A Common 9 bp Deletion in the Ataxia-telangiectasia-mutated Gene Is Not Associated with Oral Cancer

SOFIA SPYRIDONIDOU[1], CHRISTOS YAPIJAKIS[1], EMEKA NKENKE[2], TAKESHI TOYOSHIMA[2], ANTONIS VYLLIOTIS[1], ZOE SEREFOGLOU[1], FRIEDRICH W. NEUKAM[2], EFSTRATIOS PATSOURIS[3] and ELEFTHERIOS VAIRAKTARIS[1]

[1]Department of Oral and Maxillofacial Surgery, and [3]Department of Pathology, University of Athens Medical School, Athens 11521, Greece;
[2]Department of Oral and Maxillofacial Surgery, Universitat Erlangen, Klinik und Poliklinik fur Mund-, Kiefer-, Gesischtschirurgie, Erlangen D-91054, Nurnberg, Germany

Abstract. *Background: The ataxia-telangiectasia-mutated gene (ATM) product is a well-characterized tumour suppressor that plays a key role in the maintenance of genomic stability. Given the fact that the loss of heterozygosity at the ATM locus is common in head and neck tumours, we investigated the possible association of 7636del9, which is the most frequent ATM deletion, with risk for oral cancer. Patients and Methods: The 7636del9 9nt deletion was investigated in DNA samples of 67 German and Greek patients with oral cancer and 57 healthy controls of equivalent ethnicity, age and gender, by polymerase chain reaction (PCR) followed by electrophoretic analysis. Results: The anticipated deleted sequence was not detected in any of the DNA samples of oral cancer patients or controls. Conclusion: The findings of the present study indicated no association of the most common mutation in the ATM gene with risk for oral cancer.*

Oral squamous cell carcinoma (OSCC), the most common form of oral cancer, is a major cause of cancer morbidity and mortality worldwide since it is characterized by poor survival despite the development of new therapeutic approaches (1). It is widely recognized that oral carcinogenesis is a multistep process in which many factors (such as smoking and alcohol abuse) are implicated, but is also characterized by the progressive accumulation of genomic aberrations. Such genetic changes may ultimately lead to a selective growth advantage and drive tumour formation and progression by up-regulating oncogenes and/or down-regulating tumour suppressor genes (2).

Ataxia-telangiectasia-mutated (ATM) is a protein kinase which functions as a tumour suppressor by triggering appropriate cellular response to genome damage resulting from chemical carcinogen exposure or ionizing radiation (3). Exposure to ionizing radiation does not alter levels of ATM, but increases its kinase activity several fold (4-7).

Inactivation of the *ATM* gene seems to be a frequent event in the development of certain common types of cancer (8-17). Mutations in the *ATM* gene have been frequently found in patients with T-cell and B-cell leukaemias (11-16), while loss of heterozygosity of the *ATM* locus at 11q22-23 is a common event in OSCC and other cancer types (8-10, 17). Among patients with an abnormal *ATM* gene, the most frequently observed mutational change is a 9 bp deletion (7636del9) involving codons 2546-2548 in exon 54 (18-21). Although this deletion involves about 8% of patients with abnormal *ATM*, there have been no reports of its investigation in OSCC samples. Therefore, in light of the frequent loss of heterozygosity at the *ATM* locus in oral carcinomas, we investigated the possible association of this common deletion with risk for oral cancer.

Patients and Methods

The case group consisted of 67 German and Greek patients, who were diagnosed with OSCC, based on histological confirmation and operated between 1 September 1996 and 30 June 2006 at the University Departments of Oral and Maxillofacial Surgery in Athens, Greece and in Nurnberg, Germany, and 57 healthy blood donors of equivalent ethnicity, sex and age. For each patient, a family history regarding any type of cancer and thrombophilia was obtained. DNA was isolated from blood samples of patients and healthy controls using the NucleoSpin™ kit (Macherey-Nagel

Correspondence to: Professor Dr. Eleftherios Vairaktaris, MD, DDS, Ph.D., Department of Oral and Maxillofacial Surgery, University of Athens Medical School, Kifissias and Perikleous Stavrou 9, Athens 11521, Greece, Tel: +30 2106443035, Fax: +30 2106443803, e-mail: lvairakt@med.uoa.gr

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GmbH & Co, Düren, Germany). Molecular detection of the 9 bp deletion was performed by restriction fragment length polymorphism typing and agarose gel electrophoretic analysis as described elsewhere (22, 23). PCR amplification was effected using the following primers: Forward: 5'-GTTAAGCAAAATGAAAA ATATGC-3' and reverse: 5'-GGAAAGACTGAATATCACACTTC-3' (VBC Genomics, Vienna, Austria). The generated PCR product of 284 bp was digested with restriction enzyme Xba I (Takara Bio Inc., Seta 3-4-1, Otsu, Shiga, 520-2193, Japan), resulting into two fragments of 204 and 80 bp when a normal undeleted allele is present. A mutant allele, having lost the 9 bp sequence 5'-TCTAGAATT-3', is not cleaved by the enzyme and a band of 275 bp is visible after gel electrophoresis. Some individuals were studied twice in order to verify the results obtained.

Results

The 9 bp deletion (7636del9) in the *ATM* gene was not detected in any of the 124 DNA samples studied. All oral cancer patients as well as all healthy controls were homozygotes for the normal undeleted allele. Therefore, it is concluded that the 9 bp deletion is not a predisposing factor associated with risk for oral cancer.

Discussion

In light of the fact that the loss of heterozygosity at the *ATM* locus is common in head and neck tumours, we investigated the possible association of 7636del9, which is the most frequent mutation reported in the *ATM* gene (8%), with risk for oral cancer. The obtained data did not support such an association.

Despite the small number of OSCC patients studied here (N=67), the 7636del9 mutation was previously detected in one patient in a cohort of 68 unrelated familial breast cancer patients without ataxia-telangiectasia (A-T) (17). The frequency of the abnormal *ATM* alleles in Caucasians has been suggested to be 0.2-1.0%, with 0.5% being the best estimate (12). If there is no significantly increased contribution of *ATM* mutations in cancer patients compared to the general population, one would expect 0.34 mutant *ATM* carriers among 67 individuals with cancer, assuming that the average population prevalence of the disease allele is 0.5%.

Alternatively, the frequent 7636del9 mutation may have severe deleterious effects and its carriers may not survive beyond the second decade, while our cohort and that of the breast cancer study (12) involved patients with more advanced ages (>30 years). In support of this notion, the heterozygotic presence of 7636del9 in A-T patients has been associated with the poorest life expectancy among all carriers of *ATM* mutations, resulting in a mean survival of 19.2±2.2 years (24). This mutation leads to an in-frame deletion of codons 2546-2548, resulting in the omission of 3 amino acids in a region upstream from the kinase domain of the ATM protein (25). Although the ATM protein is synthesized to almost its full length, this deletion destabilizes and inactivates it, possibly through interference with its spatial conformation and/or its capacity to bind to other factors (26, 27).

However, due to the absence of data on the disease allele frequency in various age groups of the studied populations, these results should be considered preliminary. The possibility that mutant *ATM* homozygotes might not have been ascertained completely still exists, leading to an underestimation of the population frequency of abnormal *ATM* alleles. In addition, there may be an increased frequency of carriers with subtle mutations in the *ATM* gene that do not necessarily result in the major clinical features of A-T, but still predispose for neoplasia through interference with cellular functions of ATM protein. This notion would be supported by the existence of variant forms of A-T as well as the low consanguinity rate observed in A-T families. Much larger studies and pooled analyses of mutation screening will be required to establish whether the susceptibility to any type of cancer is conferred by mutant *ATM* alleles.

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