Expression of keratin 15 in dentigerous cyst, odontogenic keratocyst and ameloblastoma

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Abstract. The etiology and pathogenesis of odontogenic lesions are poorly understood. Keratin 15 (K15) is a type I cytoskeletal protein that provides structural support to the cells and has been considered to be a stem cell marker. The aim of the present study was to evaluate the expression of K15 in the epithelial lining of dentigerous cysts (DCs), odontogenic keratocysts (OKCs) and ameloblastomas (ABs). The study included 41 samples of DCs (n=13), OKCs (n=12), and AB tissues (n=16). K15 protein expression was evaluated via immunohistochemistry and data were statistically analyzed using a Kruskal-Wallis test. K15 was expressed in the majority of the studied lesions with various distributions in the different study samples. The Kruskal-Wallis test revealed non-significant differences in the expression of K15 among the three odontogenic lesions (P=0.380). The present study confirmed the high expression of K15 in the different epithelial layers of DC, OKC and AB. This type of expression excludes the reliability of regarding K15 as a stem cell marker in DC, OKC and AB. However, K15 may reflect the abnormal differentiation of pathological epithelial cells in these lesions.

Introduction

Both etiology and pathogenesis of odontogenic lesions are poorly understood. Therefore, studying odontogenic lesions is important in order to update the knowledge of these lesions. For example, after a long period of its discovery, WHO in the year 2005 re-classified odontogenic keratocyst from odontogenic cyst into an odontogenic tumor. Then after, and in its 4th edition, the WHO in the year 2017 re-classified keratocystic odontogenic tumor into odontogenic keratocysts (OKC). This controversies in the classification of OKC reflects the limitation in the knowledge and molecular basis of this lesion. OKCs are associated with unerupted tooth in 25-40% of cases, which results in a clinicoradiographical diagnosis of dentigerous cyst (1). The treatment of OKC remains also controversial. It ranges from as simple as marsupialization and decompression (2) to radical resection with subsequent bone graft reconstruction (3).

Dentigerous cysts (DCs) are odontogenic lesions arising from the crown of impacted, embedded, or unerupted teeth. Enucleation of the cyst and extraction of the cyst-associated tooth is the current standard treatment for a dentigerous cyst (4).

Ameloblastoma (AB) is a rare, benign, slow-growing but locally invasive neoplasm of odontogenic origin involving mainly the mandible and less frequently the maxilla. It has a high recurrence rate if treated in a conservative manner (5). The radical surgical option is the current standard of care for ameloblastoma and includes en bloc resection with 1-2 cm bone margins and immediate bone reconstruction to help return function (6).

Keratin 15 (K15) is a type I cytoskeletal protein. Although its main function is providing structural support to the cells, other functions of K15 in both adult stratified epithelia and different pathological lesions is not fully understood. While K15 was proposed to be a stem cell marker that presents in the basal keratinocytes of all stratified epithelia, it also has been reported to be expressed in suprabasal epithelial layers of normal and diseased epithelial tissues. This different location of K15 expression questions the status of K15 as an actual stem cell marker (7).

The expression and type of keratin is highly affected by epithelial cell differentiation and lineage (8). Therefore, expression of the keratins 1, 2, 4, 5, 6, 7, 8, 10, 13, 14, 16, 17, 18, 19 and 20 was investigated in odontogenic cysts and tumors (9-14). To the best of our knowledge, there is only one study that discussed the expression of K15 in AB (15), and no presence of any previous literature that deals with K15 expression in other

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Abbreviations: K15, keratin 15; DC, dentigerous cyst; OKC, odontogenic keratocyst; AB, ameloblastoma

Key words: keratin, K15, odontogenic, dentigerous, keratocyst, ameloblastoma

odontogenic cysts. The aim of the current study was to evaluate the expression of K15 in DC, OKC, and AB.

Materials and methods

A total of 41 formalin fixed paraffin embedded (FFPE) samples were retrieved from the archive of the Oral Pathology Department of Tongji Hospital, Huazhong University of Science and Technology (Wuhan, China). These samples were used retrospectively and were categorized into three groups, which are DC (n=13), OKC (n=12), and AB (n=16). No true tissue equivalent exists to serve as a control group, as odontogenic lesions encompass reactive tissues and tumors which replace the healthy bone. This study was approved by the Institutional Review Board of Tongji Medical College, Huazhong University of Science and Technology and followed the protocol of the World Medical Association Declaration of Helsinki.

Standard streptavidin-biotin peroxidase complex immunostaining method was used (Wuhan Boster Biological Technology, Ltd., Wuhan, China). Samples were cut as 5-μm tissue sections, then dewaxed before rehydration. After that, 3% H_2O_2 solution was used to quench the endogenous peroxidase activity before the antigen unmasking step in a microwave using 0.01 M citrate buffer heated to boiling point. After that, goat serum (Wuhan Boster Biological Technology, Ltd.) was used to treat the samples for 50 min at room temperature and then samples were incubated with 1:100 diluted primary mouse monoclonal antibody at 4˚C overnight (BM0783; Clone: 6E7, Wuhan Boster Biological Technology, Ltd.). This step was followed by using 10 μg/ml biotinylated secondary antibody for 2 h at room temperature (goat anti-mouse; BA1001 Wuhan Biological Technology, Ltd.). Then, the slides were stained with 20 μg/ml streptavidin-biotin-peroxidase complex. Finally, the sections were developed with 3,3'-diaminobenzidine substrate and counter-stained with Mayer's hematoxylin. Negative controls were passed in the same procedure, but instead the step of the primary antibodies, the sample is incubated with phosphate-buffered saline.

K15 expression was recognized as yellowish to brown cytoplasmic staining of the positive cells. Samples were semi-quantitatively scored using a standard light microscope. The positively stained cells were counted and scored as the percentage of positive cells from the total epithelial cells in 10 continuous and representative high power (magnification, x400) fields. The scoring was 0 (absent), when there was no identified staining of the odontogenic epithelium or when the staining was questionable; 1 (weak) for ≤20%; 2 (mild) for 21-40%; and 3 (strong) for >40% positivity rate of the odontogenic epithelium.

Data were analyzed using the SPSS 19.0 software (IBM Corp., Armonk, NY, USA) and are presented as the mean ± standard deviation (SD). Comparison of K15 protein expression among the studied groups was analyzed using Kruskal-Wallis statistical test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

The study composed of 41 samples. Males were 23 (56.1%) and females were 18 (43.9%). Fifteen (36.6%) of the cases were located in the maxilla, and 26 (63.4%) cases were in the mandible. The mean age was 37.24 with SD of ±18.269.

K15 protein expression was determined as yellowish to brown cytoplasmic staining in the odontogenic epithelial cells of DCs, OKCs and ABs (Fig. 1). In the DCs, positive cells were distributed mainly through the suprabasal layers and K15 expression was weak in 30.8% and strong in 69.2% of the cases (Table I). In OKC samples, K15 expression involved mostly all epithelial layers. It was absent in 16.7%, mild in 8.3%, and strong in 75% of the total OKC cases (Table I). The distribution of positive K15 stained epithelial cells in AB was more through the central stellate reticulum-like cells than the peripheral columnar cells. It was absent in 6.3%, weak in 25%, mild in 25% and strong in 43.8% of AB samples (Table I). All samples were analyzed and the photos in (Fig. 1) were the best representative of all the samples. Kruskal-Wallis test showed non-significant differences in the expression of K15 among the three odontogenic lesions (P=0.380).

Discussion

The immunohistochemical detection of different keratins has been made in many epithelial diseases. Specifically, the expression of keratins type 1, 2, 4, 5, 6, 7, 8, 10, 13, 14, 16, 17, 18, 19 and 20 was investigated in odontogenic cysts and tumors (9-14). However, very little is known about the expression of K15 in these lesions. As far to our knowledge, there is only one study that discussed the expression of K15 in AB (15), and no presence of any previous literature that deals with K15 expression in other odontogenic cysts. This is the first study that determines the expression of K15 in DC and OKC in addition to AB.

Keratins form intermediate filament proteins of the epithelial cells. They show molecular diversity and are categorized into acidic type I keratins, which include (K9, K10, K11, K12, K13, K14, K15, K16, K17, K18, K19, and K20), while the basic or neutral type II keratins involves (K1, K2, K3, K4, K5, K6, K7, and K8). Specific pairing of types I and II molecules of keratin leads to the formation of the heteropolymeric filaments. An exception is K15, which lacks a natural co-expression partner (7,8). Keratins function as an important cytoskeleton that provides mechanical integrity of the epithelium. Furthermore, some keratins are involved in intracellular signaling pathways like during wound healing, protection from stress, and apoptosis (8). Meanwhile, K15 function in both adult stratified epithelia and different pathological lesions is not fully understood. Our results showed that K15 was expressed in most of the studied cases of DC, OKC, and AB and in different epithelial layers. The high expression of K15 in the current study could indicate the importance of K15 protein for the mechanical stability of odontogenic lesions which usually exist and extend inside strong jaw bone like the mandible in addition to the maxilla.

We observed a non-significant difference in K15 expression among DC, OKC, and AB. This interprets the same limit of extensions that necessitate the almost same method of treatment which is surgical removal of these different pathologically classified lesions. On the other hand, the non-significant difference in the expression of K15 among the studied lesions is precluding the utility of the quantity of K15 expression as

Table I. Semi-quantitative analysis of K15 protein expression in the studied samples.

Cases	No. of cases	Absent expression of K15, n (%)	Weak expression of K15, n (%)	Mild expression of K15, n (%)	Strong expression of K15, n (%)
Dentigerous cyst	13	0 (0)	4 (30.8)	0 (0)	9 (69.2)
Odontogenic keratocyst	12	2 (16.7)	0 (0)	1 (8.3)	9 (75)
Ameloblastoma	16	1 (6.3)	4 (25)	4 (25)	7 (43.8)

K15, keratin 15.

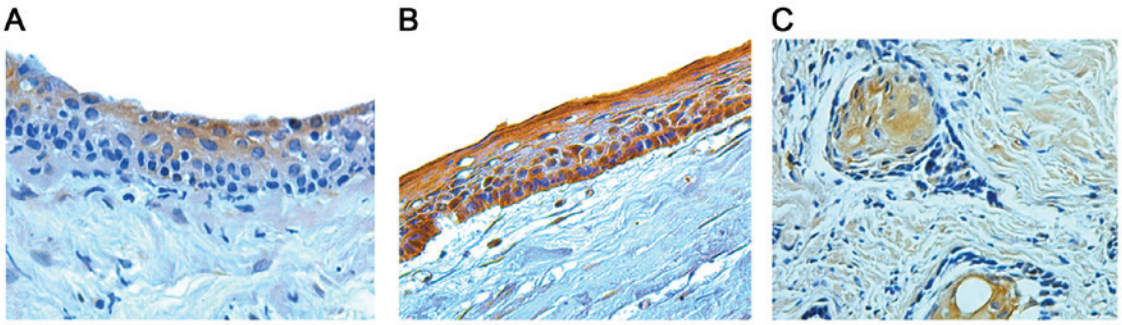

Figure 1. Immunohistochemical staining of K15 proteins that exhibited yellow-brown cytoplasmic staining in dentigerous cyst, odontogenic keratocyst and ameloblastoma (magnification, x400). K15 expression is mainly in suprabasal layers in (A) DC, (B) through all epithelial layers of OKC and (C) primarily in the central stellate reticulum-like cells than the peripheral columnar cells of AB. K15, keratin 15; DC, dentigerous cyst; OKC, odontogenic keratocyst.

an indicator to differentiate between these lesions. However, the location and distribution of K15 expression could be helpful in differentiating DC in which K15 positive cells were mostly located suprabasal than OKC which showed K15 expression throughout all epithelial layers. In the same subject, Pal *et al* (15) used K15 to differentiate between different AB histopathological types. Wassem *et al* (16) concluded that activation and proliferation of keratinocytes result in downregulation of K15. Our previous studies showed the non-different expression of proliferating epithelial cells in OKC and AB (17,18). This may explain the similar expression of K15 in odontogenic epithelium of both OKC and AB in the current study.

Tumors are hierarchically organized tissue in which tumor stem cells are responsible for the uncontrolled self-renewal and abnormal differentiation of the tumor tissues. Tumor stem cells confer the heterogeneity of tumor cellular composition. The differences in properties and response to external stimuli of benign tumor stem cells from their counterparts of normal tissue are not well known (19). The role of tumor stem cells in odontogenic lesions is essential for the better understanding of the biology and management of these lesions. Several benign and malignant odontogenic neoplasms have been reported to originate from the remnants of dental stem cells (20). In AB, different studies confirmed the expressions of stem cell markers in different locations. While the tumor stem cells are suggested to be located in the peripheral layers (20), another study demonstrate considerable expression of the P75NTR stem cell marker mostly in regions resembling the stellate reticulum (21). Further studies observed the stem cell indicators in both the stellate reticulum like cells and the peripheral basal cells of ABs (22,23). A recent study performed by Monroy *et al* (23), demonstrated a high expression of a tumor stem cell marker, namely Oct-4 in the suprabasal layers of OKC and in both stellate reticulum and peripheral columnar cells of AB, while the CD44, which is another stem cell marker used in the same study was highly expressed in all epithelium of OKC and AB (23).

K15 is mainly known to be expressed in hair follicle bulge stem cells (24). Currently, no consensus about considering K15 as a marker for stem cell population in a tissue. There are different studies indicating that K15 is a reliable marker for stem cells in a normal (24-26) and pathological tissues (27-29). Recently, a study showed that K15 marks multipotent, long-lived, and injury-resistant crypt cells. These cells may function as a cell of origin in intestinal cancer (30). Furthermore, K15 was predominantly detected in the basal cell zone of the normal oral mucosa (31). However, other studies have shown that K15 is not always restricted to the basal layers of the epithelium, but it has a variant distribution in different normal tissues. K15 expressed in the suprabasal layer in IFN-γ-treated *ex vivo* skin samples (32), esophagus (33), and in pathological conditions (34). K15 also present in uninterrupted pattern in the basal layers of oral epithelium (35). Therefore, these studies excluded the reliability of K15 to be a marker for stem cell. Bose *et al* (7), concluded that K15 could be expressed in the stem cells but also in differentiated cells. So that K15 marker may not be regarded as an indicator of the stem cell population in a tissue (7).

In the epithelium, the stem cells should represent a limited number of the total epithelial cells and usually have basal layer distribution (36). Contrary, the results of our study demonstrated high expression of K15 in different epithelial layers, including the suprabasal layer of DC, OKC, and AB. This high expression in addition to the suprabasal distribution of the K15 positive cells in our study lead to the conclusion which excludes the reliability of K15 to be a stem cell marker

in the studied odontogenic cysts and tumor. This conclusion is in agreement with other studies that doubt the use of K15 as a stem cell marker in other tissue types (7,34).

The high expression of K15 in AB could contribute to its benign activity and inability for metastasis. This suggestion is in correspondence with previous findings that connected the metastatic ability of squamous cell carcinoma to the downregulation of K15 (37). In the same line, a more pronounced expression of K15 in normal esophageal tissue occurs, compared with carcinoma tissue (38). Another observation that agree with our suggestion found a downregulation of K15 in the keratinocyte which become more mobile during wound healing (39).

Instead of being a stem cell marker, K15 staining of cells in the present study may reflect abnormal cell differentiation as the studied samples represent pathological tissues. This attribution is in accordance with a study performed by Troy *et al* (34). That study suggested that K15 expression in basal-like cells of epithelium could reflect their loss of homeostasis and probably undergoing abnormal epidermal differentiation program.

The current study, as many previous reports (9-12,15,17,18) excluded the use of control group samples in the study of odontogenic lesions. This is because no true tissue equivalent exists to serve as a negative control, as odontogenic lesions encompass reactive tissues and tumors which replace the healthy bone. The present study is limited to description of one molecular expression, which is K15. However, it is the first study to investigate and compare the expression of K15 in DC and OKC. This study will open the door for subsequent clinical applications to evaluate the pathways and related factors of K15 expression in odontogenic cysts and tumors. At the time of the diagnosis of the odontogenic cysts and tumors, investigators do not know the exact time for initiation of these lesions. Therefore, it is irrational to correlate the radiographical size of the odontogenic cysts and tumors to the biomarkers, like K15. Furthermore, correlating immunohistochemical marker, like K15 in our study to the site, age, or sex of the patient needs a relatively large study samples which is usually not present in a study of odontogenic lesions. For all these reasons, the present study did not correlate the K15 expression with the size of lesion, site, sex, or age of the patients.

The current standard management of the DC, OKC, and AB is the surgery which usually results in significant morbidity especially in large lesions and high recurrence rate as in OKC and AB. Additionally, surgery may necessitate the need for graft tissues. Overexpression of K15 in the studied odontogenic lesions could be helpful for the understanding the disease process in these pathogenically poorly understood lesions and could lead to the development of an adjuvant non-surgical treatment modality to control the disease process. One of those modalities could include the radiolabelling mAb against K15 as a tool for the therapy of DC, OKC, and AB. This suggestion is supported by several studies that formulated the initiation of radioimmunotherapy against different keratins like K19 in the management of cervical cancer (40), and against K8 in head and neck squamous cell carcinoma (41).

The present study confirmed the high expression of K15 in different epithelial layers of DC, OKC, and AB. This manner of expression excludes the reliability of regarding K15 as a stem cell marker in DC, OKC, and AB. But rather, K15 may reflect abnormal differentiation of pathological epithelial cells in these lesions.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

MAA designed the study, collected the samples, performed immunohistochemical analyses and microscopical studies, conducted statistical analysis and wrote the manuscript. AMA designed the study, performed microscopical studies and wrote the manuscript. SZ designed the study, collected the samples and wrote the manuscript.

Ethics approval and consent to participate

The present study was approved by the Institutional Review Board of Tongji Medical College, Huazhong University of Science and Technology (Wuhan, China) and followed the protocol of the World Medical Association Declaration of Helsinki. Due to the retrospective nature of the present study the requirement for informed consent was waived.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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