

Analysis of Polymorphism in the Survivin Gene Promoter as a Potential Risk Factor for Head and Neck Cancers Development

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SUMMARY

Introduction Association studies have shown that gene polymorphisms in various classes of genes can modulate cancer risk. The -31G/C polymorphism in the promoter of survivin gene, affects the expression of the anti-apoptotic protein survivin which in turn may predispose an individual to some types of cancer.

Objective The aim of the study was to determine whether the survivin promoter -31G/C polymorphism could be a susceptibility factor for squamous cell carcinoma (SCC) of the oral cavity and basal cell carcinoma (BCC) of the skin.

Methods The DNA obtained from 88 patients with SCC, 60 patients with BCC and 111 healthy individuals was subjected to polymerase chain reaction-restriction fragment length polymorphism analysis (PCR-RFLP) in order to determine genotype and allele frequencies in patients and control groups. Logistic regression was used for cancer risk assessment.

Results The following distribution of genotypes was obtained: CC genotype 15% in the SCC group, 13% in the BCC group and 12% in controls; CG genotype 41% in SCCs, 35% in BCCs, 48% in controls; GG genotype 44% in SCCs, 52% in BCCs and 40% in controls. Allelic frequencies were as follows: G allele 0.65 in SCCs, 0.69 in BCCs and 0.64 in the control group; C allele 0.35 in SCCs, 0.31 in BCCs and 0.36 in the control group. There was no statistically significant difference in allele or genotype frequencies between the patients and controls ($p > 0.05$).

Conclusion In Serbian population, -31G/C polymorphism in the promoter of the survivin gene cannot be considered as a risk factor for oral squamous cell carcinoma and skin basal cell carcinoma.

Keywords: polymorphism; survivin; promoter; head and neck cancers

INTRODUCTION

Head and neck cancers are placed among the most common neoplasm worldwide [1, 2]. They can develop in the oral cavity, pharynx, paranasal sinuses, nasal cavity, larynx, salivary glands and the skin of head and neck [3]. In our study we investigated two types of head and neck cancer with different origin, natural history and clinical outcome – squamous cell carcinomas (SCCs) of the oral cavity representing highly invasive malignancies, and basal cell carcinomas (BCCs) of the skin, a class of usually non-aggressive tumors.

Oral SCCs account for approximately 500,000 new cases worldwide, making them the 6th most common cancer type [1, 2, 4]. The overall 5-year survival rate for oral SCCs is merely 50%. Almost 85% of patients are alcohol or tobacco abusers or both [4]. Other causative agents include chewing tobacco, ill-fitting dental appliances, chronic candidiasis, viral infections (mainly involving certain HPV types) and poor oral hygiene [2, 3].

BCCs mostly occur on sun-exposed areas of the body, such as the head and neck (80% of cases). Acute and chronic skin exposure to ultraviolet radiation is supposed to be a crucial factor in their development [5, 6]. Most

of them have a superficial or nodular growth pattern. Although mortality rates are low, BCCs may grow per continuitatem and cause severe local destruction. Metastasis occurs in less than 1% [7, 5]. Although most BCCs occur in elderly persons, the incidence in young patients is increasing lately in a disproportionate mode [7, 8].

It is well known that head and neck carcinogenesis is a multifactorial process in which environmental etiologic factors are coupled with alterations in oncogenes and tumor suppressor genes [4]. Recently, research has also been focused on genetic factors that can modulate cancer susceptibility such as common DNA polymorphisms [9]. Functional single nucleotide polymorphisms (SNPs) in the survivin gene have recently emerged as potential risk factors for the development of several malignant diseases.

Survivin is a member of the inhibitor of apoptosis (IAP) gene family. IAPs are anti-apoptotic proteins that inhibit initiator (caspase-9) and effector caspases (caspase-3 and 7) and thus prevent apoptosis [10, 11]. Beside its involvement in the regulation of apoptosis, survivin is involved in cell cycle progression, and microtubule stability, thus contributing to ordered development and cellular homeostasis [12].

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Structurally, the human survivin gene comprises four exons separated by three introns spanning 14.7-kb, which encodes a 16.5-kDa protein [13, 14]. Survivin is expressed in a cell-cycle-dependent manner, with a peak in the G2/M phase of the cell cycle, when it is associated with the microtubules of the mitotic spindle, and exhibits a rapid down-regulation in the G1 phase [15]. This is controlled at the transcriptional level and mediated by cell cycle-dependent elements (CDEs) and cell cycle homology regions (CHRs) located in the proximal region of the survivin promoter [16]. Several single-nucleotide polymorphisms have been identified within the promoter region of the survivin gene, one of which is located at the CDE/CHR repressor binding site (-31G/C). This polymorphism has been associated with overexpression of survivin at both messenger RNA (mRNA) and protein levels in a number of cancer cell lines [17].

Survivin is strongly expressed in embryonic and fetal tissues but is undetectable in most terminally differentiated normal adult tissues [18]. By contrast, dramatic overexpression of survivin compared to normal tissues was demonstrated in tumors of lung, breast, colon, stomach, esophagus, pancreas, bladder, nonmelanoma skin cancers, and others [16]. In genome-wide searches, survivin constituted the fourth top "transcriptome" in cancers of colon, lung, brain, breast, and melanoma, but its expression was low or undetectable in the normal tissue of the same specimens [19].

OBJECTIVE

The aim of this study was to investigate a possible association between survivin promoter single nucleotide polymorphisms (SNP) at position -31G/C known to be a regulator of survivin expression, and the susceptibility for the development of two types of HNC in the Serbian population.

METHODS

Patients

The study population comprised 88 patients with oral squamous cell carcinoma (53 males, 27 females, mean age, 69 years) and 60 patients (26 males, 34 females, mean age 71 years) with basal cell carcinoma of the skin, histologically confirmed, who underwent surgical resection at the Clinic for Maxillofacial Surgery, Faculty of Dental Medicine, University of Belgrade. The study protocol was approved by the institutional Ethical Committee.

Survivin -31 G/C genotyping

Genomic DNA was isolated from the tissue of histologically negative margins from 88 patients with SCC and 60 patients with BCC using the organic extraction protocol. For the control group, genomic DNA was isolated from buccal swabs of 111 healthy individuals using QIAmp DNA Mini Kit (Qiagen, GmbH, Germany), as recommended

by the manufacturer. Patients and controls were sex and age matched. The survivin promoter polymorphism -31G/C genotyping was performed using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. A 151 base pair fragment, surrounding the -31 position, was amplified using the following primers: 5'-AAGAGGGCGTGCGCTCCCGACA-3' and 5'-GAGATGCGGTGGTCCTTGAGAAA-3'. The PCR was performed in a total volume of 20 µl containing 2 µl of 10X PCR buffer (MBI Fermentas, Lithuania), 1.5 µl of MgCl₂, 0.2 mM dNTPs, 0.375 µM of each primer, 200 ng of genomic DNA and 1 unit of Taq DNA polymerase (MBI Fermentas, Lithuania). The amplification conditions were as follows: initial denaturation at 95°C for 5 minutes, followed by 35 cycles consisting of denaturation at 94°C for 45 s, annealing at 60°C for 45 s, elongation at 72°C for 1 min, and a final elongation at 72°C for 10 min. The digestion was carried using 5 units of Msp I (MBI Fermentas, Lithuania), resulting in products of 90 and 61 base pairs (bp) for the CC genotype, three fragments of 151, 90 and 61 bp for the GC genotype and a 151 bp fragment for the GG genotype. Genotypes were confirmed by randomly re-genotyping 10% of samples. There were no discrepancies between genotypes determined in duplicate.

Statistical analysis

Chi square test and Fisher exact test were used to determine possible differences in the genotype and allele frequencies. The association of -31 survivin variants with risk of disease was examined by use of unconditional logistic regression analysis to calculate odds ratios (OR) and their 95% confidence intervals (CI). P values of <0.05 were considered statistically significant. The expected frequency of survivin variants in controls was analyzed by the Hardy-Weinberg equilibrium test. Calculations were performed with the statistical package Stata V6.

RESULTS

The interpretation of the characteristic electrophoretic bands following PCR-RFLP and determination of individual genotypes in healthy subjects and patients with SCC and BCC, along with observed allele frequencies and risk estimate for the -31G/C polymorphism in the survivin gene are given in Tables 1 and 2.

Genotype and allele frequencies at position -31 did not show significant difference between patients and controls.

DISCUSSION

Survivin, a crucial anti-apoptotic factor, also plays an important role in cell cycle regulation. Considerable evidence suggests that elevated expression of survivin may promote tumorigenesis, and indeed survivin is highly expressed in common human cancers [20-24].

Table 1. Genotype and allele frequencies and logistic regression analysis data for the survivin promoter -31G/C polymorphism in squamous cell carcinoma (SCC) of oral cavity

Parameter	SCC (n=60)	Control (n=111)	OR	95% CI	p
G/G	39 (44%)	45 (40%)	1.00	Reference	
C/G	36 (41%)	53 (48%)	0.78	0.43-1.43	0.261
C/C	13 (15%)	13 (12%)	1.15	0.48-2.78	0.462
C/G+C/C	49 (56%)	66 (60%)	0.86	0.49-1.51	0.348
G	0.65	0.64	1.00	Reference	
C	0.35	0.36	0.96	0.54-1.71	0.500

OR – odds ratio; CI – confidence interval; p – probability; n – number of individuals

Table 2. Genotype and allele frequencies and logistic regression analysis data for the survivin promoter -31G/C polymorphism in basal cell carcinoma (BCC) of oral cavity

Parameter	BCC (n=60)	Control (n=111)	OR	95% CI	p
G/G	31 (52%)	45 (40%)	1.00	Reference	
C/G	21 (35%)	53 (48%)	0.58	0.29-1.14	0.077
C/C	8 (13%)	13 (12%)	0.89	0.33-2.41	0.515
C/G+C/C	29 (48%)	66 (60%)	0.64	0.34-1.20	0.108
G	0.69	0.64	1.00	Reference	
C	0.31	0.36	0.80	0.44-1.44	0.275

Functional polymorphisms influencing survivin expression may be considered as risk factors for carcinogenesis. Despite the existing data on the association of survivin gene promoter polymorphism -31G/C and cancer susceptibility, this association is not universally observed. Depending on cancer types, as well as on patients' ethnic origin, published results are quite divergent. A recent meta-analysis by Srivastava et al. [25] indicates that an increased risk of cancer related to this SNP was significant only in the Asian population.

Though the comprehensive experiments of Xu et al. [17] on a large number of cancer cell lines has shown that survivin overexpression is due to the presence of the G allelic variant, strangely, the majority of clinical studies have found the opposite, i.e. that the C allele is related to survivin overexpression and to a higher risk for cancer development. Jang et al. [26] identified that individuals with at least one G allele at position -31 were at a significantly decreased risk of lung cancer compared to individuals with the -31C/C genotype. Cheng et al. [27] found that the frequency of C allele and C/C genotype were significantly higher in patients with gastric cancer than in healthy individuals. In the study of Gazouli et al. [28], -31C/C genotype and -31C allele were associated with a significantly

increased risk for colorectal cancer (CRC). They observed a strong association between -31C/C genotype and advanced stages (III and IV) of the disease and unfavorable prognosis. However, several studies, such as the one involving Brazilian patients with gastric carcinoma, showed the G allele to be responsible for an increased risk of the development of diffuse tumor types and for the early onset of the disease [29]. Similarly, Yang et al. [30] demonstrated that carriers of the G allele were at a higher risk of distal and well-differentiated gastric cancer. The results of our previous study, conducted on keratocystic odontogenic tumors (KCOTs) also showed that the GG genotype and the G allele are associated with an increased risk of KCOT development [31].

Finally, a third group of studies, including the present study on HNCs, failed to establish any influence of the -31 G/C SNP on cancer development. Wagner et al. [32], for instance, were unable to demonstrate association of -31G/C polymorphism with acute myeloid leukemia. It seems also that this SNP is irrelevant as potential risk factor for cervical carcinogenesis [33].

The fact that there is no straightforward relationship between survivin gene variants, its expression at the mRNA level and the expression at protein level, points to a very complex regulation of survivin production and function. And although survivin is recognized as a prognostic marker for some tumors, controversies are present in immunohistochemical analyses as well, regarding the prognostic significance of cytoplasmic versus nuclear survivin expression.

CONCLUSION

In Serbian population the polymorphism -31G/C in the survivin gene promoter does not modulate the susceptibility for head and neck tumor development.

NOTE

This paper is a part of PhD thesis of Marija Kostić.

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Анализа полиморфизма у промотору гена за сурвивин као могућег фактора ризика за настанак тумора главе и врата

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КРАТАК САДРЖАЈ

Увод Доказано је да полиморфизми у различитим класама гена могу да повећају ризик за развој малигну тумора, између осталих и сквамозелуларног карцинома (SCC) усне дупље и базозелуларног карцинома (BCC) коже. Сурвивин је бифункционални протеин-инхибитор апоптозе и регулатор ћелијског циклуса. Откривено је више функционалних полиморфизма у овом гену, а један од кључних је полиморфизам G/C на позицији -31, за који је показано да је модулатор експресије сурвивина и да доприноси повећању ризика од оболевања од различитих типова тумора.

Циљ рада Циљ рада је био да се анализира учесталост генотипова и алела за -31G/C полиморфизам гена за сурвивин код особа оболелих од SCC и BCC и код здравих испитаника. Логистичком регресионом анализом испитана је повезаност овог полиморфизма и ризика за настанак SCC и BCC.

Методе рада Учесталости алела и генотипова код 88 особа оболелих од SCC, 60 особа оболелих од BCC и 111 здравих испитаника одређене су ланчаном реакцијом полимеразе

и рестрикционом анализом. Логистичком регресијом процењена је склоност ка развоју SCC и BCC.

Резултати Генотип CC је утврђен код 15% испитаника са SCC, 13% са BCC и 12% здравих особа. Генотип CG је забележен код 41% испитаника са SCC, 35% са BCC и 48% здравих особа. Генотип GG је откривен код 44% особа са SCC, 52% са BCC и 40% здравих испитаника. Учесталост G-алела била је следећа: 0,65 код испитаника са SCC, 0,69 код испитаника са BCC и 0,64 у групи здравих особа. Учесталост C-алела била је: 0,35 код испитаника са SCC, 0,31 код испитаника са BCC и 0,36 у групи здравих особа. Није било статистички значајне разлике у расподели генотипова и алела између болесника с карциномима и здравих испитаника ($p > 0,05$).

Закључак Полиморфизам -31G/C у промотору гена за сурвивин не може се сматрати фактором ризика за развој ова два типа тумора.

Кључне речи: полиморфизам; сурвивин; промотор; тумори главе и врата