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Glypican-3 distinguishes aggressive from non-aggressive odontogenic tumors: a preliminary study

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BACKGROUND: Glypican-3 is a cell surface proteoglycan that is found in embrionary tissues, and there are no studies investigating this protein in odontogenic tumor. Thus, the aim of this study was to investigate glypican-3 in a series of aggressive and non-aggressive odontogenic tumors.

METHODS: Fifty-nine cases of tumors were divided into aggressive odontogenic tumors (20 solid ameloblastomas, four unicystic ameloblastoma, 28 KOTs including five associated with Gorlin–Goltz syndrome) and non-aggressive odontogenic tumors (five adenomatoid odontogenic tumors and two calcifying cystic odontogenic tumors) and analyzed for glypican-3 using immunohistochemistry.

RESULTS: Glypican-3 was observed in seven solid ameloblastoma and eighteen keratocystic odontogenic tumors including three of the five syndromic cases, but there was no significant difference between syndromic and sporadic cases ($P > 0.05$; Fisher's exact Test). All cases of unicystic ameloblastoma ($n = 4$), adenomatoid odontogenic tumor ($n = 5$), and calcifying cystic odontogenic tumor ($n = 2$) were negative.

CONCLUSIONS: This provided insights into the presence of glypican-3 in odontogenic tumors. This protein distinguished aggressive from non-aggressive odontogenic tumors.

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Introduction

Ameloblastomas and keratocystic odontogenic tumors (KOTs) are benign tumors derived from the odontogenic epithelium that contain fibrous stroma without odontogenic ectomesenchyme (1). These tumors are characterized by their locally aggressive behavior and capacity for recurrence (1, 2). In contrast, adenomatoid odontogenic tumors and calcifying cystic odontogenic tumors are non-invasive tumors characterized by low recurrence rates. Although these tumors are derived from the odontogenic epithelium, they differ in terms of their histogenesis (1, 3, 4).

Glypicans (GPC) are a family of proteoglycans that consists of six members (GPC 1-6), highlighting glypican-3 (GPC-3). The GPC-3 gene encodes a 70-kDa surface protein (5) that consists of two subunits (N-terminal and C-terminal subunit) (6, 7). High levels of the protein are found in embryonic tissues, while its expression is variable in tissues such as breast, ovary, and mesothelium (8, 9). Glypican-3 is widely used as a diagnostic marker for hepatocellular carcinoma and has therefore been considered a therapeutic target of this tumor (10). Furthermore, glypican-3 acts as a negative regulator of Hh activity by competing with PTCH1 for SHH binding (10, 11) and components of the SHH pathway have been investigated in many odontogenic tumors (12).

Previous studies have shown that immunohistochemical markers help distinguish between tumors and odontogenic cysts (13–16). However, to our knowledge, there are no studies investigating glypican-3 in odontogenic tumors. Therefore, the objective of this study was to determine whether this immunohistochemical marker could be used to distinguish aggressive from non-aggressive odontogenic tumors.

Materials and methods

The study was approved by the Ethics Committee of Federal University of Bahia. Fifty-nine cases of tumors were divided

into aggressive odontogenic tumors (20 solid ameloblastomas, four unicystic ameloblastoma, 28 KOTs including five associated with Gorlin–Goltz syndrome) and non-aggressive odontogenic tumors (five adenomatoid odontogenic tumors and two calcifying cystic odontogenic tumors), obtained from the archive of the Pathological Anatomy Service, School of Dentistry, Federal University of Bahia (FOUFBA), were submitted to morphological analysis according to the WHO classification (1) and to immunohistochemical analysis.

For immunohistochemistry, the samples were cut into 3-μm-thick sections. The histological sections were deparaffinized in xylene and rehydrated in absolute alcohol. A polymer system (EnVision Advance™; Dako, Carpinteria, CA, USA) was used for immunostaining. For exposure of the antigen epitopes, antigen retrieval was performed in citrate buffer, pH 6.0, in moist heat at 96°C for 45 min. Endogenous peroxidase was blocked by immersing the sections in 3% hydrogen peroxide for 10 min. Non-specific protein binding was blocked using Protein Blocking Solution™ (Dako). The sections were then incubated overnight with the primary antibody (glypican-3, clone 1312; Santa Cruz, CA, USA) diluted 1:100 in Tris–HCL buffer with background-reducing solution (Dako), followed by incubation with the polymer detection system (EnVision Advance™; Dako). The reaction was developed with diaminobenzidine (Dako) and the sections were counterstained with Harris hematoxylin for 1 min. Finally, the sections were dehydrated in absolute alcohol, cleared in xylene, and mounted on slides with Canada balsam. The negative control consisted of replacing the primary antibody with non-immune serum. Sections obtained from a hepatocarcinoma served as positive control.

Cells exhibiting brown staining in a membrane and/or cytoplasmic location were defined as positive. The cytoarchitectural pattern was also analyzed in different tumors.

For immunohistochemical analysis, the images were captured with a light microscope (Axiostarplus; Zeiss, Göttingen, Germany, 2008) under a bright-field fixed focus coupled to a digital camera system (Axiocam ICc3; Zeiss, 2008). The following scores were attributed: score 0, absence of staining; score 1, weak staining; score 2, moderate staining; score 3, strong staining. The proportion of staining was defined as the percentage of stained cells (0, 0%; 1, 1% to 25%; 2, 25% to 75%; 3, >75%). Multiplication of the intensity of immunostaining (0–3) by the proportion of stained cells (0 > 75%) resulted in the following score: When <25% of the cells were stained or the staining intensity was weak, the maximum product of the two scores was 3 and immunostaining was classified as low; when at least 25% of the cells were stained or the staining intensity was moderate or strong, the maximum product of the two scores was at least 4 and immunostaining was classified as high (adapted from Dultra et al. 17).

The data were analyzed using the Statistical Package for the Social Science (SPSS), version 13.0. Fisher's exact test was used to evaluate possible associations. A level of significance of 5% was adopted.

Results

Positive immunostaining for glypican-3 was observed in 25 of the 59 odontogenic tumors. Seven (35%) of the 20 solid ameloblastomas were positive vs. 18 (64.28%) of the 28 KOTs, with this difference being significant ($P = 0.045$). Three of the five syndromic cases of KOT were positive, but there was no significant difference between syndromic and sporadic cases ($P > 0.05$). All cases of unicystic ameloblastoma ($n = 4$), adenomatoid odontogenic tumor ($n = 5$), and calcifying cystic odontogenic tumor ($n = 2$) were negative.

In ameloblastomas, cytoplasmic and granular staining was generally observed in peripheral cells resembling ameloblasts and in central cells resembling the stellate reticulum of the enamel organ (Fig. 1A). Areas of differentiation were immunonegative for glypican-3. In KOTs, immunostaining was frequently cytoplasmic (Fig. 1B,C), but membranous staining was eventually observed (Fig. 1D). Staining was detected particularly in the suprabasal cells of KOTs and was absent in areas of inflammation. In some cases, immunostaining appeared as clusters. No immunostaining was observed in stromal fibroblasts of KOT, but it was present in four solid ameloblastomas, but it was not frequent (Fig. 1E).

Discussion

To our knowledge, this is the first study that investigated glypican-3 immunostaining in odontogenic tumors and particularly differentiated between aggressive and non-aggressive tumors. Glypican-3 is a marker commonly used for the diagnosis of hepatocellular carcinoma (18–21), the differentiation between neoplastic and non-neoplastic urothelium (22), the distinction between squamous carcinomas and adenocarcinomas of the lung (23), and even the differentiation between low- and high-grade tumors (22).

In the present study, despite the observation of a significant difference between ameloblastomas and KOTs, these aggressive odontogenic tumors exhibited an immunohistochemical distinction of glypican-3 compared to adenomatoid odontogenic tumors and calcifying odontogenic tumors. Thus, glypican-3 seems to have contributed to invasiveness of those tumors. However, expression was significantly higher in KOTs than solid ameloblastomas, but this difference was not significant between syndrome KOT and non-syndrome KOT. Other authors also tried to establish differences between benign odontogenic tumors by showing that calretinin was expressed differently in ameloblastomas and other odontogenic tumors such as adenomatoid odontogenic tumor, ameloblastic fibroma, and odontogenic myxoma (16). Calretinin has been suggested to be important for the differential diagnosis between solid and unicystic ameloblastomas (13). Differential expression of calretinin in unicystic ameloblastomas compared to residual cysts, dentigerous cysts, and odontogenic keratocysts has been reported (24). According to Koneru et al. (14), calretinin is expressed in ameloblastomas and keratocysts, but not in adenomatoid odontogenic tumors.

The present results showed the absence of glypican-3 staining in unicystic ameloblastomas. However, despite this difference compared to solid ameloblastomas, these results may not be consistent considering the small number of unicystic cases, although unicystic tumors are less aggressive (1). Recently, Bologna-Molina et al. (25) demonstrated the presence of another member of the glypican family,

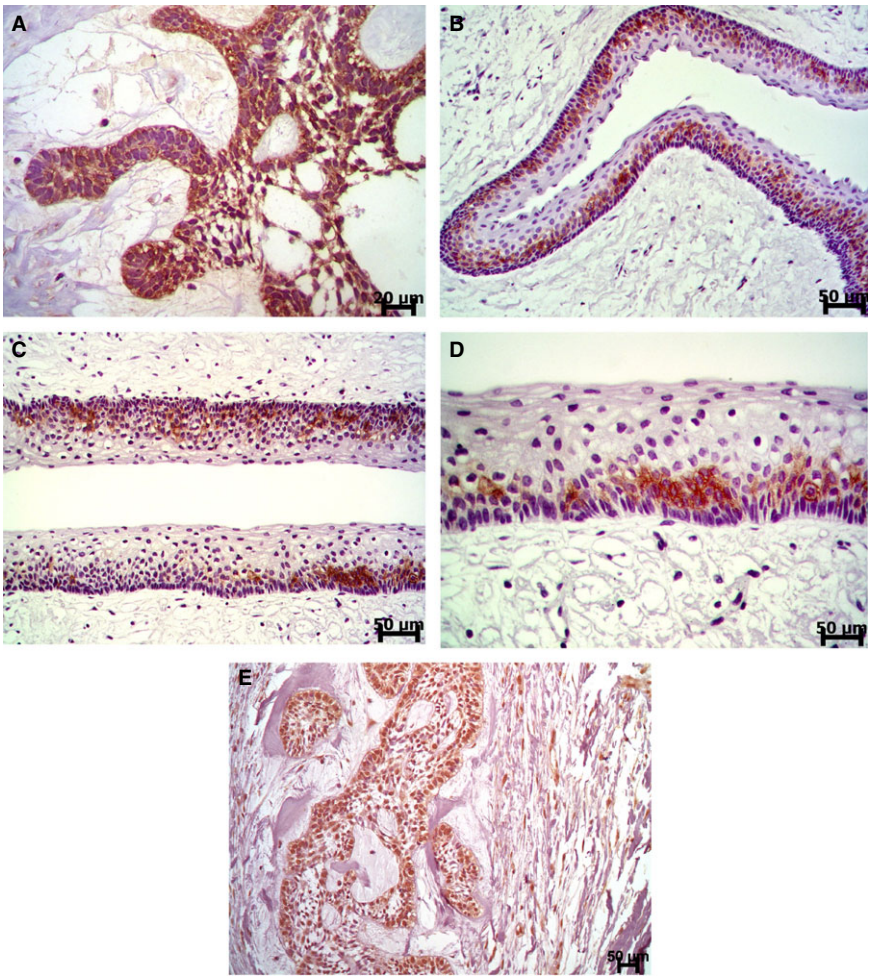

Figure 1 Ameloblastoma: (A) epithelial island exhibiting columnar cells and stellate reticulum-like cells immunopositive for glypican-3. Keratocystic odontogenic tumor: (B, C) suprabasal cells immunopositive for glypican-3, (D) note membranous immunostaining in suprabasal cells. (E) Solid ameloblastoma: stromal fibroblasts positive for glypican-3; note that they are close to tumoral parenchyma.

glypican-1, in different types of ameloblastomas, but found no differences between solid and unicystic cases.

Previous studies have investigated the immunohistochemical distinction between syndromic and non-syndromic keratocysts (26). Similar to the present study, glypican-3 was not differentially expressed in these cases. It is possible that a larger number of cases would permit to better distinguish between syndromic and non-syndromic keratocysts, as other authors found a difference between these groups of tumors (26). Other aspects including recurrence and size of KOTs and ameloblastomas could make clear differences between aggressive and non-aggressive odontogenic tumors as well.

In this study, predominantly cytoplasmic staining was observed in ameloblastomas and KOTs, in agreement with previous studies (27). Glypican-3 is a heparan sulfate proteoglycan linked to the cytoplasmic surface of the plasma membrane by a glycosyl-phosphatidylinositol (GPI) anchor (28). In ameloblastomas, the different morphological components of the tumors were stained, similar to the finding of Bologna-Molina et al. (25) for glypican-1. In contrast, in KOTs, immunostaining was observed in the suprabasal and intermediate layers, which seem to correspond to the proliferative compartment of these tumors as reported in the literature.

In addition, the epithelial tissue organization and its histopathological changes could explain the reason for the absence of reactivity in inflammatory areas of KOTs. With respect to ameloblastomas, we hypothesized that glypican-3 is not useful to detect squamous metaplasia in these tumors, although it was described in squamous carcinoma of lung as reported by Aviel-Ronen et al. (23). However, further studies could clarify these aspects.

An interesting finding was the detection of stromal staining, especially in fibroblasts of ameloblastomas, which was not observed in KOTs. Bologna-Molina et al. (25) also detected glypican-1 immunostaining in solid and desmoplastic ameloblastomas and attributed its presence to the accumulation of heparin-binding growth factors that would be protected from proteolytic activity. According to Ng et al. (29), glypican-3 serves as a co-receptor for fibroblast growth factor (FGF)-9, demonstrating that FGF receptors are regulated by heparan sulfate proteoglycans as suggested by Matsuo and Kimura-Yoshida (30). Furthermore, one can hypothesize that

fibroblasts devoid of glypican-3 in KOTs store other growth factors involved in myofibroblast differentiation.

This study provided insights into the presence of glypican-3 in odontogenic tumors, which distinguished aggressive from non-aggressive odontogenic tumors. Other studies involving a larger number of cases should contribute to the understanding of the role of glypican-3 in these tumors and in other odontogenic tumors.

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Conflict of interest

None.