

Genetic and epigenetic alterations of the blood group ABO gene in oral potentially malignant lesions and squamous cell carcinoma

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The PhD dissertation was accepted by the Faculty of Health Sciences of the University of Copenhagen, and defended on May 19, 2004.

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Dan Med Bull 2004;51:238.

ABSTRACT

This work was carried out at the Department of Oral Diagnostics, School of Dentistry, University of Copenhagen, Denmark, from 2001 to 2003.

Loss of A/B antigen expression in oral carcinomas is a frequent event that is due to lack of the A and B gene-encoded glycosyltransferase activity. The aim of the present study is therefore to study the genetic and epigenetic alterations in the *ABO* locus in oral carcinoma and potentially malignant lesions, in order to understand better the molecular mechanisms leading to loss of A/B antigen expression. Seventythree formalin-fixed, paraffin-embedded samples and 25 frozen samples that expressed A/B antigen in normal epithelium were investigated by immunohistochemical (IHC) staining and genotype analysis. The samples included 17 leukoplakias without dysplasia, 24 dysplasia, and 57 squamous cell carcinomas (25 frozen and 32 paraffin-embedded samples). Both tumor and normal cells were collected by laser microdissection. DNA was extracted and analysed by PCR coupled with restricted digestion analysis in order to establish the *ABO* genotype. Total and patchy loss of A/B antigen expression was frequent in carcinomas (79%) and in potentially malignant lesions (54%). The paraffin-embedded tissues were genotyped and were shown that 8/24 (33.3%) oral carcinomas and 3/22 (13.6%) potentially malignant lesions had a specific *ABO* allelic loss related to loss of A/B antigen expression. There was no difference between results obtained from formalin-fixed and frozen carcinomas. O allele loss was found in 13 cases involving all four groups of lesions. Thirty frozen samples of oral squamous cell carcinomas were investigated for A/B glycosyltransferase expression by IHC staining, LOH by microsatellite analysis, and methylation status analysis by methylation-specific PCR (MS-PCR) and fluorescence melting curve analysis (MS-MCA). In unfixed frozen tumor samples loss of A/B antigen expression was consistently linked to loss of A/B glycosyltransferases expression. LOH at 9q34 was found in 7/27 cases (26%), and one case showed microsatellite instability (MSI). Hypermethylation of the *ABO* proximal promoter was shown in 10/30 tumors (33.3%), and also found in hyperplastic or dysplastic tissue adjacent to the tumors, suggesting that the hypermethylation is an early event in oral carcinoma. Both LOH and the hypermethylation were associated with negative staining of A antigen except for one case with LOH. No correlation was found between hypermethylation of the distal *ABO* promoter and loss of antigen expres-

sion. Hypermethylation of the death-associated protein kinase gene (*DAPK1*) at 9q34 was found in 5/30 tumors (16.7%), but there was no association between *ABO* and *DAPK1*-methylation. Collectively, we have identified genetic and epigenetic events, including hypermethylation of the *ABO* gene proximal promoter, LOH at 9q34 region and specific allelic alteration, that may account for loss of A/B antigen expression in 66.7% (14/21) of frozen samples of oral carcinomas. In order to explain the loss of A/B antigen expression in the other oral carcinoma patients, information is needed on negative regulatory factor(s) and alternative promoters that control the activity of glycosyltransferases in normal and tumor tissues.