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Research Article

THE IMMUNOHISTOCHEMICHAL EXPRESSION OF HER2/NEU IN AMLOBLASTOMA AND DENTAL FOLLICLE

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Abstract

Introduction: The odontogenic cysts and tumors are an important part of the oral and maxillofacial pathology. Ameloblastoma is the most common odontogenic tumor of the epithelial origin and in most cases it has a benign behavior. This study aimed to assess HER2/neu expression in ameloblastoma and dental follicles. **Materials and methods:** 10 solid ameloblastoma paraffin blocks and 15 dental follicle samples were stained for the HER2/neu immunohistochemically. The results were analyzed statistically and P.value < 0.05 was considered significant. **Results:** Only one ameloblastoma showed slight weak immunopositivity for HER2/neu in the epithelial component and in other cases of ameloblastoma and in all cases of dental follicles, staining was negative. The staining results of ameloblastomas and dental follicles were not statistically different. **Conclusion:** Due to the negative staining of HER2/neu in most of the ameloblastoma samples, it seems that this marker does not play an essential role in the development and progression of ameloblastoma.

Keywords: Ameloblastoma, Dental follicle, HER2/neu expression.

Introduction

The odontogenic cysts and tumors are an important part of the oral and maxillofacial pathology. While the cysts can be seen in abundance, but odontogenic tumors are rare lesions. Today, molecular markers, including odontogenic lesions are widely studied in pathology, and it seems that these markers can be useful in predicting the rate of progression of odontogenic tumors and cysts. Ameloblastoma is the most common odontogenic tumor of the epithelial origin. Its prevalence in the lower jaw is more and mainly is associated with an impacted tooth and have three clinical subgroups, including multi-cystic (86%), unicystic (13%) and environmental (1%). Ameloblastoma is a slow growing, painless swelling. In the microscopic view, islands of epithelium have a core of angular cells. A single layer of elongated cylindrical cells of pseudo-ameloblast with reverse polarity surrounds the central mass. Ameloblastoma has a local invasion, which in most cases is a benign behavior. While the tumor gets rarely malignant and metastatic, but can cause major malformations of the jaw and face. The treatment of these tumors is surgery and if the tumor is not removed, the risk of recurrence is high.

Dental follicles composed of fibrous tissue, remnants of odontogenic epithelium and reduced enamel epithelium (1).

Growth factors and their receptors play an important role in the growth of normal tissue in the body and in the evolution and development of neoplasms. Epithelial growth factor (EGF) is an important factor in the regulation of normal and neoplastic cell proliferation. By epithelial growth factor receptors (EGFR), this factor is attached to target cells and stimulates the cellular protein kinase that in turn causes the tyrosine phosphorylation that in fact is the first step to mitosis. EGFR is in category tyrosine kinase and includes 4 subgroups: HER4; HER3; HER2; and EGFR (2). Several studies have assessed the increased expression of these receptors in the human tumor growth of breast

(3-5), ovary (6), lung (7), bladder (8), and Osteosarcoma (9), and several results have been reported.

HER2/neu or ErB-2 is a known proto-oncogene with molecular weight of 185kDa, which is located on the long arm of human chromosome 17 and it is very similar to the EGFR structure (10). Due to the growth cycle phosphorylation, this protein starts and advances the differentiation and migration of cells. Previous studies suggest that the oncogene HER2/neu is involved in pathogenesis of many malignancies in humans. In breast cancer cells, high levels of this protein indicate a poor prognosis of the disease, and hence it is used as a marker to determine the prognosis and to select the type of treatment in breast cancer (11).

Given that ameloblastoma is an epithelial tumor, it seems that the growth factor receptors are involved in the development or progression of the tumor. In addition, previous studies have reported different results in this area, so, in this study we decided to investigate the expression rate of HER2/neu in ameloblastoma tumor and dental follicle.

Materials and Methods

Generally, 25 samples were studied. A total of 10 solid ameloblastoma paraffin blocks and 15 dental follicle samples were stained for HER2/neu immunohistochemically.

Immunohistochemical (IHC) staining method of paraffin block as follows:

IHC staining was performed by standard methods of Envision. In summary, after preparation of slices, samples were deployed on slides stained with Poly-L-Lysin for 24 hours at 37°C until were dried. Then, the samples were deparaffinized in Xylene and were rehydrated in varying degrees of ethanol. Consequently, in order to stop the internal peroxidase activity, the samples were placed in methanol containing peroxide (H2O2) 0.3% for 30 minutes at room temperature and then were rinsed by dissolving Phosphate buffered saline (PBS) PH = 7.2. Immunohistochemical staining by anti-mouse antibodies and monoclonal of HER2/neu (manufactured in Dako / Denmark) was performed according to the manufacturer's protocol. After the incubation with the primary antibody, Envision technique was used. Samples were incubated for 30 minutes in polymer solution (anti-mouse) and washed with PBS. In the next stage, the dye of 3, 3'-Diaminobenzidine (DAB) Hydrochloride (DAB), which gives a brown color to a complex antigen–antibody, was used. Then, the samples were counter stained with hematoxylin and after winemaking, plates were placed on them. Finally, immunohistochemical staining was performed under light microscope by two pathologists. In this study, a sample of breast cancer was used as a positive control, and without adding the primary antibody an ameloblastoma sample was used as a negative control. The staining results were statistically analyzed and P.value <0.05 was considered as significant.

Results

Only one ameloblastoma (acanthomatose type) exhibited weak staining of HER2/neu in squamous cells of the center of ameloblastoma Islands (Figure 1) and in other cases of ameloblastoma (Figure 2) and in all cases of dental follicles (Figure 3), epithelial tissue staining was negative. The staining results of ameloblastomas and dental follicles were not statistically different.

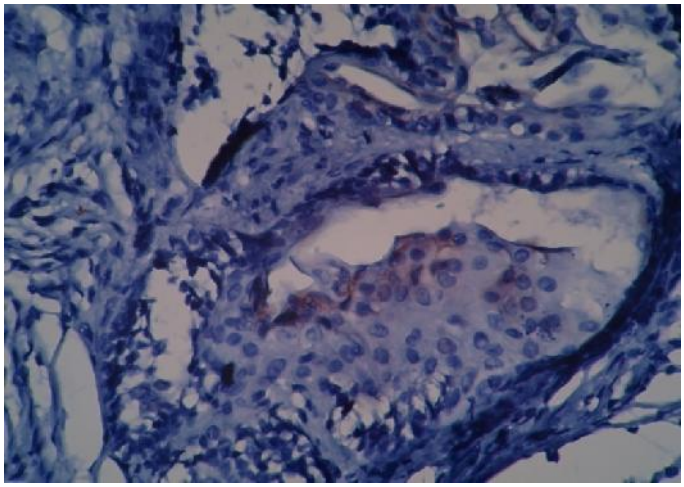

Figure 1. Weak expression intensity of HER2/neu in the epithelial lining of acanthomatous ameloblastoma

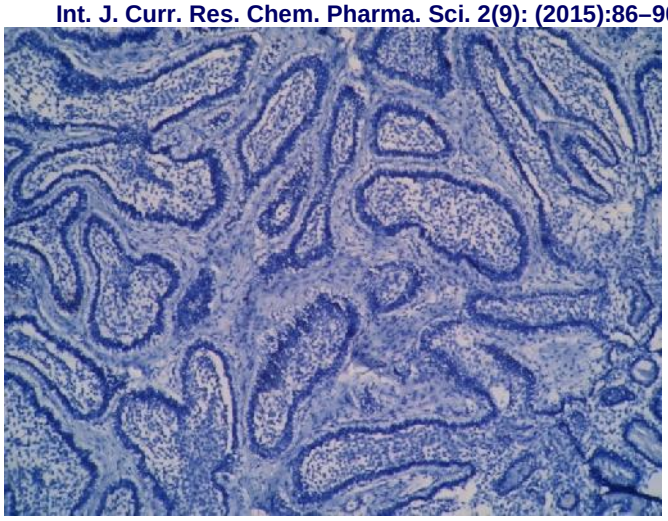

Figure 2. In other cases of ameloblastoma showing no reactivity for HER2/neu

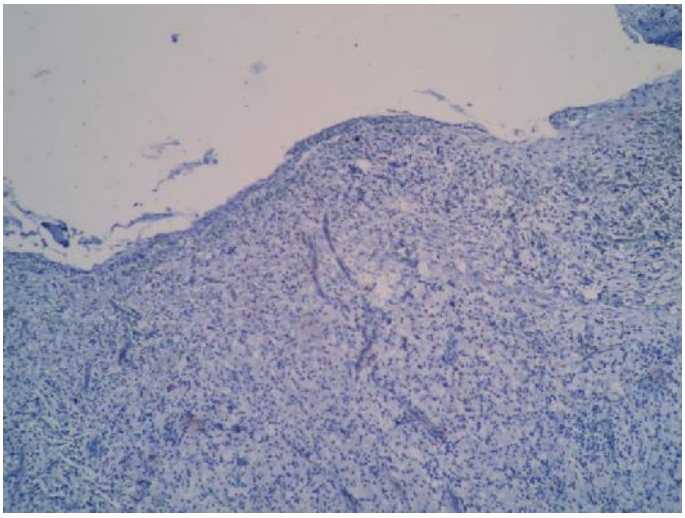

Figure 3. . Dental follicle showing no reactivity for HER2/neu.

Also, in two amloblastomas and four dental follicles, a weak stating of Her2/neu was observed in the stroma. According to Mann-Whitney test, no statistically significant relationship existed between two groups of amloblastoma and dental follicle in the intensity of staining HER2/neu in stroma (P = 0.62).

Discussion

EGF as a mitogenic factor is important for epithelial cells that are involved in the regulation of normal and neoplastic cell proliferation. This factor is attached by specific surface receptors to the target cell and induces the cell proliferation. ErbBs are membranous cell receptors from the category of tyrosine kinase. This category includes the four subgroups, one of which is HER2/neu. Due to the Phosphorylation of cell growth and proliferation cycle, this subgroup starts and advances the differentiation and migration of cells. An increase happens in the expression of this receptor in the process of the growth of human tumors, especially breast cancer (2).

In this study, two groups of ameloblastoma and dental follicle were studied. The results of the HER2/neu in all 15 dental follicles (100%) and in 9 ameloblastoma samples (90%) were negative, and only in one ameloblastoma were positive; so, the results were not statistically significant. The staining of one ameloblastoma was seen in *a*canthomatous *type* squamous epithelium. The incidence of HER2/neu in the stroma of ameloblastoma in 8 samples (80%) was negative, and only in 2 samples, was positive. In addition, in the dental follicle, 11 samples (73.3%) were negative and 4 samples (26.7%) were positive.

Evaluation of HER2/neu in odontogenic lesions has been done before in just two studies.

In the study by Oikawa et al., staining of HER2/neu in dental follicle and dentigerous was fully positive (100%) and in ameloblastoma was positive (57%). In this study, the incidence of this protein was not seen in *a*canthomatous ameloblastoma (10). The results of this study were inconsistent with our study. In this study, HER2/neu expression in the dental follicle epithelium was completely negative and was seen in just one acanthomatous ameloblastoma. In a study by Oikawa et al., polyclonal antibody made by Nichirei Company had been used (10) whilst in our study, polyclonal antibody made of Dako company was used. Despite the antibodies made by different companies can have different results, monoclonal antibodies are generally more specific while in polyclonal antibodies, the likelihood of non-specific reactions is more. In addition, in the present study only a variety of initial solid ameloblastoma was used while in a study by Oikawa et al., recurrent types and unicystic also were assessed and this can also be partially reason of the difference in the final result.

Based on the study conducted by Esfahani et al., increased expression in HER2/neu was seen in 41.7%, 3.48%, and 2.88% of cases of odontogenic keratocyst, dentigerous cyst, and radicular cyst, respectively. They stated that increased expression of this protein in carcinomas important and its high levels in radicular cyst compared with dentigerous and odontogenic keratocyst could explain the higher rate of changes in the remaining radicular cyst (2). A major component in radicular cyst is inflammation that can lead to increased cell proliferation indifferent ways, whereas in examples in the present study, inflammation was slight or minimal. On the other hand, the factors involved in the pathogenesis of cysts compared with tumors are different and it is expected that the results of staining of odontogenic cyst will vary with ameloblastoma.

In a study by Abdel-Aziz et al. and in a study by Vered et al., the expression of EGFR in all cases was a positive amloblastoma. At the end of these studies, it was suggested that the anti-EGFR factors can be used in the treatment of large-size tumors or inoperable cases that are close to vital structures (12,13). Cetuximab and Panitumumab monoclonal antibodies are used as a main strategy in the treatment of colon adenocarcinoma that is positive EGFR (10). Since in most previous studies, the expression of EGFR has been seen in the ameloblastoma and many of the researchers know this lesion as a positive EGFR tumor, perhaps this family of medications also can be considered in the treatment of ameloblastoma.

In the studies conducted by de-Vicente et al., Goncalves et al., and Oliveira et al., EGFR expression was observed mainly in odontogenic cysts (14-16). As previously mentioned, in the study by Esfahani et al. also HER2/neu expression was found often in odontogenic cysts. According to the study, it seems that HER2/neu have little role in the development and progression of ameloblastoma. By examining the stained EGFR, HER2, HER3 andHER4, Oikawa et al. have emphasized role of HER4, In addition to EGFR, in tissue odontogenic.

EGFR (HER1) is the head of the group of the epithelial growth factor receptor family (HER) and it has an expression in the epithelial component, including epithelial odontogenic. The expression of other members of the family, such as HER2, HER3 and HER4 in odontogenic types of lesions may be different that needs to be investigated further.

Conclusion

Due to the negative staining of HER2/neu in most of the ameloblastoma samples, it seems that this marker does not play an essential role in the development and progression of ameloblastoma.

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