Death-Associated Protein Kinase is Underexpressed in High-Grade Oral Squamous Cell Carcinoma

La Expresión de la Proteína Quinasa Asociada a Muerte Celular (DAP Quinasa) está Reducida en Carcinoma de Células Escamosas Oral de Alto Grado

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SUMMARY: An immunohistochemical analysis of 40 cases of oral squamous cell carcinoma was performed to evaluate the relationship between the expression pattern of death-associated protein kinase (DAPk) positive cells with the histological malignancy grading of these lesions. According to our results, eleven cases (27.5%) were high-grade malignancy tumours and 29 (72.5%) were low-grade ones. We found that 92.86% of the low-grade tumours were positive to anti-DAP kinase antibody whereas only 7.14% of the high-grade tumours presented positivity, and this difference was statistically significant (p<0.01). Sixteen (55.2%) of the low-grade carcinomas exhibited moderate immunoreactivity whereas ten cases (34.5%) showed weak staining and three cases (10.3%) were negative tumours. Immunostaining was lacking in nine (81.8%) of the high-grade carcinomas and "weak" in the two (18.2%) remaining cases. Thus, DAPk expression is significantly decreased in high-grade oral carcinomas, and evidences indicate that it might be related to the severity of cytological atypia.

KEY WORDS: Immunohistochemistry; DAP kinase; Apoptosis; Squamous cell carcinoma.

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the most common malignant tumour occurring in the oral cavity. In the majority of the cases, it is diagnosed at an advanced stage and consequently present with a poor prognosis (Biazevic et al., 2006; Borges et al., 2008). In the last few years relevant advancements in the diagnosis and treatment of oral cancer has been observed. Nevertheless, despite this recent improvement, there are still many difficulties in evaluating the prognosis of OSCC. Thus, various studies attempting to establish the role of protooncogenes, antioncogenes and apoptosis-regulating genes in the tumoral progression have recently been performed in order to find more significant information to evaluate and predict the biological behavior of this neoplasm (Albuquerque et al., 2003).

The Death-associated protein kinase (DAP kinase) is a 160kDa cytoskeletal-associated calcium/calcmodulin-dependent serine/threonine kinase which plays an important role in programmed cell death (Chen et al., 2006; Zalckvar et al., 2009). Overexpression of DAP kinase has been demonstrated to induce apoptosis in various cell line (Yamamoto et al., 2002; Pelled et al., 2002; Pulling et al., 2004). On the other hand, research has demonstrated that DAP kinase protein expression is frequently reduced or absent in tumour cell lines (Raveh & Kimchi, 2001; Bai et al., 2004). In addition, low expression of this protein, particularly in response to hypermethilation, has been associated to high invasiveness or metastatic potential of malignat neoplastic cells (Simpson et al., 2002; Lehmann et
There are only few reports looking at the possible role of DAP kinase in the pathogenesis of oral or head and neck cancer (Rosas et al., 2001; Ogi et al., 2002). Thus the goal of this study is to investigate the relation between the immunohistochemical expression of DAP kinase and the histological grading of malignancy in squamous cell carcinoma of the oral mucosa.

MATERIAL AND METHOD

Forty cases of OSCC were retrieved from the files of the Oral Pathology Service of the School of Dentistry of the University Tiradentes (Aracaju, Brazil). The tumors were graded as high or low score according to the criteria of histological malignancy grading established by Bryne et al. (1989). For immunohistochemical reactions, 3mm thick sections were prepared from formalin-fixed paraffin-embedded surgical specimens. Immunohistochemical staining was carried out using the Labelling Streptoavidin Biotin method (LSAB) (Large Volume Detection System antipolyvalent, Dako, LSAB2 kit peroxidase, Dako Corporation, Capinteria, CA, USA). The sections were deparaffinized in toluene and submitted to antigen recovery by immersion of the slides in citrate buffer, 0.01M, pH 6.0 treated in microwave oven (three cycles for five minutes each at 650W). After rehydratation in phosphate buffered saline (PBS, pH 7.2), the sections were incubated with the primary antibody anti-DAP kinase (clone V55, Abgent, CA, USA, 1:50, 18h), followed by biotinylated antirabbit immunoglobulins (Dakopatts) and finally with streptoavidin – peroxidase conjugate (Detection Kit – peroxidase/DAB, Dakopatts). The last two incubations were performed for 30 min at room temperature. The reaction was revealed with 0.03% diaminobenzidine (Sigma) in buffered solution for 12 min and then the sections were counterstained with Meyer’s haematoxilin. Non-specific reactivity of the antibodies was checked by omitting the primary antibody.

Two observers evaluated all slides independently, and the immunostaining pattern of the antibody anti-DAP kinase was analyzed by light microscopy, considering the following parameters: (+++), when more than 50% of the tumour cells were labeled, (++), when 10 to 50% were positive; and (+) when less than 10% of positive cells could be seen. Carcinomas lacking immunostained cells were considered negative (-). After the immunohistochemical analysis, these data were correlated with the malignancy histological grading of the tumours. Significance was statistically determined using c2 statistics. A p-value < 0.05 was regarded as significant.

RESULTS

Morphological analysis showed that eleven cases (27.5%) were high-grade malignancy tumours and twenty-nine (72.5%) were low-grade malignancy tumors. Positive cytoplasmatic immunolabelling could be verified in twenty-eight cases (70.0%). Two (7.14%) of the twenty-eight DAP kinase-positive carcinomas were high-grade tumors whereas twenty-six (92.8%) were low-grade tumours. On the other hands, nine (75%) of the twelve DAP kinase-negative carcinomas were high-grade tumours and three of them low-grade lesions (Fig. 1).

Sixteen (55.2%) of the low-grade carcinomas exhibited moderate immunoreactivity whereas ten cases (34.5%) showed weak staining. Three cases (10.3%) were negative tumours. Immunostaining was lacking in nine (81.8%) of the high-grade carcinomas and weak in the two remaining cases (18.2%) (Table I, Fig. 2). Our results indicated an inverse relationship between DAP kinase immunoreactivity and the histological grading of tumoral malignancy (p<0.01). Positive staining was found in isolated cells or small cell groups within larger tumoral islands and sheets. Immunoreactivity showed to be more intense in cells adjacent to keratinization areas (diskeratotic cells and keratin pearls) but extensively absent in areas of severe cytological atypia.
DISCUSSION

Various studies have demonstrated that DAPk gene transcription is involved in apoptosis phenomenon and therefore may play a relevant role in suppressing oncogenic transformation (Raveh & Kimchi, 2001; González-Gómez et al.; Bai et al.). DAPk protein downregulates the expression of integrins, and consequently blocks cellular adhesion, thereby activating a particular pathway of apoptosis known as anoikis (Wang et al., 2002). Nevertheless, the capacity to promote apoptosis through DAPk pathway is frequently lost in anoikis-resistant cells, resulting in anchorage independence, a hallmark of malignant tumour cells closely associated to metastasis (Frisch & Screaton, 2001). Loss of
DAP kinase RNAm and protein expression was found in human cancer, often as a result of silencing by DNA hypermethylation (Lehmann et al.; Simpson et al.; Pulling et al.). In addition, DAPk appears to be underexpressed in metastatic cell lines (Lehmann et al.; Simpson et al.; Pulling et al.).

We investigated the relationship between the immunohistochemical expression of DAPk and the histological grading of malignancy in forty cases of squamous cell carcinoma of the oral mucosa. According to our results, the intensity of the immunohistochemical expression of DAPk is inversely proportional to the histological malignancy grading of oral squamous cell carcinoma. This theory is supported by the fact that immunoreactivity was more intense in cells adjacent to keratinization areas, hallmarks of well-differentiated tumors, but lacked in areas of severe cytological atypia. Likewise, loss of DAPk expression has been reported in a wide range of tumor types (Raveh & Kimchi; Lehmann et al.; Simpson et al.; Bai et al.). Surprisingly, three cases of low-grade tumors failed in expressing DAPk. However, it must be emphasized that all those tumors presented marked cytological atypia and minimum keratinization, despite they were classified as low-grade tumors. Thus, these findings seem to point at a link between the phenomenon of “dedifferentiation” and loss of DAPk expression in OSCC. Nevertheless, the mechanism responsible for silencing DAPk gene is not completely clear yet. It has been suggested that hypermethylation of CpG islands (segments of DNA within the gene promoter characterized by extensive repetition of residues of cytosine followed by residues of guanine) might suppress gene transcription (Lehmann et al.; González-Gómez et al.; Pulling et al.), but its role in the carcinogenesis of oral carcinoma is still unknown. In addition, the relationship between loss of DAK expression and worse overall survival prognosis was recently demonstrated in breast cancer (Lévy et al.) but further investigations are necessary to assess the role of DAPk in evaluating the survival rates and prognosis of carcinomas of the oral mucosa.

In conclusion, we have found that DAPk expression decreased significantly in high-grade oral carcinomas, which means that this expression might correlate with the histological malignancy grading of these tumors.

REFERENCES


