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Original research

Expression of cytokeratin 14, cytokeratin 19 and E-Cadherin in ameloblastoma correlates with the cytodifferentiation of enamel organ

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ABSTRACT

Objective: To analyze the bell stage of human tooth germ and ameloblastoma to determine the level of morphological differentiation in the constituting cells of the enamel organ and ameloblastoma.
Study design: The study included 10 tooth germs from 5 human fetuses (20–24 weeks) and 20 tissue samples of ameloblastoma retrieved from the archives. The morphological characteristics of both tooth germs and ameloblastomas were evaluated by routine sections and immunohistochemistry using CK14, CK19 and E-cadherin.
Results: All the sections of the tooth germs and ameloblastomas positively stained with the markers employed in this study. The CK14 stained all cellular elements within the enamel organ, but expression of CK19 was limited to the outer enamel epithelium and inner enamel epithelium cell lineage. The reaction with E-cadherin was variable in the tooth germs examined. All tissue samples of ameloblastoma reacted to CK14, CK19 and E-cadherin. However, CK14 reacted intensely in all the tumors cells while the reaction with other markers was variable depending on the cell types in a given tumor.
Conclusion: The present study demonstrates that expression of CK14, CK19 and E-cadherin in the enamel organ and ameloblastoma share similar cytodifferentiation features.

1. Introduction

Ameloblastoma is a benign epithelial odontogenic tumor having close resemblance to the enamel organ of tooth germ, but, unlike the latter, the tumor cells of ameloblastoma do not differentiate to the point of enamel formation. Previous studies were conducted to delineate histogenesis and cytodifferentiation of the constituting cells of enamel organ and ameloblastoma using a number of immunomarkers [1–12]. Some of these studies claimed that the constituting cells of the enamel organ and ameloblastoma are distinct [3,7], whereas others believed that the tumor cells of ameloblastoma retain cytodifferentiation similar to the enamel organ [4,6]. Nevertheless, no logical and reasonable conclusion could be drawn from these studies due to inadequate description of the staining reaction and inconsistent reporting of the constituting cells of the enamel organ, especially with regard to the IEE lineage. As CK14 and CK19 are regarded as phenotypic marker of the stage of differentiation in tooth germs and E-cadherin has been shown to reflect aggressiveness of ameloblastoma, it is believed that these markers may lend credible information regarding cytodifferentiation of the tumor cells of ameloblastoma. Therefore, the purpose of the present study was to evaluate the expression of pattern of CK14, CK19 and E-cadherin in the bell stage of human tooth germs and ameloblastoma to ascertain the level of cytodifferentiation of tumor cells.

☆ AsianAOMS: Asian Association of Oral and Maxillofacial Surgeons; ASOMP: Asian Society of Oral and Maxillofacial Pathology; JSOP: Japanese Society of Oral Pathology; JSOMS: Japanese Society of Oral and Maxillofacial Surgeons; JSOM: Japanese Society of Oral Medicine; JAMI: Japanese Academy of Maxillofacial Implants.

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2. Material and methods

2.1. Human fetus and ameloblastoma

This study utilized ten tooth germs obtained from five human fetuses and twenty tissue samples of ameloblastoma retrieved from the archives. None of the fetuses were live, but spontaneously aborted and unclaimed, which were obtained from the Institute of Obstetrics and Gynaecology, Madras Medical College, Chennai. The study protocol was duly approved by the institutional ethical committee and the procedure for using and handling the fetal tissue was in consonance with the guidelines of the Indian Council of Medical Research [13]. The gestational ages of the fetuses roughly represents 20–25 weeks which was based on the measurement of the length of the fetus. The fetuses were macroscopically healthy and the selected fetuses were fixed in 10% neutral buffered formalin for at least 48 h, followed by careful dissection of the maxillary and mandibular processes for decalcification in 8% hydrochloric acid for an hour and then routinely processed. The paraffin embedded blocks of twenty cases of ameloblastoma were retrieved from the archives.

For both fetus and ameloblastoma, serial sections (3 μm) were made for haematoxylin and eosin (H & E) staining and simultaneously the sections for immunohistochemistry were transferred to 3-aminopropyltriethoxysilane coated slides. The H & E sections of the ameloblastomas were evaluated with reference to the outer or peripheral cell morphology of the follicles or islands corresponding to the inner enamel epithelial cell lineages of the enamel organ, such as inner enamel epithelium (IEE), preameloblast (PA), presecretory ameloblast (PSA) and secretory ameloblast (SA).

2.2. Immunohistochemsitry

For immunohistochemistry, the sections were dewaxed in xylene and rehydrated in graded alcohol. For antigen retrieval, the sections were placed in the pressure cooker containing Tris/EDTA buffer at pH 9.0 for 15 min and then cooled for 10 min at room temperature. The sections were then washed in 0.5 mM Tris-buffered (TBS) saline at pH 7.4 for 5 min and blocked with PolyExcel H_2O_2 for 5 min. The sections were then washed with TBS buffer for 5 min and incubated with pre-diluted (1:100) primary antibodies to cytokeratin-14 (clone LL002), cytokeratin-19 (clone EP72) and E-cadherin (clone EP6), [PathnSitu Biotech Laboratories, Livermore, CA] for 30 min at room temperature.

The primary antibody was removed by washing with TBS buffer for 5 min and treated with PolyExcel target binder for 10 min. The sections were again washed with TBS buffer for 5 min and covered with PolyExcel HRP conjugated with secondary antibody (PathnSitu Biotech Laboratories, Livermore, CA) for 15 min at room temperature, followed by washing in PolyExcel DAB buffer for 5 min and placed in PolyExcel Stunn DAB solution for 5 min at room temperature. Finally, the sections were washed with TBS buffer for 5 min and counterstained with Mayer's Haematoxylin. The sections were treated with graded alcohol and one change of xylene. The positive controls were used as indicated by the manufacturer (normal skin tissue for CK14 and E-cadherin, and colon tissue for CK19). In addition, the oral epithelium within the test section was considered as the positive internal control for tooth germs. Negative controls were performed as described above but by replacing the primary antibody and substituting with an immunoglobulins G isotype, The expression of CK14, CK19 and E-cadherin was assessed based on the presence of brown colour staining (cytoplasmic and/or membranous) and the intensity of staining reaction was graded semi-quantitatively as mild, moderate and intense. The slides were observed with Olympus BX43 microscope. The photomicrographs were captured with the infinity camera attached to the Olympus microscope and from smart phones camera using free-hand technique. The captured images were adjusted using photoshop for brightness and contrast.

3. Results

3.1. Histological features of human tooth germs and ameloblastoma with routine sections

Of the ten tooth germs employed in this study, two tooth germs represented early bell stage (EBS) and the rest consisted of late bell stage (LBS) of development. The EBS was characterized by the presumptive keratinized and non-keratinized oral epithelium, dental lamina and enamel organ, dental papilla (DP) and dental follicle (DF) along with the associated bony crypts. The enamel organ comprised of outer enamel epithelium (OEE), stellate reticulum (SR), stratum intermedium (SI) and inner enamel epithelium (IEE). The latter cell layer extends from the cervical loop to the cusp tip and is divided into IEE, preameloblasts (PA) and transitional presecretory ameloblasts (PSA) based on their location and morphological differentiation (Fig. 1**A–D** and Table 1).

The LBS was distinguished from the EBS by the presence of dental lamina fragments and by functional differentiation as evidenced by the formation of dentin matrix/dentin and/or enamel matrix/enamel. As in the EBS, the enamel organ was comprised of OEE, SR, SI, IEE, PA and transitional PSA, but additional differentiating lineages of IEE such as PSA and secretory ameloblasts (SA) were also evident (Fig. 1**E–H** and Table 1). The OEE showed indentation and conspicuous vascularity. The zones of IEE and PA are similar in extent and morphology as in the EBS. However, the zone of transitional PSA extends from the zone of PA to the zone of PSA.

The ameloblastoma in this study was characterized by follicles or islands with the outer or peripheral cells that varied in morphology from cuboidal to columnar in shape. The cells in certain fields closely resembled the various differentiating lineages of IEE found in the tooth germ. The central cells of the follicles or islands resembled SR of the tooth germ. The central cells are further characterized, in some follicles or islands, by squamous or acanthomatous differentiation.

3.2. The immunohistochemical features of the human tooth germ and ameloblastoma

All the immunohistochemical markers employed in this study showed positive reaction in both EBS and LBS of the examined tooth germs – Table 2.

3.3. CK14 expression in the tooth germ (EBS and LBS)

In both the EBS and LBS, the staining reaction with CK14 was intense in the oral epithelium, primary dental lamina, succession dental lamina, succession tooth bud and in the cell layers of the enamel organ (Fig. 2**A).** However, in EBS, the staining reaction was mild or negative in the IEE and intense in the PA followed by reduced intensity of staining in the transitional PSA (Fig. 2**B & C).** In contrast, IEE lineage cells reacted intensely to CK14 in the LBS (Fig. 2**G–I).**

3.4. CK19 expression in the tooth germ (EBS and LBS)

During the EBS, apart from the expression in the basal cells of the oral epithelium, the reaction to CK19 was positive in the succession dental lamina but negative in the succession tooth bud and primary dental lamina (Fig. 2**D).** Within the enamel organ, in both EBS and LBS, the expression of CK19 was negative in the SR, SI and

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