



Original article

Survivin gene polymorphism association with papillary thyroid carcinoma

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ABSTRACT

Survivin expression is correlated with suppression of apoptosis in human solid tumors. A polymorphism at position –31 (G/C) (rs 9904341) has been associated with cancer risk in several studies. We evaluated the correlation of this polymorphism with the risk of papillary thyroid carcinoma (PTC) in an Iranian population.

The cases consisted of patients with PTC ($n = 123$) and normal controls, composed of non-related healthy people ($n = 131$).

The frequency of GC or CC genotype in patients with PTC was significantly higher than in the controls [GC + CC vs GG, $p = 0.02$ OR; 1.7, 95%CI (1.05–3.04)]. There was a significant difference between patients with more aggressive clinical manifestations, including lymphatic involvement compared to the controls [GC + CC vs GG, $p = 0.0006$, OR; 3.7, 95%CI (1.6–9.2)].

The presence of C allele was significantly associated with the presence of more profound manifestations, including lymph node involvement, vascular involvement and multifocality.

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Introduction

Thyroid carcinoma is the most common malignancy of the endocrine system, which is becoming increasingly prevalent [11]. The rising incidence might be explained by improvements in the clinical detection of small tumors or as a result of increased exposure to a known or emerging risk factor. Several factors have been reported to confer the risk of thyroid cancer development, such as environmental risk factors (e.g. sex, age and radiation) and genetic factors. Familiar aggregation has been reported in 6% of patients diagnosed with thyroid carcinoma, and a 4- to 10-fold increase in the risk of developing papillary thyroid carcinoma has been reported in their first degree relatives [25]. Genetic polymorphisms may contribute to the susceptibility of thyroid carcinoma [3,14]. Therefore, identifying genetic markers associated with thyroid carcinoma might be helpful in early diagnosis and decreasing disease mortality [8,10].

The majority of thyroid tumors are well differentiated with a follicular cell origin classified as papillary thyroid carcinoma (PTC), follicular thyroid carcinoma (FTC), and Hurthle cell carcinomas

(HCC). The prevalence of PTC has been estimated as 79%, FTC as 13%, and HCC as 3% [7].

Apoptosis and unbalanced cell proliferation play a critical role in the development of cancers [22,23]. The human inhibitor of apoptosis protein (IAP) family consists of eight members of baculoviral repeat containing (BIRC 1 to BIRC 8) [16,21]. High expression of survivin (BIRC 5) and XIAP (BIRC 4) is critical for apoptosis suppression in human solid tumors [19]. Survivin has a unique propriety because of its bifunctional role as a protein that exhibits cell cycle regulation and inhibition of apoptosis [18]. It is expressed in the G2/M phase of the cell cycle to support the rapidly dividing cell machinery [17]. Importantly, survivin is generally expressed in embryonic tissues. Therefore, lack of survivin function results in disorganized and embryonic death [1,20].

Although several previous studies have reported that survivin protein is undetectable in differentiated adult tissues [15–17], some investigators have recently found evidence of survivin expression in normal adult cells. Therefore, it has been speculated that it may also have a role in normal cellular function [4,32].

Survivin gene in human spans 14.7 kb located at the telomeric region of chromosome 17q25 [2]. Several single nucleotide polymorphisms (SNPs) were identified within the promoter region of the survivin gene. A polymorphism at position –31, which involves the substitution of G for C (rs 9904341), has been the most documented one in previous reports. This polymorphism is located

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at the cell cycle-dependent elements (CDE) and cell cycle homology region (CHR) repressor binding motif of the promoter [22]. This mutation (G/C) seems to be correlated with increased expression of survivin at both transcription and translation levels [29,31]. Also, many other survivin promoter polymorphisms, including –644T>C, –625G>C, and –241T>C, have been reported to be in linkage disequilibrium with –31G>C [33].

Several case–control studies have examined the association between –31G/C polymorphism and cancer risk [26], including nasopharyngeal carcinoma [32], esophageal cancer [34,28], gastric cancer [5,32], hepatocellular carcinoma [4], pancreatic cancer [27], and urothelial carcinoma [30]. Immunohistochemical staining for survivin expression in tissue samples of thyroid carcinoma showed increased expression of survivin in thyroid carcinoma [13]. According to the critical role of survivin in carcinogenesis with prognostic and therapeutic implications [6], we evaluated the correlation of this polymorphism with thyroid cancer risk in an Iranian population.

Materials and methods

Study population

The groups of cases consisted of patients with PTC (n = 123), whose diagnoses were based on the final pathology report after thyroidectomy. Control groups were composed of non-related, healthy people (n = 131). Approximately 5 ml of venous blood sample was collected from each subject. The study was approved by the Ethics Committee of Tehran University. Informed consent was obtained from all of the patients attending the study.

DNA extraction and genotyping

Blood samples were anticoagulated with ethylenediamine tetraacetic acid (EDTA).

The 151 bp DNA fragment containing the –31 polymorphic site was amplified using pairs of primers as follows: 5'-AAGAGGGCGT-GCGTCCCACACA-3' and 5'-GAGATGCGGTGgTCCTTGAGAAA-3'. The PCR was performed in a total volume of 20 µl containing 2 µl 10× PCR buffer (Fermentas), 2.5 mM MgCl₂, 0.2 mM dNTPs, 0.375 µM of each primer, 200 ng genomic DNA, and 1 u of Tag DNA polymerase (Fermentas).

The amplification conditions were as follows: denaturation at 95 °C for 10 min, five cycles at 95 °C for 45 s, and 72 °C for 60 s for primer annealing, followed by 30 cycles at 94 °C for 45 s, 62 °C for 45 s, and 72 °C for 45 s, with a final elongation at 72 °C for 10 min. PCR product size was 151 bps.

8 µl of PCR product was digested with 5 u MspI at 37 °C overnight. Digested products yielding 151 bps of uncut fragment for the GG genotype, two fragments of 61 and 90 bps for the CC

Table 1
Clinical characteristics of patients with papillary thyroid carcinoma.

| Variable | No. of patients (%) (n = 123) |
|--------------------------|-------------------------------|
| Mean age ± SD | 39 ± 14 |
| Gender(female/male) | 96/27 |
| Mixed follicular variant | 11(8.9) |
| Tall cell variant | 5 (4) |
| Microcarcinoma | 14(11.3) |
| Lymphatic involvement | 43(34.9) |
| Vascular involvement | 9(7.3) |
| Multifocality | 14(11.3) |
| Cervical recurrence | 2(1.6) |
| Distant metastasis | 1(0.08) |

genotype, and three fragments of 151 bp, and 90 and 61 bp for the CG genotype were visualized on a 4% agarose gel stained with ethidium bromide.

Statistical analysis

The strength of the association between different groups and alleles or genotypes of survivin gene polymorphism was estimated using odds ratios (OR) and 95% confidence intervals (CI). Levels of significance were determined using contingency tables by either the Chi-square or Fisher's exact analysis.

All analyses were carried out using the SPSS version 11.5 and STATA version 8. *p* ≤ 0.05 was considered as significant statistical difference.

Results

The mean age of patients in the PTC group was 39 ± 14 years. The females accounted for 75% and males for 25% of the study population. The mean age of healthy controls was 53 ± 10, comprising 50% males and 50% females (Table 1).

Survivin gene –31G/C polymorphism allele and genotype frequencies in patients with papillary thyroid carcinoma and controls

The allele and genotype frequencies of the survivin gene –31G/C polymorphism conformed to Hardy–Weiberg equilibrium both in patients and in control populations. When comparing the frequency of survivin gene polymorphism in the patients with PTC and controls, we observed that the frequency of GC or CC genotype in patients with PTC was significantly higher than in the controls [GC + CC vs GG, *p* = 0.02 OR; 1.7, 95%CI (1.05–3.04)]. Also, Allele C frequency was significantly increased in patients with PTC compared to the controls [GC + CC vs GG, *p* = 0.003, OR; 1.7, 95%CI (1.1–2.6)] (Table 2).

Table 2
Survivin –31G/C polymorphism allele and genotype frequencies in patients with PTC and various clinical variables compared to the controls.

| Survivin –31G/C | Controls n (%) | PTC n (%) | Micro carcinoma n (%) | Lymphatic involvement n (%) | Vascular involvement n (%) | Multifocality n (%) |
|--------------------------------|----------------|---------------|-----------------------|-----------------------------|----------------------------|---------------------|
| Genotype | | | | | | |
| GG | 70(53.4) | 48 (39) | 5(35.7) | 10(23.2) | 3(33.3) | 4(28.5) |
| GC | 54(41.2) | 56(45.5) | 6(42.8) | 25(58.1) | 3(33.3) | 6(42.8) |
| CC | 7(5.3) | 19(15.4) | 3(21.4) | 8(18.6) | 3(33.3) | 4(28.5) |
| <i>p</i> value (GC + CC vs GG) | | 0.02* | 0.2 | 0.0006* | 0.2 | 0.07 |
| Odd ratio (95%CI) | | 1.7 (1.05–3) | 2.06 (0.5–8.2) | 3.7 (1.6–9.2) | 2.2 (0.4–4.6) | 2.8 (0.7–13) |
| Allele | | | | | | |
| G | 194(74.1) | 152(61.7) | 16(57.2) | 45(52.3) | 9(50) | 14(50) |
| C | 68(25.9) | 94(38.2) | 12(42.8) | 41(47.6) | 9(50) | 14(50) |
| <i>p</i> value | | 0.003* | 0.05 | 0.0002* | 0.02* | 0.007* |
| Odd ratio (95%CI) | | 1.7 (1.1–2.6) | 2.1 (0.8–5.08) | 2.5 (1.5–4.4) | 2.8 (0.9–8.4) | 2.8 (1.1–6.7) |

* *p* < 0.05 has been considered as significant.

Also, we examined the association between survivin polymorphism and clinical variables, including lymphatic involvement, vascular involvement, microcarcinoma, and multifocality. We observed that –31G/C allele and genotype frequencies were significantly different in patients with lymphatic involvement compared to the controls [GC+CC vs GG, $p=0.0006$, OR; 3.7, 95%CI (1.6–9.2)] (Table 2). There were no significant differences in survivin polymorphism genotype frequencies between patients with microcarcinoma, vascular involvement, and multifocality, which might be due to the small number of cases examined. However, the frequency of allele C was significantly increased in patients with vascular involvement and multifocality (Table 2).

Discussion

This study was the first attempt to examine survivin gene –31G/C polymorphism in thyroid carcinoma. Our results suggest that survivin C–31G polymorphism is associated with a risk of PTC in an Iranian population.

Functional studies have previously investigated the effect of –31G/C polymorphism using a luciferase assay, indicating that G allele decreases promoter activity as compared with the C allele in HeLa and CHO cells [15]. Allele C was shown to be correlated with over-expression of survivin at both mRNA and protein levels and also with cell cycle-dependent transcription in various cancer cell lines [12].

Survivin overexpression in human cancers has been indicated as an important marker for tumor aggressiveness and is chemoresistant with poor prognosis [1,9,24].

Our finding is in keeping with previous studies in which the C allele has been reported to carry a risk of developing various carcinomas. The presence of C alleles was not only a predisposing factor for PTC in our study. A more significant increase in the frequency of allele C was observed in patients with profound manifestations, including lymph node involvement, vascular involvement, and multifocality, highlighting the importance of our finding, which might have both therapeutic and diagnostic implications.

More studies investigating a larger number of samples of patients with different types of thyroid carcinoma, including thyroid follicular carcinoma, might reveal the role of survivin in the development and prognosis of various types of thyroid carcinoma. In addition, the study of other groups of patients with benign thyroid lesions, including lymphocytic thyroiditis or nodular hyperplasia, will be helpful. Although this must be confirmed in larger populations, as seen in our study, the relation between survivin and tumor aggressiveness might indicate survivin as a potential marker for the differential diagnosis between different types of thyroid carcinoma.

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