

## Loss of A, B, and H antigens in oral cancer

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### **PÉRDIDA DE LA EXPRESIÓN DE LOS ANTÍGENOS ABH EN CÁNCERES ORALES**

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#### RESUMEN

Los antígenos ABH, productos de la interacción de dos sistemas genéticos Hh y ABO, están sujetos a leyes de herencia y pueden estar localizados no sólo en los eritrocitos sino también en la mayoría de las células humanas.

El objetivo del este trabajo fue investigar la expresión de antígenos ABH en pacientes con lesiones orales pre-malignas y malignas orales.

Se trabajó con muestras incluidas en tacos de parafina de pacientes con lesiones orales. Los pacientes fueron clasificados en 2 grupos: a) Pacientes con lesiones premalignas y malignas diagnosticadas clínica y anatopatológicamente y b) pacientes con lesiones benignas. Se investigaron los antígenos ABH por la técnica de inmunoadherencia específica modificada. Se utilizó la adherencia al tejido vascular como control positivo y al tejido adiposo como control negativo. Los resultados fueron valorados de forma semicuantitativa desde adherencia fuertemente positiva a negativa. Hemos encontrado una significativa relación entre la expresión antigénica ABH y el grado de malignidad de las lesiones analizadas ( $P_{\text{Yates}} = 0.005$ ). Una pérdida de reactividad ABH en los sitios de mayor invasividad tumoral se correlaciona con el grado del desarrollo del tumor, el grado histológico y su malignidad.

**PALABRAS CLAVE:** Antígenos de grupo sanguíneo ABO / Pre-cáncer oral / Cáncer oral.

#### ABSTRACT

In most human carcinomas, including oral carcinoma, a significant event is decreased expression of histo-blood-group antigens A, B and H. The mechanisms of aberrant expression of blood-group antigens are not clear in all cases. The aim of this work was to investigate the association of ABO blood groups with oral cancer. We studied the expression of ABH antigen in tissues of premalignant lesions and in diagnosed malignant tumors. In total, 132 patients were examined, half of whom suffered from oral pre-cancerous and cancerous lesions, while the other half were the control group (benign lesions). All tumors were histologically confirmed. We found a significant relationship between antigen expression and the malignancy of the lesions analyzed ( $P_{\text{Yates}} = 0.005$ ). A loss of ABH reactivity within the most invasive sites of the tumors correlated significantly with the stage of tumor development and histological grade of malignancy.

These findings support the view that features regarding the cells of deeper parts of the carcinomas are very important for the clinical behavior of the tumors and that loss of ABH-antigen expression is linked to the stage of the tumor and invasion of carcinoma cells.

**KEY WORDS:** ABO blood-group antigens / Oral precancer / Oral cancer.

## INTRODUCTION

Histo-blood group ABH antigens are major alloantigens in humans. These antigens are widely distributed in human tissues and undergo changes in expression during cellular differentiation and malignant development<sup>(1)</sup>.

The A, B, and H antigens are complex carbohydrate structures found on glycoproteins and glycolipids present on the surface of erythrocytes, endothelial cells, and on most epithelial cells. Alleles of the *ABO* gene code for glycosyltransferases that act on the precursor H antigen<sup>(2)</sup>. In red blood cells (RBC), the H antigen is determined by a fucosyltransferase coded for by the *FUT1* gene. If the H antigen is absent, there is no substrate for the ABO glycosyltransferases to modify. Homozygous deficiency of *FUT1* gives rise to the Bombay phenotype where there is no A or B antigens on the red cell due to lack of the H precursor.

Alteration of ABH antigens in hematologic malignancy was first reported by van Loghem et al, who described very weak A antigen expression on the red cells of a patient with acute myeloid leukemia who had previously shown normal A antigen expression. Loss of A, B, or H antigens from the surface of red blood cells is now recognized as a recurrent observation in hematologic malignancy<sup>(3,4)</sup>.

Disappearance of the antigens is ascribed to the absence of A or B transferase gene expression. In patients with loss of ABH antigens, a varying proportion of red blood cells do not agglutinate, giving a characteristic mixed-field reaction. Mixed-field reactions can also occur in healthy individuals where the reactions are associated with rare alleles of the *ABO* gene such as A and B<sup>(5)</sup>.

Tumor progression is often associated with altered glycosylation of the cell-surface proteins and lipids. The peripheral part of these cell-surface glycoconjugates often carries carbohydrate structures related to the ABO and Lewis blood-group antigens. The expression of histo-blood-group antigens in normal human tissues is dependent on the type of differentiation of the epithelium. In most human carcinomas, including oral carcinoma, a significant event is decreased expression of histo-blood-group antigens A and B. The mechanisms of aberrant expression of blood-group antigens are not clear in all cases. A relative down-regulation of the glycosyltransferase that is involved in the biosynthesis of A and B antigens is seen in oral carcinomas in association with tumor development<sup>(4,6)</sup>. However, several recent studies have shown that altered glycosylation plays a major role in most aspects of the malignant phenotype, including signal transduction and apoptosis.

Studies of associations between various cancers and the ABO blood groups have shown elevated relative risks for some categories of disease<sup>(7,8)</sup>.

To investigate the association of expression of ABH antigens and oral cancer, we conducted a study of premalignant lesions and diagnosed malignant tumors.

## MATERIALS AND METHODS

The patients analyzed in this study presented to the Stomatology Department of The Odontology Faculty of the National University of Rosario, Argentina during the period 2006-2007.

In total 132 subjects were examined, half of whom suffered from oral pre-cancerous and cancerous lesions, while the other half were the control group (benign lesions). All of them were subjected to clinical oral examinations. In the group of patients with oral pre-cancerous and cancerous lesions (experimental group), a pathohistological examination of the oral mucosa was performed.

### Tissue preparation

All biopsies were fixed in 4% buffered formaldehyde, paraffin embedded, sectioned at 4  $\mu$ m, and stained with hematoxylin and eosin.

Sections (4  $\mu$ m) from the tumor biopsies were placed on gelatine-coated slides. Sections were deparaffinized in xylene and brought to water through graded ethanol (100%).

### Specific red cell adherence test

Specific red cell adherence test was performed on paraffin embedded sections to detect the intensity of isoantigens A, B and H (O) on the epithelial cell surface by a three layer sandwich technique, as described in<sup>(9,10)</sup>. Commercially available Anti A, Anti B, and Anti AB antisera from Span Diagnostic Limited and *Ulex europaeus* lectin (Anti H) were used. Slides of 4-5 micron section were deparaffinized and brought to water, immersed in Tris buffered saline 0.05 M (pH 7.4) for 30 minutes, covered with isologous antisera according to patients' blood group, and incubated for one hour with Anti-A, -B and -O antisera in a moist chamber at room temperature. The slides were then dipped in Tris buffered saline three times with occasional stirring to remove the unreacted antisera. A few drops of 2-5% isologous indicator RBC's suspension were added to the sections and incubated for 20 minutes in group A or B and one hour for group O. The slides were inverted over a support in a petridish containing Tris buffered saline such that the undersurface of the slide just touched the solution, and kept for five minutes to settle unreacted RBCs down. The slides were observed under low power magnification and photographed immediately.

Normal tissues containing blood group antigens, endothelium of blood vessels and RBCs acted as inbuilt

positive controls, and adipose tissues acted as inbuilt negative controls.

### Interpretation

In the present study the isoantigenicity of the epithelium was graded according to degree of adherence of indicator RBCs as strongly positive adherence (++++), to negative adherence (-). Intermediate levels were graded as + for 25% of adherence, ++ for 50% of adherence, and +++ for 75% of adherence.

### RESULTS

The immunoadherence reaction to tissue sections using antibodies and red blood cells showed a loss of A, B or H antigens related to the stage of tumor (Table I).

A loss of ABH reactivity within the most invasive sites of the tumors correlated significantly with the stage of tumor development and histological grade of malignancy.

In the tissue sections studied, the endothelium of blood vessels was reactive with the erythrocytes (positive control), and adipose tissues did not react with the red blood cells (negative controls).

Loss of A, B, and H antigens from the surface of red blood cells was observed in patients with oral malignancy (89.4%), while the other 10.6% conserved the ABH expression. 39.4 % of the benign lesions which were diagnosed anatomopathologically lost the antigenic reactivity.

### DISCUSSION

Blood-group antigens can be present on key receptors controlling cell proliferation, adhesion, and motility, such as epidermal growth factor receptor, integrins, cadherins, and CD44<sup>(7)</sup>. The expression patterns of these various receptors differ according to the type of normal epithelium and the type of cancer, and therefore the role of ABH antigens in the biology of human cancers may also vary. The function of the expression of ABO antigens in normal stratified oral epithelium is unclear

In routine diagnostic histopathology, classification of tumor type is based on the histologic appearance of the most differentiated parts of the tumor. The prognosis of the tumor, on the other hand, is based partly on properties within the less differentiated parts. In most cases, the degree of differentiation is determined by cellular and tissue morphology and by the ability of the cells to synthesize certain specific products such as keratin and mucins. It has previously been demonstrated that the expression of cell surface carbohydrates in oral stratified epithelium is related

**TABLE I.** Expression of the ABH antigens in fixed tissue sections of oral lesions

	Precancerous Cancerous lesions	Benign lesions
Partial or total deletion	59	26
Antigenic conservation	7	40
<i>P</i> Yates = 0.005		

to cell differentiation<sup>(11,12)</sup>. In tumors, changes in glycosylation are found in both glycolipids and glycoproteins<sup>(6)</sup>. Most studies have dealt with alteration of carbohydrates at the cell surface.

In the present work, therefore, we used the loss of the expression of ABH antigens as a marker of differentiation. As the expression of these antigens can be detected by monoclonal antibodies, they are a better objective marker of differentiation than the more commonly used subjective histologic assessment. The presence or absence of blood-group antigens have been used to predict the clinical course of patients with superficial transitional cell carcinoma of the bladder<sup>(13,14)</sup>. The red-cell adherence test has been the most widely accepted method of antigen determination, but this technique has inherent weaknesses. Recently, the immunoperoxidase assay has been used to detect antigens on tumor cells. We compared patients using the red-cell adherence and immunoperoxidase methods on adjacent micro cut sections. The red-cell adherence and immunoperoxidase methods performed similarly (89%) when assessing the presence or absence of antigen<sup>(15)</sup>.

Immunohistochemical studies of oral squamous cell carcinomas have shown loss of expression of A or B antigens in more than 80% of cases, all of which showed concomitant loss of A/B transferase<sup>(16,17)</sup>. Studies of potentially malignant lesions have shown loss of A/B antigen in most lesions with epithelial dysplasia and in half of the lesions clinically. In the normal oral cavity, keratinized epithelium in the palate or gingiva shows little or no expression of A or B blood-group antigen. Since a change from a non-keratinized to a keratinized differentiation pattern is a characteristic of many oral carcinomas and potentially malignant lesions, the lack of expression of blood-group antigens in such lesions could be due to a change in the differentiation pattern of the epithelium<sup>(19,20)</sup>. However, it has been demonstrated that half of the leukoplakias that developed in buccal mucosa show expression of A antigen, even though they histologically appear as keratinized lesions<sup>(18)</sup>. Similar A expression was found in mechanically induced hyperkeratinized lesions

of buccal mucosa<sup>(20)</sup>. These findings indicate that loss of antigen is not invariably associated with hyperkeratinization.

It is generally accepted that tumors are composed of heterogeneous cell populations with different biological behaviors. To obtain optimal prognostic information about the tumor, therefore, the entire tumor cell population should be studied. Despite the somewhat nonrepresentative nature of the biopsy material, it was possible to show that loss of ABH antigens was associated with the spread of tumor (stage). This could be of diagnostic and prognostic value. Similar loss of ABH antigens in bladder cancers was associated with a poor prognosis in some studies<sup>(13-15)</sup>.

Most investigations concerning the tissue localization of the histo-blood-group antigens have shown that the antigens in the tissues correspond to the erythrocyte blood group<sup>(2,16)</sup>. Furthermore, the expression of histo-blood-group antigens in normal human tissues is dependent on the type of differentiation of the epithelium and the degree of maturation of the single cell within the epithelium. In stratified epithelium, the expression of histo-blood-group antigens depends on the state of cellular differentiation (maturation), and there is a sequential elongation of the terminal carbohydrate chain during the life span of the cell. Basal cells express short carbohydrate chains that are A/B precursors, whereas A, B or H antigens may be seen in the spinous cell layer.

The results obtained in this work have demonstrated that the patients examined showing benign lesions expressed the ABH antigens in the tissues analyzed but there were significant differences in the experimental group. We also found a higher intensity of oral disease in the group with total ABH deletion, and the occurrence of epithelial dysplasia was most frequently found in this group.

In a previous work, we examined the differences in the saliva secretor status by comparing patients with oral pre-cancerous and cancerous lesions on the one hand, and the healthy population on the other, in relation to the expression of ABH antigens in fixed tissue sections of these patients. The results obtained demonstrated that most people examined in the healthy group were secretor, and there was a significant difference between secretors and non-secretors in the experimental group. We also found a higher intensity of oral disease in the non-secretor group, and the occurrence of epithelial dysplasia was mostly found in the non-secretor group<sup>(21)</sup>.

The studies of patients with pre malignant and malignant oral lesions in which non-secretor status predominates, indicates it as a risk marker for the development of oral cancer.

We also observed a reduction or complete deletion of A, B or H antigen expression in sections of tissues of patients

with oral pre carcinomas or carcinomas. Disappearance of the antigens is ascribed to the absence of A or B transferase gene expression. These findings are consistent with several studies showing that loss of A and B antigen expression is associated with increased tumorigenicity in syngenic animals.

In summary, our results indicate that at the same time as the morphological changes that occur during the process of oral carcinogenesis, another series of events occurs. Further follow-up studies are required to clarify the role of predictive markers of risk in precursor lesions of oral cancer.

However, as it is generally accepted that cancer cells must undergo a whole series of changes to become metastatic, it is remarkable that the degree of expression of a single carbohydrate structure was significantly correlated with an aggressive clinical behaviour of the tumor. It is therefore possible that further prognostic information can be obtained by detecting a group of other related carbohydrate structures at the cancer cell membranes.

## DISCLOSURES

The authors declare no financial conflicts of interest.

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