Interleukin 1 β Gene Single-Nucleotide Polymorphisms and Susceptibility to Pancreatic Neuroendocrine Tumors

Maja Cigrovski Berković,¹ Tina Catela Ivković,² Jasminka Marout,¹ Vanja Zjajić-Rotkvić,¹ and Sanja Kapitanović²

Pancreatic neuroendocrine tumors (pNETs) are rare neoplasms with not fully understood etiology. Interleukin 1 β (IL1 β) plays an important role in pancreatic pathology, especially carcinogenesis, but its role in pNET development remains unknown. The aim of this study was to analyze the association between *IL1 β* polymorphisms and susceptibility to pNETs. *IL1 β* –511 C/T and +3954 C/T single-nucleotide polymorphisms (SNPs) were analyzed by real-time polymerase chain reaction–SNP analysis. IL1 β serum values in pNET patients were also determined. Association between high-expression C/T –511 *IL1 β* genotype and susceptibility to pNET ($p=0.042$) was found, especially with functional pNET ($p=0.014$), where it was associated with the T allele ($p=0.016$). Combination of genotype analyses confirmed carriers of –511/+3954 CTCT to be at risk of developing functional pNETs ($p=0.006$) and carriers of –511/+3954 CTCC at risk of developing nonfunctional pNETs ($p=0.019$). IL1 β serum levels of all patients were below the limit of detection. Our results suggest IL1 β involvement in pNET development, and we also found association between the *IL1 β* –511 SNP and susceptibility to pNET, especially functional pNETs. Nonfunctional pNETs seem to have inferior prognosis when compared with functional pNETs. It is possible that they differ in tumor microenvironment and that nonfunctional tumors share similarities with adenocarcinoma. We believe that our findings will contribute to understanding of the etiology and possible novel prognostic markers for pNETs when future studies investigating the serum and tumor tissue IL1 β levels are done.

Introduction

PANCREATIC NEUROENDOCRINE TUMORS (pNETs) are uncommon, mostly well-differentiated and indolent neuroendocrine neoplasms. They are largely nonfunctional, but when associated with hormone oversecretion they can cause hypoglycemia (insulinoma), Zollinger-Ellison syndrome (gastrinoma), Verner-Morrison (VIP-oma), and carcinoid syndrome (Massironi *et al.*, 2008). Molecular mechanisms of pNET genesis are poorly understood but have been the focus of many recent reports (Chan *et al.*, 2003; House *et al.*, 2003; Franko *et al.*, 2010). Chronic inflammation has been suggested as a contributor in development of pancreatic diseases such as diabetes and pancreatic cancer. Interleukin 1 β (IL1 β), a key proinflammatory cytokine encoded by the *IL1 β* gene, has been associated with chronic inflammation and plays an important role in pancreatic inflammatory diseases, including pancreatic cancer (Chen *et al.*, 2001). Elevated levels of IL1 β protein enhances the intensity of the inflammatory response, damages pancreatic beta cells through nitric oxide production leading to their apoptosis, and has also been found to inhibit insulin release in pancreatic islets cultured *in vitro* or in insulinoma cells (Corbett *et al.*, 1993; Ankarcona

et al., 1994). IL1 β levels are regulated at the transcriptional level, and polymorphisms in the *IL1 β* gene have been found not only to influence levels of IL1 β but also to change susceptibility to different diseases (Pociot *et al.*, 1992; Chen *et al.*, 2006). The *IL1 β* gene is located in the *IL1* cluster on chromosome 2q. A single-nucleotide polymorphism (SNP) in the proximal promoter region of the *IL1 β* gene, –511C/T, is associated with gastrointestinal cancers such as gastric (El-Omar *et al.*, 2000), hepatocellular (Wang *et al.*, 2003), and pancreatic carcinoma (Barber *et al.*, 2000). SNP +3954 in exon 5 of the *IL1 β* gene was also recently demonstrated to influence survival of patients with unresectable pancreatic cancer (Barber *et al.*, 2000; Hamacher *et al.*, 2009). Functionally, IL1 β was shown to mediate adhesion and invasion of pancreatic cancer cells, and through autocrine and paracrine production it contributes to chemoresistance of pancreatic cancer cells (Ebrahimi *et al.*, 2004; Muerkoster *et al.*, 2004; Greco *et al.*, 2005; ten Kate *et al.*, 2006). The roles of *IL1 β* –511C/T and +3954 C/T genotypes in the development of pNETs remain unknown. pNETs represent a heterogeneous group with varying tumor biology and prognosis; they can either occur as solitary tumors, and up to 15% of pNETs are a part of inherited syndromes. The aim of this study was to

¹Department of Endocrinology, Diabetes, and Metabolism, University Hospital “Sestre milosrdnice,” Zagreb, Croatia.

²Laboratory for Personalized Medicine, Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia.

investigate the association between *IL1*β -511 C/T and +3954 C/T polymorphisms and susceptibility to functional and nonfunctional pNETs. The secondary aim was to investigate the serum values of *IL1*β protein in pNET patients.

Patients and Methods

Patients

The study included 60 consecutive patients diagnosed with pNETs and 60 healthy unrelated individuals as controls. Patients as well as controls were Caucasian and of Croatian nationality. They were recruited from the Department of Endocrinology, Diabetes, and Metabolism, University Hospital "Sestre milosrdnice" and all gave written informed consent for the participation in this study. Among patients, 37 had nonfunctional and 23 functional tumors, associated with hypoglycemia (13) (Barber *et al.*, 2000), Verner-Morrison (1) (Massironi *et al.*, 2008), carcinoid (6) (Ankarcrona *et al.*, 1994), and Zollinger-Ellison (3) (House *et al.*, 2003) syndromes, respectively. pNETs were diagnosed by standard procedures (first imaging-endoscopic ultrasound [EUS], computed tomography, and when possible, octreoscan). Diagnosis was confirmed by either immunocytochemistry following fine needle aspiration (FNA) or pathohistological analysis after surgical removal. The study was approved by the Ethics Committees of the University Hospital "Sestre Milosrdnice" and School of Medicine University of Zagreb. DNAs from 60 healthy volunteers were obtained from the Croatian Tumor and DNA Bank for Basic Research (Spaventi *et al.*, 1994).

Methods

DNA isolation. Genomic DNAs were isolated from peripheral ethylenediaminetetraacetic acid-treated blood of patients and healthy controls using proteinase K digestion and phenol-chloroform extraction.

SNP genotyping. Real-time polymerase chain reaction (PCR)-SNP analysis of *IL1*β -511C/T (rs16944) and +3954C/T (rs1143634) was performed using an ABI PRISM 7000 SDS (Applied Biosystems) and predeveloped TaqMan SNP genotyping assays C_1839943_10 (-511CT) and C_9546517_10 (+3954CT) (Applied Biosystems). PCR was carried out according to the manufacturer's protocol. For quality control, 15% of randomly selected samples of both cases and control were analyzed a second time, without finding any discrepancies. Control samples covering three possible SNP genotypes and no template control were run in parallel with tested samples in each experiment.

***IL1*β serum levels.** Serum *IL1*β levels were determined for all patients by double-sided chemiluminescent immunometric method using *IL1*β monoclonal antibody with the IMMULITE system. Lower limit of detectability for the *IL1*β serum levels was 5 pg/mL (Diagnostic Products Corporation, Corporate offices), and there was no possibility to measure values lower than that mentioned. All patients had serum levels below the lower limit of detection. Results were not influenced by serum bilirubin or lipid levels or by hemoglobin concentration in case of hemolytic sera. Serum samples were stored at -20°C for up to 6 months prior to analysis.

Statistics

This study was planned as a study of independent cases and controls with one control per case, and the power test was calculated. Prior data indicate that the probability of exposure among controls is 0.5. If the true odds ratio (OR) for disease in exposed subjects relative to unexposed subjects is 3, we will need to study 58 case patients and 58 control patients to be able to reject the null hypothesis that this OR equals 1 with probability (power) 0.8. The type I error probability associated with this test of this null hypothesis is 0.05. We used an uncorrected chi-squared statistic to evaluate the null hypothesis.

The distribution of *IL1*β -511C/T and +3954 C/T polymorphisms was compared between pNET patients and controls. For the mentioned analyses, OR was used. For the analysis of distribution of genotypes and alleles between the patients with functional and nonfunctional pNETs, χ^2 and Fisher's test were used when appropriate. The OR and 95% confidence interval (CI) with Haldan's correction was performed for the analysis of -511/+3954 genotype/haplotype differences between patients with functional and nonfunctional pNETs and healthy controls. The level of significance was 0.05. Genotype frequencies were distributed in accordance with the Hardy-Weinberg equilibrium.

Results

The present study included 60 consecutive patients diagnosed with pNETs by standard diagnostic procedures and 60 controls. Patients' as well as controls' characteristics are given in Table 1.

DNAs were genotyped using real-time PCR-SNP method and allelic discrimination analysis (Fig. 1). *IL1*β -511 C/T and +3954 C/T distribution differences between patients with pNETs and healthy controls are given in Table 2. There was a statistically significant association between the high-expression C/T genotype and risk of pNET development (OR, 2.217; 95% CI, 1.022–4.812; $p=0.042$; Table 2), and combined C/T and T/T high-expression genotypes had a tendency for statistical significance (OR, 2.000; 95% CI, 0.956–4.182; $p=0.063$). The statistical significance was especially associated with functional pNETs where high-expression *IL1*β -511 C/T (OR, 3.913; 95% CI, 1.241–12.342; $p=0.014$; Table 3) and high-expression C/T and T/T -511 *IL1*β genotypes were associated with the risk of functional pNET development (OR, 3.600; 95% CI, 1.183–10.952; $p=0.016$; Table 3). Among patients with functional pNETs, we observed the tendency of the more frequent T allele (OR, 1.8843; 95% CI, 0.9379–3.7856;

TABLE 1. CHARACTERISTICS OF PANCREATIC NEUROENDOCRINE TUMOR PATIENTS

	Pancreatic neuroendocrine tumor cases	Healthy controls
Number (n)	60	60
Mean age, years (range)	55.12 (22–85)	50.33 (26–75)
Gender (n)	Male: 24 Female: 36	31 29
Tumor functional status (n)	Functional: 23 Nonfunctional: 37

FIG. 1. Real-time polymerase chain reaction–single-nucleotide polymorphism allelic discrimination analysis of *IL1 β* –511 C/T polymorphism.

$p=0.0762$; Table 3). On the other hand, the +3954 C/T genotype was similarly distributed between functional pNET patients and healthy controls (Table 3).

Table 4 presents distribution differences in –511 C/T and +3954 C/T genotypes between patients with nonfunctional pNETs and healthy controls. There were no statistically significant associations between the analyzed *IL1 β* SNPs and risk of developing nonfunctional pNETs. In Table 5, *IL1 β* –511 C/T and +3954 C/T distribution differences between patients with functional and nonfunctional pNETs are given. There were no statistically significant differences between the patients with functional and nonfunctional pNETs with respect to *IL1 β* –511 C/T and +3954 C/T genotypes.

Tables 6 and 7 show at risk population for developing functional and nonfunctional pNETs according to –511/

+3954 genotype combinations. There was a statistically significant association between CTCT –511/+3954 genotype combination and risk of functional pNET development (OR, 8.556; 95% CI, 1.526–47.958; $p=0.006$; Table 6), whereas patients with the CTCC –511/+3954 genotype combination were associated with risk of nonfunctional pNET development (OR, 5.026; 95% CI, 1.169–21.590; $p=0.019$; Table 7). Serum *IL1 β* levels analyzed for all patients were lower than 5 pg/mL (threshold set by manufacturer), with no possibility to approximate the *IL1 β* levels.

Discussion

pNETs are uncommon neoplasms with an incidence of <1 per 100,000 person-years in population-based studies. They

TABLE 2. GENOTYPE FREQUENCIES OF *IL1 β* –511 C/T AND +3954 C/T POLYMORPHISMS IN PANCREATIC NEUROENDOCRINE TUMOR PATIENTS AND HEALTHY CONTROLS

SNP genotype		Controls n=60 (%)	Patients n=60 (%)	OR (95% CI)	p-Value
<i>IL1β</i> –511C/T	C/C	30 (50)	20 (33.3)	1	
	C/T	23 (38.3)	34 (56.7)	2.217 (1.022–4.812)	0.042
	T/T	7 (11.7)	6 (10)	1.286 (0.376–4.392)	0.689
	C/T+T/T	30 (50)	40 (66.7)	2.000 (0.956–4.182)	0.063
	C	83 (69.2)	74 (61.7)	1	
<i>IL1β</i> +3954C/T	T	37 (30.8)	46 (38.3)	1.394 (0.817–2.379)	0.222
	C/C	32 (53.3)	28 (46.7)	1	
	C/T	25 (41.7)	31 (51.6)	1.417 (0.682–2.945)	0.349
	T/T	3 (5)	1 (1.7)	0.381 (0.038–3.874)	0.387
	C/T+T/T	28 (46.7)	32 (53.3)	1.306 (0.638–2.676)	0.465
	C	89 (74.2)	87 (72.5)	1	
	T	31 (25.8)	33 (27.5)	1.089 (0.614–1.930)	0.770

SNP, single-nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

TABLE 3. GENOTYPE FREQUENCIES OF *IL1β* -511 C/T AND +3954 C/T POLYMORPHISMS IN FUNCTIONAL PANCREATIC NEUROENDOCRINE TUMOR PATIENTS AND HEALTHY CONTROLS

SNP genotype		Controls n=60 (%)	FP patients n=23 (%)	OR (95% CI)	p-Value
<i>IL1β</i> -511C/T	C/C	30 (50)	5 (21.7)	1	
	C/T	23 (38.3)	15 (65.2)	3.913 (1.241–12.342)	0.014
	T/T	7 (11.7)	3 (13.1)	2.571 (0.493–13.402)	0.274
	C/T+T/T	30 (50)	18 (78.3)	3.600 (1.183–10.952)	0.016
	C	83 (69.2)	25 (54.3)	1	
<i>IL1β</i> +3954C/T	T	37 (30.8)	21 (45.7)	1.884 (0.938–3.786)	0.076
	C/C	32 (53.3)	8 (34.8)	1	
	C/T	25 (41.7)	15 (65.2)	2.400 (0.879–6.557)	0.082
	T/T	3 (5)	0 (0)	0.546 (0.026–11.626) ^a	0.679 ^a
	C/T+T/T	28 (46.7)	15 (65.2)	2.143 (0.791–5.806)	0.128
	C	89 (74.2)	31 (67.4)	1	
	T	31 (25.8)	15 (32.6)	1.389 (0.663–2.911)	0.387

^aWith Haldan correction.

FP, functional pancreatic neuroendocrine tumor.

are usually more indolent than pancreatic adenocarcinoma and have a better prognosis. The origin of these tumors is not fully known, but proinflammatory cytokines were found in pNET tissue (Massironi *et al.*, 2008; Chan *et al.*, 2003; Franko *et al.*, 2010). *IL1β* acts as autocrine and paracrine stimuli in carcinogenesis of different tumor types (El-Omar *et al.*, 2000). In this study we examined the association between the *IL1β* gene SNPs with neuroendocrine pancreatic tumors in Croatian population. The main drawback of this study is the small sample size of pNET patients, but according to power test, the present result is enough to give an appropriate answer for the role of *IL1β* -511C/T and +3954 C/T in pNET susceptibility.

Our results showed statistically significant association between the *IL1β* -511C/T genotype and functional pNET risk. We observed a significantly increased prevalence of the -511C/T genotype in functional pNET cases with an OR of 3.913. Similar to our results, the -511C/T genotype contributed to an increased risk for pancreatic carcinoma, with an OR of 1.42, and also NSCLC and gastric cancer, with an OR of 1.9 and 1.8, respectively (El-Omar *et al.*, 2000; Zienoldindiy *et al.*, 2004).

Recently, it was found that an exon 5 SNP (+3954) of the *IL1β* gene predisposes to pancreatic cancer. Homozygosity

for the minor *IL1β* +3954 T allele correlates with increased *IL1β* secretion and shortens survival of patients with advanced pancreatic cancer (Barber *et al.*, 2000). We observed a higher prevalence of the +3954 C/T genotype in patients with functional pNETs (65.2% vs. 43.2%) than in nonfunctional pNETs, but the difference did not reach statistical significance ($p=0.220$). *IL1β* is a pleiotropic cytokine acting at multiple levels during carcinogenesis. It participates in hormone secretion; in the setting of pancreas it influences susceptibility to tumor development, tumor invasiveness, metastasis, and angiogenesis (Corbett *et al.*, 1993; Apte *et al.*, 2006).

Studies investigating *IL1β* serum levels in patients with different solid tumors suggest that *IL1β* is a product of tumor cells, and as such can be used as a prognostic marker (Yoshida *et al.*, 2002). According to Deans *et al.* (2006), *IL1β* serum levels in gastroesophageal carcinoma correlate with systemic inflammation and are a marker of worse prognosis, whereas in colorectal carcinogenesis, *IL1β* positively correlated with carcinoembryonal antigen (CEA), a marker of tumor exocrine differentiation leading also to a worse prognosis (Abdul and Hoosein, 2002). In our patient population, serum *IL1β* levels were below the limit of detectability (5 pg/mL). In other available research, serum levels of *IL1β*

TABLE 4. GENOTYPE FREQUENCIES OF *IL1β* -511 C/T AND POLYMORPHISMS IN NONFUNCTIONAL PANCREATIC NEUROENDOCRINE TUMOR PATIENTS AND HEALTHY CONTROLS

SNP genotype		Controls n=60 (%)	NFP patients n=37 (%)	OR (95% CI)	p-Value
<i>IL1β</i> -511C/T	C/C	30 (50)	15 (40.5)	1	
	C/T	23 (38.3)	19 (51.4)	1.652 (0.694–3.936)	0.255
	T/T	7 (11.7)	3 (8.1)	0.857 (0.194–3.795)	0.838
	C/T+T/T	30 (50)	22 (59.5)	1.467 (0.640–3.359)	0.363
	C	83 (69.2)	49 (66.2)	1	
<i>IL1β</i> +3954C/T	T	37 (30.8)	25 (33.8)	1.145 (0.617–2.124)	0.669
	C/C	32 (53.3)	20 (54.1)	1	
	C/T	25 (41.7)	16 (43.2)	1.024 (0.442–2.372)	0.956
	T/T	3 (5)	1 (2.7)	0.533 (0.052–5.488)	0.582
	C/T+T/T	28 (46.7)	17 (45.9)	0.971 (0.427–2.209)	0.945
	C	89 (74.2)	56 (75.7)	1	
	T	31 (25.8)	18 (24.3)	0.923 (0.472–1.804)	0.814

NFP, non-functional pancreatic neuroendocrine tumor.

TABLE 5. GENOTYPE FREQUENCIES OF IL1 β -511 C/T AND +3954 C/T POLYMORPHISMS IN PATIENTS WITH FUNCTIONAL AND NONFUNCTIONAL PANCREATIC NEUROENDOCRINE TUMORS

Polymorphism		NFP n=37 (%)	FP n=23 (%)	p-Value
IL1 β -511	C/C	15 (40.5)	5 (21.7)	0.334
	C/T	19 (51.4)	15 (65.2)	
	T/T	3 (8.1)	3 (13.1)	
	C/T+T/T	22 (59.5)	18 (78.3)	
	C	49 (66.2)	25 (54.3)	
IL1 β +3954	T	25 (33.8)	21 (45.7)	0.220
	C/C	20 (54.1)	8 (34.8)	
	C/T	16 (43.2)	15 (65.2)	
	T/T	1 (2.7)	0 (0)	
	C/T+T/T	17 (45.9)	15 (65.2)	
	C	56 (75.7)	31 (67.4)	0.323
	T	18 (24.3)	15 (32.6)	

in different epithelial tumors were usually around 1 pg/mL, and in healthy subjects the IL1 β levels were lower than 0.2 pg/mL (Yoshida *et al.*, 2002). This leaves the possibility that other, more sensitive laboratory methods would be needed to determine precise IL1 β levels, which would give insight into their value as an additional tumor marker. *In vitro* studies of pancreatic cell lines have detected accumulation of IL1 β -511C/T in locally advanced, undifferentiated, and metastatic pancreatic cancers. Further, the probability of pancreatic cancer resectability was significantly lower in the IL1 β high-expression genotype -511C/T (Hamacher *et al.*, 2009). Additionally, new insight into the role of IL1 β in pNET tumorigenesis would be obtained through cell culture research as well as immunohistochemistry studies of pNET tissues.

Our results showed statistically significant association between the IL1 β -511C/T genotype and CTCT -511/+3954 genotype combination and susceptibility to functional pNET development and patients with CTCC -511/+3954 genotype combination were associated with risk to non-

TABLE 6. COMBINATIONS OF IL1 β -511 AND +3954 GENOTYPES IN FUNCTIONAL PANCREATIC NEUROENDOCRINE TUMOR PATIENTS AND HEALTHY CONTROLS

IL1 β genotypes/ haplotypes -511, +3954		Controls n=60 (%)	FP patients n=23 (%)	OR (95% CI)	p- Value
CC CC	14 (23.3)	2 (8.7)	1		
CC CT	15 (25)	3 (13.0)	1.400 (0.203–9.663)	0.731	
CC TT	1 (1.7)	0 (0)	1.933 (0.060–62.175)	0.721 ^a	
CT CC	13 (21.7)	4 (17.4)	2.154 (0.336–13.804)	0.407	
CT CT	9 (15)	11 (47.8)	8.556 (1.526–47.958)	0.006	
CT TT	1 (1.7)	0 (0)	1.933 (0.060–62.175)	0.721 ^a	
TT CC	5 (8.3)	2 (8.7)	2.800 (0.307–25.525)	0.365	
TT CT	1 (1.7)	1 (4.3)	7.000 (0.302–162.212)	0.238	
TT TT	1 (1.7)	0 (0)	1.933 (0.060–62.175)	0.721 ^a	

^aWith Haldan correction.

TABLE 7. COMBINATIONS OF IL1 β -511 AND +3954 GENOTYPES IN NONFUNCTIONAL PANCREATIC NEUROENDOCRINE TUMOR PATIENTS AND HEALTHY CONTROLS

IL1 β genotypes/ haplotypes -511, +3954		Controls n=60 (%)	NFP patients n=37(%)	OR (95% CI)	p- Value
CC CC	14 (23.3)	3 (8.1)	1		
CC CT	15 (25)	11 (29.7)	3.422 (0.787–14.881)	0.083	
CC TT	1 (1.7)	1 (2.7)	4.667 (0.223–97.502)	0.332	
CT CC	13 (21.7)	14 (37.8)	5.026 (1.169–21.590)	0.019	
CT CT	9 (15)	5 (13.5)	2.593 (0.494–13.613)	0.252	
CT TT	1 (1.7)	0 (0)	1.381 (0.046–41.665)	0.856 ^a	
TT CC	5 (8.3)	3 (8.1)	2.800 (0.420–18.690)	0.289	
TT CT	1 (1.7)	0 (0)	1.381 (0.046–41.665)	0.856 ^a	
TT TT	1 (1.7)	0 (0)	1.381 (0.046–41.665)	0.856 ^a	

^aWith Haldan correction.

functional pNET development. All these results suggest IL1 β involvement in pNET development.

Acknowledgments

This study was supported by grants (098-0982464-2508 and 134-1342428-0491) from the Ministry of Science, Education, and Sports of the Republic of Croatia.

Disclosure Statement

The authors confirm that no conflict of interest exists in this study.

References

- Abdul, M., and Hoosein, N. (2002). Relationship of the interleukin-1 system with neuroendocrine and exocrine markers in human colon cancer cell lines. *Cytokine* **18**, 86–91.
- Ankarcrona, M., Dypbukt, J.M., Brune, B., and Nicotera, P. (1994). Interleukin-1 β -induced nitric oxide production activates apoptosis in pancreatic RINm5F cells. *Exp Cell Res* **213**, 172–177.
- Apte, R.N., Dotan, S., Elkabets, M., White, M.R., Reich, E., Carmi, Y., Song, X., Dvozkin, T., Krelin, Y., and Voronov, E. (2006). The involvement of IL-1 in tumorigenesis, tumor invasiveness, metastasis and tumor–host interactions. *Cancer Metastasis Rev* **25**, 387–408.
- Barber, M.D., Powell, J.J., Lynch, S.F., Fearon, K.C., and Ross, J.A. (2000). A polymorphism of the interleukin-1 beta gene influences survival in pancreatic cancer. *Br J Cancer* **83**, 1443–1447.
- Chan, A.O., Kim, S.G., Bedeir, A., Issa, J.P., Hamilton, S.R., and Rashid, A. (2003). CpG island methylation in carcinoid and pancreatic endocrine tumors. *Oncogene* **22**, 924–934.
- Chen, G., Hohmeier, H.E., and Newgard, C.B. (2001). Expression of the Transcription Factor STAT-1 α in Insulinoma Cells Protects against Cytotoxic Effects of Multiple Cytokines. *J Biol Chem* **276**, 766–772.
- Chen, H., Wilkins, L.M., Aziz, N., Cannings, C., Wyllie, D.H., Bingle, C., Rogus, J., Beck, J.D., Offenbacher, S., Cork, M.J., Rafie-Kolpin, M., Hsieh, C.M., Kornman, K.S., and Duff, G.W. (2006). Single nucleotide polymorphisms in the human

- interleukin-1B gene affect transcription according to haplotype context. *Hum Mol Genet* **15**, 519–529.
- Corbett, J.A., Sweetland, M.A., Wang, J.L., Lancaster, J.R., and McDaniel, M.L. (1993). Nitric oxide mediates cytokine-induced inhibition of insulin secretion by human islets of Langerhans. *Proc Natl Acad Sci U S A* **90**, 1731–1735.
- Deans, D.A., Wigmore, S.J., Gilmour, H., Paterson-Brown, S., Ross, J.A., and Fearon, K.C. (2006). Elevated tumour interleukin-1beta is associated with systemic inflammation: a marker of reduced survival in gastro-oesophageal cancer. *Br J Cancer* **95**, 1568–1575.
- Ebrahimi, B., Tucker, S.L., Li, D., Abbruzzese, J.L., and Kurzrock, R. (2004). Cytokines in pancreatic carcinoma: correlation with phenotypic characteristics and prognosis. *Cancer* **101**, 2727–2736.
- El-Omar, E.M., Carrington, M., Chow, W.H., McColl, K.E., Bream, J.H., Young, H.A., Herrera, J., Lissowska, J., Yuan, C.C., Rothman, N., Lanyon, G., Martin, M., Fraumeni, J.F., Jr., and Rabkin, C.S. (2000). Interleukin-1 polymorphisms associated with increased risk of gastric cancer. *Nature* **404**, 398–402.
- Franko, J., Feng, W., Yip, L., Genovese, E., and Moser, A.J. (2010). Non-functional neuroendocrine carcinoma of the P pancreas: incidence, tumor biology, and outcomes in 2,158 patients. *J Gastrointest Surg* **14**, 541–548.
- Greco, E., Basso, D., Fogar, P., Mazza, S., Navaglia, F., Zambon, C.F., Falda, A., Pedrazzoli, S., Ancona, E., and Plebani, M. (2005). Pancreatic cancer cells invasiveness is mainly affected by interleukin-1beta not by transforming growth factor-beta1. *Int J Biol Markers* **20**, 235–241.
- Hamacher, R., Diersch, S., Scheibel, M., Eckel, F., Mayr, M., Rad, R., Bajbouj, M., Schmid, R.M., Saur, D., and Schneider, G. (2009). Interleukin 1 beta gene promoter SNPs are associated with risk of pancreatic cancer. *Cytokine* **46**, 182–186.
- House, M.G., Herman, J.G., Guo, M.Z., Hooker, C.M., Schulick, R.D., Cameron, J.L., Hruban, R.H., Maitra, A., and Yeo, C.J. (2003). Prognostic value of hMLH1 methylation and microsatellite instability in pancreatic endocrine neoplasms. *Surgery* **134**, 902–908.
- Massironi, S., Sciola, V., Peracchi, M., Ciafardini, C., Spampatti, M.P., and Conte, D. (2008). Neuroendocrine tumors of the gastro-entero-pancreatic system. *World J Gastroenterol* **14**, 5377–5384.
- Muerkoster, S., Wegehenkel, K., Arlt, A., Witt, M., Sipos, B., Kruse, M.L., Sebens, T., Kloppel, G., Kalthoff, H., Folsch, U.R., and Schafer, H. (2004). Tumor stroma interactions induce chemoresistance in pancreatic ductal carcinoma cells involving increased secretion and paracrine effects of nitric oxide and interleukin-1beta. *Cancer Res* **64**, 1331–1337.
- Pociot, F., Molvig, J., Wogensen, L., Worsaae, H., and Nerup, J. (1992). A TaqI polymorphism in the human interleukin-1 beta (IL-1 beta) gene correlates with IL-1 beta secretion *in vitro*. *Eur J Clin Invest* **22**, 396–402.
- Spaventi, R., Pećur, L., Pavelić, K., Pavelić, Z.P., Spaventi, S., and Stambrook, P.J. (1994). Human tumor bank in Croatia: a possible model for a small bank as a part of the future European Tumor Bank Network. *Eur J Cancer* **30A**, 419.
- ten Kate, M., Hofland, L.J., van Koetsveld, P.M., Jeekel, J., and van Eijck, C.H. (2006). Pro-inflammatory cytokines affect pancreatic carcinoma cell. *Endothelial Cell Interact* **7**, 454–464.
- Wang, Y., Kato, N., Hoshida, Y., Yoshida, H., Taniguchi, H., Goto, T., Moriyama, M., Otsuka, M., Shiina, S., Shiratori, Y., Ito, Y., and Omata, M. (2003). Interleukin-1beta gene polymorphisms associated with hepatocellular carcinoma in hepatitis C virus infection. *Hepatology* **37**, 65–71.
- Yoshida, N., Ikemoto, S., Narita, K., Sugimura, K., Wada, S., Yasumoto, R., Kishimoto, T., and Nakatani, T. (2002). Interleukin-6, tumour necrosis factor alpha and interleukin-1beta in patients with renal cell carcinoma. *Br J Cancer* **86**, 1396–1400.
- Zienolddiny, S., Ryberg, D., Maggini, V., Skaug, V., Canzian, F., and Haugen, A. (2004). Polymorphisms of the interleukin-1 beta gene are associated with increased risk of non-small cell lung cancer. *Int J Cancer* **109**, 353–356.

Address correspondence to:
 Sanja Kapitanović, M.D., Ph.D.
 Laboratory for Personalized Medicine
 Division of Molecular Medicine
 Ruđer Bošković Institute
 Bijenička c. 54
 Zagreb 10000
 Croatia
 E-mail: kapitan@irb.hr

Received for publication May 9, 2011; received in revised form August 16, 2011; accepted August 16, 2011.