that the effect of the *p21* polymorphism was more pronounced in *p27* wild-type genotype carriers (OR_{adjusted}, 2.20; 95% CI, 1.32–3.68) (Table 2). The *p21* variant genotype did not exhibit increased cancer risk in *p27* variant genotype carriers (OR_{adjusted}, 1.08; 95% CI, 0.55–2.14) (Table 2).

Genotypes, smoking status and cancer risk

We further analyzed the effects of these polymorphisms on cancer risk stratified by smoking status. We found that the effect of the *p21* polymorphism on cancer risk was only exhibited in non-smokers (OR_{adjusted}, 2.16; 95% CI, 1.14–4.10), and not in smokers (OR_{adjusted}, 1.44; 95% CI, 0.85–2.45) (Table 3). The *p27* polymorphism did not exhibit any effect on cancer risk in either smokers or non-smokers (data not shown).

Discussion

In this hospital-based case-control study, we found that heterozygous and homozygous variant genotypes of the *p21* polymorphism were associated with increased risk of pancreatic cancer compared with the homozygous wild-types. This increased risk was more pronounced in *p27* homozygous wild-type carriers as well as in nonsmokers. The reason for the failure of the *p21* SNP to influence risk for pancreatic cancer in the *p27* SNP carriers is not known, but perhaps only when the *p27* wild-type genotype is present, can the *p21* SNP influence the effect of *p21* on stabilizing *p27* protein⁶, resulting in decreased cellular levels of this CDK inhibitor and failure in cell cycle arrest. Smoking is a major risk factor for pancreatic cancer. The observation that the influence of the *p21* SNP on risk of pancreatic cancer was only evident in never smokers supports the notion that heavy carcinogen exposure may overwhelm the influence of the SNP on risk for pancreatic cancer.

The frequency of the *p21* A allele was 0.05 in our controls, which is the same as reported for Caucasians in the National Center for Biotechnology Information (NCBI) SNP database. The frequency of the *p27* G allele was 0.22 in our controls, which is similar to 0.24 that was reported for Caucasians in the NCBI SNP database. These findings indicate that the samples in this hospital-based study are unlikely to produce significant bias in estimating the genotype-specific ORs.

Recent studies suggest that the *p21* polymorphism is likely to contribute to genetic susceptibility to cancer^{13, 19, 21–24}, although some studies found no association between the polymorphism and cancer risk, including lung cancer^{25, 26} and breast cancer²⁷. This discrepancy could be due to different carcinogenic mechanisms among different cancer types, as well as differences in the underlying genetic backgrounds and/or environmental and social factors in different populations studied. Our findings suggest that the *p21* polymorphism was associated with an increased risk of pancreatic cancer.

It is biologically plausible that the *p21* polymorphism might influence cancer risk, as *p21* plays an important role in cell cycle arrest at the G1 to S phase checkpoint, allowing cells to repair damaged DNA and thereby inhibit carcinogenesis²⁸. The *p21* C-to-A polymorphism in codon 31 causing a serine-to-arginine substitution in the DNA-binding zinc-finger motif could

encode functionally distinct proteins^{29, 30}. The increased risk was more pronounced in carriers with the *p27* wild-type genotype as well as in nonsmokers. It is likely that only when the *p27* wild-type genotype was present or without exposure to smoking, the effect of the *p21* polymorphism on cancer risk can be adequately manifested.

There was no significant difference in pancreatic cancer risk between the variant genotypes (TG/GG) and wild-type genotype (TT) for the *p27* polymorphism. Our results are consistent with other studies that did not detect associations with risk for oral squamous cell carcinoma²⁰, breast cancer²⁷, nor prostate cancer³¹.

In conclusion, our case-control study provides evidence that the *p21* SNP was associated with risk of pancreatic cancer in Caucasian population, particularly among the *p27* homozygous wild-types as well as nonsmokers. This observation deserves further replication. Further functional studies on the SNPs are warranted to elucidate the underlying molecular mechanisms of the observed association.

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Table 1

Selected variables among cases and controls

Variables	Cases N=509 (%)	Controls N=462 (%)	OR* (95% CI)	P-value (χ^2)
Age				
<50	86 (16.9)	90 (19.5)	1.00	
50–59	161 (31.6)	164 (35.5)	1.03 (0.70–1.51)	
60–69	173 (34.0)	142 (30.7)	1.27 (0.87–1.88)	
≥70	89 (17.5)	66 (14.3)	1.41 (0.89–2.23)	0.229
Sex				
Men	335(65.8)	313 (67.8)	1.00	
Women	174 (34.1)	149 (32.2)	1.09 (0.83–1.44)	0.523
Smoking status				
Never	198 (38.9)	181 (39.2)	1.00	
Ever	311 (61.1)	281 (60.8)	1.01 (0.78–1.30)	0.929
p21 (C-to-A)				
CC	436 (85.7)	420 (90.9)	1.00	
CA/AA	73 (14.3)	42 (9.1)	1.67 (1.12–2.50)	0.011
p27 (T-to-G)				
TT	299 (58.7)	280 (60.6)	1.00	
TG/GG	210 (41.3)	182 (39.4)	1.08 (0.84–1.40)	0.554

OR = crude odds ratios; CI = confidence interval.

Table 2
Association of the p21 polymorphism with risk of pancreatic cancer stratified by p27 genotypes.

Genotype	p27 wild-type genotypes			p27 variant genotypes		
	Case patients No. (%)	Control subjects No. (%)	OR (95% CI)	Case patients No. (%)	Control subjects No. (%)	OR (95% CI)
p21 (C-to-A)						
CC	247 (82.6)	255 (91.1)	1.00 (referent)	189 (90.0)	165 (90.7)	1.00 (referent)
CA/AA	52 (17.4)	25 (8.9)	2.20 (1.32–3.68)	21 (10.0)	17 (9.3)	1.08 (0.55–2.14)

OR = odds ratios adjusted for age sex, and smoking status; CI = confidence interval.

Table 3
Association of the p21 polymorphism with risk of pancreatic cancer stratified by smoking status.

Genotype	smokers			Non-smokers		
	Case patients No. (%)	Control subjects No. (%)	OR (95% CI)	Case patients No. (%)	Control subjects No. (%)	OR (95% CI)
p21 (C-to-A)						
CC	272 (87.5)	255 (90.8)	1.00 (referent)	164 (82.8)	165 (91.2)	1.00 (referent)
CA/AA	39 (12.5)	26 (9.2)	1.44 (0.85–2.45)	34 (17.2)	16 (8.8)	2.16 (1.14–4.10)

OR = odds ratios adjusted for age and sex; CI = confidence interval.