Low incidence of H-, K- and N-ras oncogene mutations in cytological specimens of laryngeal tumours

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Abstract

Laryngeal cancer is a rare type of neoplasia, constituting approximately 2% of all human cancers. Mutations of the ras gene family is one of the main activating mechanisms in human cancer. Their involvement in head and neck cancer has been mainly demonstrated at the level of the overexpression whereas ras mutations in these cancers are rare in the Western world. In the present study we explored the incidence of codon 12-point mutation in the H-, K- and N-ras genes, in 41 laryngeal cytological specimens. These specimens corresponded to 19 benign and 22 malignant lesions of the larynx. Only two specimens carried a codon 12-point mutation in the K-ras gene (4.8%) while no mutation was detected in the H- and N-ras genes. K-ras mutations were detected in one benign and one malignant specimen. These results indicate low incidence of ras oncogene mutations in laryngeal cytological specimens. © 1999 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Laryngeal cancer constitutes approximately 2% of all cancers and 90–95% are squamous cell carcinomas. Alcohol and tobacco abuse seem to be closely related to laryngeal cancer. Oncogenes are involved in a wide range of human tumours, including laryngeal tumours. Loss of heterozygosity in several chromosomal arms is also a common feature in these tumours, indicating involvement of putative tumour suppressor genes in the development of the disease [1].

Ras family genes (H-, K- and N-ras) encode for a 21-kDa (p21ras) protein which possesses GTPase activity and participates in a signal transduction pathway [2]. Activated ras oncogenes have been detected in several human cancers and this alteration has been associated with the development of the disease [3]. Mutations in codons 12, 13 and 61 constitute one of the most frequent alterations in human cancer [3,4]. Overexpression of the ras genes has also been shown to cause a step towards malignant transformation [5]. The role of the ras genes in squamous cell carcinomas of the head and neck (SCCHN) has been investigated at the level of the RNA and protein expression, as well as by mutational analysis [6–8]. The rate of ras gene mutations has been reported as 4–14% in head and neck carcinomas but in SCCHN it is less than 5% in the Western world [8–13]. A high frequency of ras gene mutations has been found in tumours diagnosed in India, probably related to the action of specific exogenous factors [14,15].

Another important feature in head and neck tumour cells is loss of heterozygosity (LOH) and microsatellite instability (MI) of a hexanucleotide repeat located in intron 1 of the H-ras gene, providing further evidence for the involvement of the H-ras gene in this disease [16]. LOH of H-ras gene in human tumours may indicate that this gene has a tumour suppressor role, as suggested previously [17,18], since normal H-ras suppresses the malignant phenotype of transformed cell lines, acting as a tumour suppressor gene.

In the present study, we analyzed 41 laryngeal cytological specimens for mutations in codon 12 of H-, K- and N-ras genes. We employed a non-radioactive polymerase chain reaction (PCR)-based method for our
analysis. Only two point mutations in K-ras and none in the H- and N-ras genes were detected, suggesting that ras mutations are a relatively rare phenomenon in laryngeal tumours.

2. Materials and methods

2.1 Tumour specimens and DNA extraction

Forty-one laryngeal cytological specimens were obtained from the General Hospital Nikias, Piraeus, Greece, and from the Otorhinolaryngology Department of the University Hospital Heraklion, Crete, Greece (22 squamous cell carcinomas of the larynx and 19 benign neoplasias). The biological material was obtained through direct laryngoscopy, by a brush. Part of the material was smeared on slides and fixed by alcohol for Papanicolaou stain and conventional cytological diagnosis. The remaining part was rinsed in normal saline and was stored at −70°C for DNA extraction. DNA was extracted and stored at 4°C until PCR amplification.

2.2 DNA extraction, oligonucleotide primers and PCR amplification

DNA was extracted according to standard protocols using organic detergents. The oligonucleotides used for K- and H-ras codon 12 and N-ras [19] have been previously described. 200 ng of the extracted DNA of each sample was amplified in a volume of 50 μl containing 200 mM Tris–HCl, pH 8.4, 500 mM KCl, 1.5 mM MgCl₂, 150–200 μM of each dNTP, 0.5 μM of each primer and 1.25 U Taq DNA polymerase. The mixture was heated for 1 min at 95°C and samples were subjected to 35 cycles of amplification at 94°C for 55 s, 58°C for 45 s and 72°C for 45 s (K-ras); 94°C for 55 s, 54°C for 45 s and 72°C for 30 s (N-ras); 94°C for 55 s, 61°C for 45 s and 72°C for 45 s (H-ras). PCR products were analysed on a 2% agarose gel and were photographed on a UV light transilluminator.

2.3 RFLP analysis: K-ras, N-ras

Aliquots (10–40 μl) of the amplification products were digested for 16 h with 30 U BstNI in conditions recommended by the supplier.

2.4 H-ras

Aliquots (10–40 μl) of the amplification products were digested for 16 h with 30 U Msp I in conditions recommended by the supplier.

RFLP products were analysed on an 8% polyacrylamide gel and silver stained.

3. Results

Among the 41 cytological laryngeal specimens analysed, only two were found to carry a ras mutation, in codon 12 of K-ras. (Table 1). Representative examples of specimens carrying a K-ras mutation are shown in Fig. 1. The K-ras mutations were detected in one benign and one malignant lesion of the larynx. No mutations were found in codon 12 of H- or N-ras. These results are in agreement with previous studies, which show less than 5% ras mutations in SCCHN in the Western world [8–13]. As the number of samples carrying K-ras codon 12-point mutations was limited, no analysis with the clinicopathological parameters was undertaken.

4. Discussion

Genetic alterations in oncogenes [20] and tumour-suppressor genes have been implicated in the initiation, promotion and progression of cancer. The ras family of genes (H-, K- and N-ras) encodes a 21-kDa membrane protein (p21ras) which possesses GTPase activity and participates in a signal transmission pathway [2,3,21]. Hot spots for ras mutations are found in codons 12, 13 and 61, causing the mutant protein to lose its activity to exchange GTP with GDP, which is related to the promotion of the laryngeal cancer. Head and neck cancers are the sixth commonest cancers in the world but have a
wide geographical variation which is most likely due to specific environmental risk factors [22]. This is reflected in the different incidence of ras mutations in the Western world compared with Southeast Asia and India.

In the Western world, ras mutations in SCCHN are very rare (<5%), whereas in India 35% of SCCHN patients harbour a mutation in H-ras, and this has been associated with tobacco chewing. In Taiwan, 18% of oral cancer patients investigated were found to have a K-ras mutation and these patients chew betel quid but do not use tobacco [14,15]. In this study, 41 laryngeal cytological specimens were analysed for the detection of point mutations in codon 12 of K-, H- and N-ras, but only two of these specimens contained ras mutations, both of which were found in codon 12 of K-ras (4.8%). The results confirm that ras mutations in SCCHN are rare in the Western world.

This is the first study to our knowledge, investigating the incidence of ras gene mutations in cytological laryngeal specimens. Laryngeal cytological specimens are relatively easy to obtain and molecular analysis of the samples may prove to be useful for the early detection of laryngeal lesions. Previous investigation had revealed the existence of genetic alterations, at the level of microsatellite DNA, in both benign and malignant laryngeal tumours [1]. We provide evidence that codon 12-point mutations in the ras family genes is not a common event in this type of lesion using cytological specimens, although the activation of ras genes is well established in solid SCCHN tumours [7–9]. The main activating mechanism of ras genes seems to be the aberrant expression of all three members of ras family genes [7,8]. Abnormal expression of ras genes may be attributed either to mutations, not in the coding regions but within the promoters of these genes, or to imbalance of chromosomes carrying these genes, resulting in the gene amplification [23–25]. More studies are required, including a large number of samples, for the detection of hot spots for mutations within the non-coding regions of the ras family genes, providing clues for their role in the development of laryngeal lesions.

References