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# Cytokeratin profile in mucoepidermoid carcinoma is not related to its histological grading of malignancy

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**Abstract**

The objective of this experiment was to determine the relationship between the expression of cytokeratins (CKs) and histologic grading in MEC. Eleven cases of MEC were selected and graded as low, intermediate and high-grade tumors. The expression of CKs 7, 8, 10, 13 and 14 was assessed immunohistochemically using streptavidin–biotin complex method. The results showed that the studied CKs were expressed in most cases of MEC, independently of histologic grading. Nonetheless, low-grade tumors demonstrated intense staining of CK 7 and 8; additionally, CK 10 and 13 were more pronounced in this grade. The immunoexpression was variable according to cellular type and organization pattern of the tumor. Mucous cells were positive for CK 7 and 8; epidermoid cells were stained for CK 10, 13 and 14; CK 7, 8, 10 and 14 were observed in intermediate cells, and CK 7 was occasionally seen in clear cells. Cystic structures and duct-like elements in MEC were positive for CK 7 and 8, whereas solid nests showed positivity for all CKs. These results suggest that expression profile of these proteins does not reflect the biological behavior of MCE, however, it guides the detection of cellular types and differential diagnosis from other salivary gland tumors.


*Keywords:* Salivary gland tumor; Mucoepidermoid carcinoma; Cytokeratin; Immunohistochemistry

## Introduction

Mucoepidermoid carcinoma (MEC) is the most common malignant tumor of all carcinomas developing in the major and minor salivary glands (Ostman et al., 1997; Figueiredo et al., 2001; Kokemuller et al., 2005). This neoplasm was first recognized by Masson and Berger in 1924 (Prgoment et al., 2003). Since its first description, several studies have attempted to identify the MEC well-known variable biological behavior. This neoplasm can be highly aggressive; however, it shows sometimes a slow growth resembling a benign lesion.

Some factors can be related to biological behavior and patient's outcome in MEC such as anatomical site, clinical stage, invasion of adjacent structures, presence of distant metastasis (Good et al., 1998; Pires et al., 2004; Kokemuller et al., 2005) and histologic grading of malignancy (Guzzo et al., 2002; Spight and Barret, 2002; Perez-Ordonez, 2003).

Histologically, MEC is composed of variable cell types including mucous, epidermoid and intermediate cells that can be arranged in solid nests or cystic structures (Dardick, 1996). Not only the proportion and organization of these cells but also the degree of atypia and number of mitosis serve as criteria for histologic grading (low, intermediate and high grade), which is correlated with poor or good clinical outcome of this tumor (Ellis and Auclair, 1996).

The great structural and cellular diversity of MEC is responsible for intense discussion concerning its histogenesis. The role of several immunohistochemical markers has been studied in order to reach a better understanding about the factors related to histogenesis and prognosis in MEC.

Cytokeratins (CKs) constitute the major component of the cytoskeleton of all epithelia; the use of these intermediate

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Table 1
Primary antibodies specifications

| Antibody specificity [a] | Clone | Dilution | Incubation period (min) | Retrieval |
|---|---|---|---|---|
| CK 7 | OV-TL12/30 | 1:50 | 60′ | Citrate buffer 29′ |
| CK 8 | TS-1 | 1:50 | 60′ | Citrate buffer 29′ |
| CK 10 | LHP1 | 1:50 | 60′ | Trypsin 0.1% 39′ |
| CK 13 | KS-1$^{A}$3 | 1:150 | 60′ | Citrate buffer 29′ |
| CK 14 | LL002 | 1:20 | 60′ | Citrate buffer 29′ |

[a] Novocastra Laboratories.

filaments in pathology has been assessed in the diagnosis of undifferentiated neoplasms and determination of tumoral subtype (Chu and Weiss, 2000; Upsani et al., 2004). Indeed, there has been demonstrated an association between CKs expression with biological behavior and tumor differentiation in oral squamous cell carcinoma. Moreover, the study of these proteins has clarified relevant aspects regarding the histogenesis of several salivary gland tumors.

Few studies have been carried out to verify CKs profile in MEC and the relationship with its differentiation. Thus, the goal of the present experiment was to investigate CKs expression and the relation with histologic grading in MEC in order to provide data for a better understanding of tumor biological behavior. Furthermore, aspects related to MEC histogenesis were analyzed by comparing CKs pattern in normal salivary gland adjacent to the tumor.

## Materials and methods

A total of 11 cases of MEC were retrieved from the files of Oral Pathology Service, School of Dentistry, Federal University of Rio Grande do Norte. The diagnosis of MEC was confirmed by two oral pathologists based on light microscope of histologically stained slides. Hematoxylin/eosin-stained specimens were examined under a light microscope by two pathologists, and tumors were graded according to the criteria reported by Hicks et al. (1995) into low, intermediate and high grade. Specimens from the paraffin blocks were cut into 3 μm and mounted on glass silanesed microscope slides previously cleaned (3-aminopropyltriethoxysilane, Sigma Chemical, Carpenter, USA). The expression of CKs was assessed immunohistochemically using streptavidin–biotin complex method. Origin and type of monoclonal antibodies, clone, antigen retrieval treatment, incubation period and dilution are described on Table 1. Adjacent normal salivary gland tissue was used for comparative parameter. Immunostained slides were analyzed on light microscope, and each CK expression was scored according to its degree of intensity such as strongly positive (++), positive (+) or negative (−). Then, the description of the stained cells was performed. Finally, immunoprofile exhibited by the lesions in each histologic subtype was taken into consideration, and a comparison between the groups was realized.

Table 2
CKs immunoexpression according to MEC histologic grading of malignancy

| MEC grade | CK 7 | CK 8 | CK 10 | CK 13 | CK 14 |
|---|---|---|---|---|---|
| Low | ++ | ++ | ++/− | ++ | + |
| Intermediate | + | ++/− | +/− | +/− | ++ |
| High | +/− | +/− | +/− | +/− | + |

++ = strongly positive; + = positive; − = negative; +/− = cases positive and negatives.

Table 3
CKs immunoexpression according to MEC cellular type

| Cellular type | CK 7 | CK 8 | CK 10 | CK 13 | CK 14 |
|---|---|---|---|---|---|
| Mucous | ++ | ++ | − | − | − |
| Epidermoid | +/− | − | ++/− | +/− | + |
| Intermediate | +/− | +/− | +/− | − | + |
| Clear | +/− | − | − | − | − |

++ = strongly positive; + = positive; − = negative; +/− = positive and negatives.

## Results

### *Clinicopathologic features of patients*

In the present study, 8 patients diagnosed with MEC were female, and three were male. The median age was 50 years (range 9–85). Seven tumors were from minor salivary gland and 4 cases from major salivary gland.

### *Histologic grading of malignancy*

After histologic grading, the cases of MEC were distributed as follows: 4 low-grade tumors due to the presence of numerous cystic structures and mucous cells; 4 high-grade tumors inasmuch as the significant detection of solid nests tumor and squamous cells; finally, 3 intermediate-grade tumors because the appearance revealed features between low- and high-grade lesions.

### *Immunohistochemical*

Immunohistochemical results of CKs profile in relation to histologic grading, cellular type and tumor organization are listed on Tables 2, 3 and 4, respectively.

In general, most cases of MEC were immunopositive for CK 7, 8, 10, 13 and 14 independently of histologic grading. Nevertheless, CK 7 and 8 were highly expressed in low-grade tumors, especially in the cystic structures, mucous and intermediate cells. Furthermore, CK 10 and 13 were more pronounced in this grade.

CKs immunoexpression was variable according to cellular type and pattern of organization in MEC. Mucous cells were positive for CK 7 and 8; epidermoid cells were stained for CK 10, 13 and 14; CK 7, 8, 10 and 14 were observed in intermediate cells, and CK 7 was occasionally seen in clear cells. Cystic structures and duct-like elements in MEC were positive for CK

Table 4
CKs immunoexpression according to pattern of organization in MEC

| Organization pattern | CK 7 | CK 8 | CK 10 | CK 13 | CK 14 |
|---|---|---|---|---|---|
| Cystic structures | ++ | + | − | − | − |
| Solid nests | +/− | +/− | ++/− | + | ++ |
| Duct-like elements | ++ | ++ | − | + | +/− |

++ = strongly positive; + = positive; − = negative; +/− = cases positive and negatives.