



## Ras oncogenes polymorphism in Turkish thyroid cancer patients

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### Abstract

Molecular mutations to proto-oncogene sequences may be involved in the pathogenesis of human thyroid neoplasm. Problems on oncogenes and tumor suppressor genes activation in cell cycle could cause tumor. Many oncogenes and tumor suppressing genes exist in varying percentages in various types of thyroid cancers. **Ras**, **Gsp**, **Ret** or **Trk** oncogenes can be involved in thyroid tumors. Members of the **Ras** gene family (**H-ras**, **K-ras**, and **N-ras**) are signal transferring proteins. These genes codes for 21 kDa GTP binding proteins. We studied 24 thyroid cancer and 77 control for **ras** gene point mutations in two different codons (12 and 13) using a restriction fragment length polymorphism technique. According to enzyme digesting, no c-K-**ras** gene codon 12/13 and N-**ras** gene codon 12 point mutation were observed in any of the samples we studied.

Key words: *ras* gene, thyroid cancer, mutation, RFLP

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### Introduction

Mutations in the genes controlling cell growth and differentiation are considered to be the main cause of cancer (Bos, 1989). The most common cancer in Turkey is lung cancer. Thyroid cancer is not very common in general; however some people are affected in Black Sea and Central region of Turkey. The number of thyroid cancer patients in Trabzon is 6 in 2002 and 22 in 2003 (Tuncer, 2007). This type of cancer is characteristic of a group of malignant tumors related to thyroid gland. There are different types of thyroid cancer which are papillary, follicular, medullar and anaplastic (Donis-Keller, 1993). Radiation exposure, chronic goiter, family history, and environmental factors could cause thyroid cancer (De Groot, 1996).

The thyroid gland is located in the throat, below the larynx. It is made of two lobes. The thyroid gland secretes very important hormones that regulate so many metabolic processes, such as growth and energy expenditure. Women population slightly more affected than men. Approximately 10 % of thyroid cancers are present in patients younger than 21 years of age, representing 3 % of all cancers of children and adolescents, with predominance in females 2:1 in relation to males. Thyroid cancers group in this age are usually papillary (90 %), bilateral, multifocal and bigger in size compared to adults (Monte *et al.*, 2007). Anaplastic carcinoma is the most aggressive and malignant form of thyroid cancer. The most common type of thyroid carcinoma is papillary carcinom. The most frequent mutations in papillary carcinomas are point mutations of the BRAF, Ras genes and RET/PTC rearrangement (Nikiforov, 2008).

The Ras-Raf-Mek-MAP kinase-signaling pathway is activated in human carcinomas, particularly in adenocarcinomas. The pathway is responsible for transmittion of mitogenic signal to the nucleus. The constitutive activation of the pathway is thought to reason of uncontrolled cell division (Fukushima *et al.*, 2003). There are three human **RAS** genes which are N-**Ras**, **H-Ras**, and **K-Ras**. The *ras* gene family encode membrane-associated guanine nucleotide-binding

**Table 1.** Primer used in PCR

Primer	Nucleotide sequences 5'- 3'
<i>c-K-ras</i> (first round amplification)	F: GAAGCTTATGTGTGACATGTCTA R: AGGCACTCTTGCCTACGTCA
<i>c-K-ras</i> codon 12 ( <i>Hph</i> I)	F: CCTGGTGAAAATGACTGAAT R: AGGCACTCTTGCCTACGTCA
<i>c-K-ras</i> codon 13 ( <i>Hae</i> III)	F: GCCTGCTGAAAATGACTGAA R: CGTCAAGGCATCTTGCCTAGG
<i>N-ras</i> (first round amplification)	F: TAAAGTACTGTAGATGTGGCT R: TCACCTCTATGGTGGGATCAT
<i>N-ras</i> codon 12 ( <i>Bst</i> NI)	F: CAAACTGGTGGTGGTTGGACCA R: AGTGGTCCTGGATTAGCTGGAT

proteins (p21<sup>ras</sup>) (Ginesa *et al.*, 2003). Mutations in codon 12, 13, or 61 of gene of guanine nucleotide-binding proteins, convert these genes into active oncogenes (Bos, 1989)

In order to find biomarkers for thyroid cancer, it is necessary to study the mechanism of cancer progression. Mutations in cellular *ras* gene have been reported in various thyroid cancer types. In tumors, the point mutations which have been identified have been localized to codon 12, 13 or 61 of three *ras* genes (Leon *et al.*, 1987).

In this study, we evaluate the frequency of *RAS* mutations in thyroid carcinoma by PCR-RFLP method.

## Materials and methods

### DNA isolation

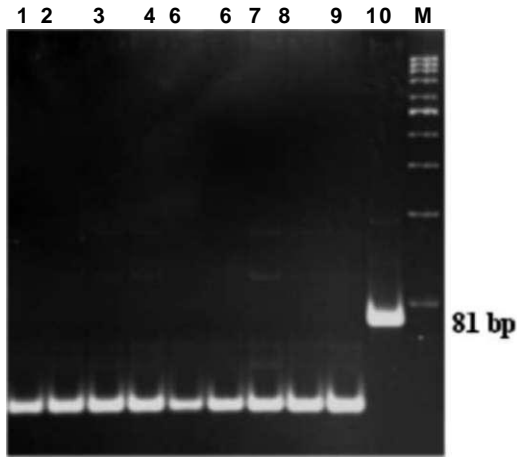
In this study total 111 DNA samples were isolated from 34 patients with histologically verified thyroid cancer (Ankara Numune Education and Investigation Hospital in Turkey) and 77 control group blood samples. DNA isolation from blood samples was isolated as described by Maniatis *et al.* (1989). 30 ml RBC lyses buffer was added to 9 ml blood sample and shaken gently. The mixture was incubated on ice for 20 min and centrifuged at 4000 rpm for 20 min at 4°C. The supernatant was removed 25 ml RBC lyses buffer was added and this process was repeated till all the red cells were removed. 20 ug/ml proteinase K, 10 % SDS (final concentration 0.5 %) and 2,5 volume STE were added and incubated overnight at 56°C in a water bath. 1:1 phenol: chloroform: Isoamylalcohol (25:24:1) was

added and shaken by hand for 10 min. Then the mixture was incubated on ice for 20 min and centrifuged at 4000 rpm for 20 min at 4°C. Upper phase was transferred into a new tube, 1:10 volume 2 M sodium acetate (pH 5.2) and 95 % ethanol (2 fold of total volume) were added and shaken gently until the DNA was precipitated, incubated overnight at -20°C. DNA was centrifuged at 4000 rpm for 20 min at 4°C. Supernatant was removed; DNA was washed in 500 ul 70 % ethanol and dissolved in 0.5-1 ml TE-buffer overnight at 37°C in a water bath.

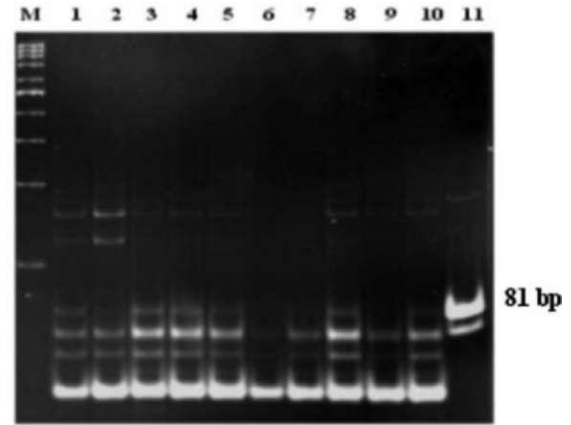
### PCR Amplification and RFLP

The procedure to detect any mutation at codon 12 and codon 13 of the *c-K-ras* gene and *N-ras* gene has been described by Capella *et al.* (1996). DNA sequences of the first coding exon of the *c-K-ras* gene were amplified using the primers 5' GAAGCTTATGTGTGACATGTCTA 3' and 5' AAGGATCCTGCAGTAATATGCA 3' for 15 cycles (92°C for 1 min and 4 s; 50°C for 35 s; 72°C for 1 min and 25 s; PCR 1). Then 1 ml of the amplified product was reamplified by means of nested PCR using primers in Table1 for 30 cycles (92°C for 15 s; 50°C for 15 s; 72°C for 30s; PCR 2). PCR was performed (Biometra, Germany) using 1 mg genomic DNA, 2.5U *Taq* polymerase (Fermentas) 0.2 mM each dNTP, 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 2 mM MgCl<sub>2</sub>, 0.1 % Triton X-100, and 20 pmol primers in total volume 50 ul.

Codon 12 and 13 mutations were detections by digesting 70 bp amplified fragment with *Hph*I and *Hae*III, respectively. A similar approach was used to detect mutations at codon 12 of the *N-ras* gene. The



**Figure 1.** RFLP (HaeIII) of PCR products of codon12 for the c-K-ras gene. M- DNA marker (100bp), lane1-5 control samples, lane 6-9 thyroid cancer samples, lane 10 uncut samples.

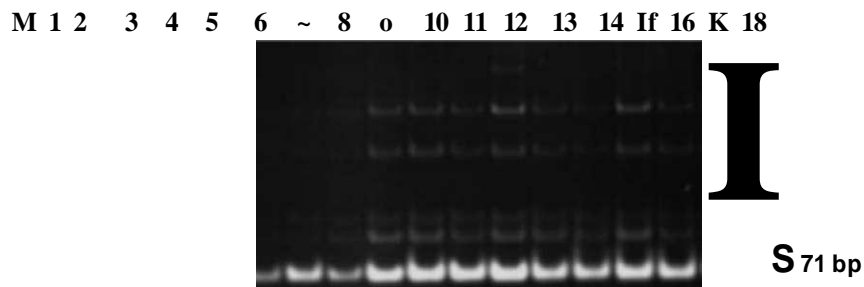


**Figure 2.** RFLP (HphI) of PCR products of codon13 for the c-K-ras gene. M- DNA marker(100bp), lane1-5 control samples, lane 6-10 thyroid cancer samples, lane 11 uncut samples.

first round amplification was performed in the same conditions as for the c-K-ras gene using the primers described in Table 1. Codon 12 mutations were detections by digesting 81 bp amplified fragment with BstNI. A 10-ul aliquot of amplified material is taken for restriction enzyme digestion using *HphI*, *HaeIII* and *BstNI* (GeneMark) and respective buffer for 2 hr at 37°C in a total volume of 20 ul. After enzymatic digestion, the digested fragments were subjected to electrophoresis in a 12% polyacrylamide gel electrophoresis and stained with ethidium bromide (Fig 1-3).

## Results and discussion

In this study, we examined 111 samples with thyroid tumors and without tumors. DNA samples were analyzed for mutations at codons 12/13 of c-K-ras gene and codons 12 of N-ras using Nested Polymerase Chain Reaction and Restriction Fragment Polymorphism (PCR-RFLP). Selective amplification of the region around the studied codons of the *ras* oncogene was performed by PCR. At two stages PCR reaction, amplification products size were 81 bp for c-K-ras gene and 71 bp for N-ras gene. The Amplification



**Figure 3.** RFLP (BstNI) of PCR products of codon12 for the N-ras gene. M- DNA marker(100bp), lane1-8 control samples, lane 9-17 thyroid cancer samples, lane 18 uncut samples.

products were digested with restriction enzymes Hph1 and HaeIII for *c-K-ras* (Figure 1-2) and *BstnI* for *N-ras* gene (Figure 3). According to enzyme digesting, no mutation at codon 12 and codon 13 was observed. Similarly, Bouras *et al.* (1998) were found any point mutations at the same codon for 128 thyroid tissue samples.

Mutations in all three families of *ras* oncogenes have been detected in both benign and malignant thyroid tumors (Du Villard *et al.*, 1995; Manenti *et al.*, 1994). Mutations of the *ras* (Ha-, Ki-, *N-ras*) proto-oncogene have been reported in 20-60 % of thyroid tumors, particularly in follicular types (Namba *et al.*, 1990; Wright *et al.* 1989) and more frequently in iodine-deficient areas (Shi *et al.*, 1991). For this reason, *ras* mutations have been suggested as an important prognostic marker for thyroid cancer (Karga *et al.*, 1991, Shi *et al.*, 1991; Namba *et al.*, 1990, Shi *et al.*, 1991). The reason we could not find any mutations could be that the number of patients studied is low. We need to increase the number of patients for future studies.

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