(Short Communication)

Immunohistochemical Expression of CD44 in Ameloblastoma and Keratocystic Odontogenic Tumor

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ABSTRACT

Ameloblastoma (AM) is most commonly encountered in the jaw bones. The etiopathogenesis is idiopathic. Keratocystic odontogenic tumor (KCOT) is controversially considered a cyst and a tumor. According to WHO, KCOT is a benign, unicystic, or multicystic, intraosseous tumor of odontogenic origin. Either syndromic or non-syndromic, the PTCH gene is a proven participant in the etiology. This study contrasts the expression of AM and KCOT for CD44. CD44, a transmembrane glycoprotein, is a homing cell adhesion molecule and is a stem cell marker for breast, prostate, pancreatic cancers, and for several head and neck benign and malignant tumors. The results show that KCOT demonstrates a stronger positivity than AM. In conclusion, the breakdown in the cellular adhesion, as a clue to osteoclastogenesis, is stronger in KCOT than in AM. The strong expression of KCOT for the monoclonal antibody of CD44 defies the cystic nature and supports the neuroplasticity of KCOT.

1-Introduction

Ameloblastoma (AM) is a common odontogenic tumor in the oral cavity whose idiopathic nature manipulates scholars and clinicians. The etiopathogenesis of AM is also

controversial. AM usually affects young adults in the fourth and fifth decades of life, causing local discomfort and bony expansion [1, 2]. Ameloblastoma has, according to the taxonomy of the World Health Organization, four histological phenotypes: conventional, peripheral, desmoplastic, and unicystic types. The conventional (solid/multicystic) AM reveals, in turn, other subtypes including, among many, follicular, plexiform, acanthomatous, keratoameloblastoma, hemangiomatous, adenoid, and granular AM. Yet, several subtypes may be intermingled at the same lesion [3].

Keratocystic odontogenic tumor (KCOT) is a benign, unicystic or multicystic, intraosseous tumor of odontogenic origin. Its etiopathogenesis involves an active role of the PTCH gene. Clinically, KCOT may be solitary or multiple. The latter is usually one of the stigmata of the inherited nevoid basal cell carcinoma syndrome. According to the WHO Working Group, the term KCOT is recommended because it better reflects its neoplastic nature. KCOT is, moreover, encountered in the mandible more frequently than in the maxilla [4].

Recently, a clinicopathological association of KCOT and ameloblastoma has been reported [5]. Moreover, Gurgel et al concluded transcriptional profiles of SHH pathway genes in both KCOT and in ameloblastoma. Recruiting qPCR, they detected overexpression of upstream (PTCH1 and SMO) and downstream (GLI1, CCND1, and BCL2) genes in the SHH pathway which proposed a constitutive activation of this pathway in both KOT and ameloblastoma. This also prospected a shared mechanism for the development of these types of tumors [6] This prompted speculations about any shared pathways between the above-mentioned lesions. Toward a better understanding of the pathogenesis of ameloblastoma and of KCOT, this study uses a pair of immunohistochemical markers, which are expected to highlight a previously unexplored pathway in both ameloblastoma and KCOT.

CD44 is a transmembrane glycoprotein that participates in many cellular processes, including growth, survival, differentiation, and motility [7,8]. It is ubiquitously expressed on the surface of many mammalian cells including endothelial cells, epithelial cells, fibroblasts, keratinocytes, and leukocytes [9]. Moreover, some CD44-specific isoforms are present in cancer stem cells (CSCs) which can be proven effective in pharmaceutically targeting such tumors.

Accordingly, CD44 is a homing cell adhesion molecule and is a stem cell marker for breast, prostate, pancreatic cancers and for several head and neck benign and malignant tumors [10,11].

Sathi et al. [11] indicated that CD44 is expressed and is possibly involved in the recurrence of AM. They also specified central stellate reticulum-like cells, situated in the close vicinity of the peripheral ameloblast-like cells, to be candidate cancer stem-like cells in AM.

2. Materials and Methods

Specimen selection

A total of 40 cases of non-syndromic KCOTs (n=20) and ameloblastoma (n=20) were submitted in this study to be stained for CD44. All were contrasted to five archival cases of normal mucosa. Of the 20 cases of AM, there were 3 unicystic, 8 plexiform, 7 follicular, and 2 basaloid AM. All the submitted cases of KCOT were parakeratinized.

Immunohistochemical procedures

From the archival formalin-fixed paraffin tissue blocks, 4 µg sections were cut and mounted on positively charged glass slides. Sections were deparaffinized with xylene and rehydrated in graded ethyl alcohol, sections were immersed in a citrate buffer solution of PH 4.8 and were put in the microwave oven before staining procedures. For immunostaining, a universal kit (R&D Systems; USA) was used. Peroxidase anti-peroxidase method of immunostaining using the streptavidin-biotin system was carried out where 3% hydrogen peroxide was applied to the sections to block the endogenous peroxidase activity. The sections were immunostained for CD44 antibody (dilution of 1: 100, clone VFF-7; Novocastra Laboratories Ltd, Newcastle, UK)

The tissue sections were incubated overnight at room temperature. Sections were then covered by the link antibody followed by streptavidin labeling antibody. After rinsing with PBS, DAB chromogen was applied to the sections followed by counter stain. Sections were then dehydrated in graded alcohol and were cleared in xylene to be mounted.

Image analysis

For each positive section, four microscopic fields showing the highest immunopositivity were selected and photomicrographs were captured at a magnification of 40×. Images were then

transferred to the computer system for analysis using the image analysis software (Image J, 1.43r, NIH, USA), to measure the area fraction of immunopositivity for CD44. Area fraction was calculated as the ratio of immunopositive area to the total area of the microscopic field.

Statistical Analysis

Statistical analysis was carried out on the tabulated data using Statistical Package for Social Sciences (SPSS) software (version 19, SPSS, Inc., Chicago, IL, USA). One-way ANOVA with post-hoc Tukey HSD tests was calculated to compare the immunoexpression of CD44 in both lesions. The corresponding p-value was calculated to determine the significance level where $p < 0.05$ was considered significant and $p < 0.01$ was considered highly significant.

3-Results

The five archival cases of normal mucosa were negative for CD44. However, homogenous membranous labeling for CD44 was observed in twelve cases (60%) of AM and sixteen cases (80%) of KCOT. The palisading basal cells of the positive cases of KCOT as well as the suprabasal epithelial cells revealed stronger immunoreactivity than in stromal cells (Figures 1 &2). Also, the membranous immunostaining in the positive cases of AM was stronger in the peripheral columnar cells than in the central stellate-reticulum-like cells. Of these, the plexiform pattern demonstrated the strongest immunoreactivity for CD44 (Figure 3) while the unicystic type showed the weakest immunostaining (Figure 4).

In AM, on the one hand, the mean area fraction was 5.961 (±1.49) in the epithelial cells and 1.23 (±0.035) in the stromal cells. On the other hand, the mean area fraction was 10.803 (±1.86) in the epithelial cells and 1.593 (±0.035) in the stromal cells as shown in Table 1.

The p-value corresponding to the F-statistic (45.8133) of one-way ANOVA between the immunoexpression of AM and KCOT is less than 0.05. The post-hoc Tukey HSD test was performed to compare with the appropriate critical value of the studentized range distribution.

As shown in Table 2, there is a highly significant difference between the immunoexpression of AM and KCOT for CD44. Also, the statistical differences between the immunoreactivity of the epithelial and stromal components were highly significant in AM and

KCOT. Nevertheless, there was no statistical significance between the immunoreactivity of the stromal cells in the AM and KCOT.
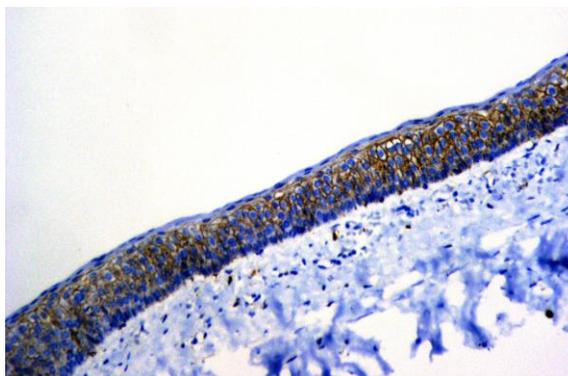
Figure 1. Immunohistochemical staining for CD44 demonstrating strong homogenous membraneous labeling in the lining epithelial cells of the KCOT (Original magnification: 40x).
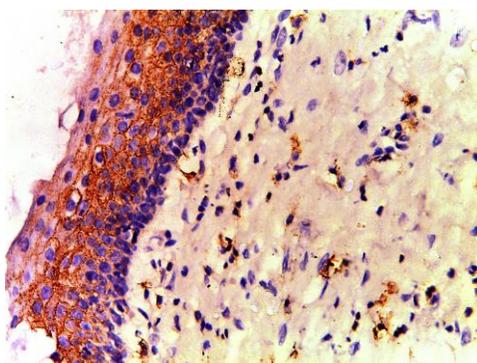
Figure 2. Immunohistochemical staining for CD44 representing moderate homogenous membraneous labeling in the lining epithelial cells of the KCOT (Original magnification: 40x).

Table 1. Expression of AM and KCOT for CD44

	Ameloblastoma	**KCOT**
Degree of positivity	12 out of 20	16 out of 20
	3 week (unicystic)	2 week
	4 moderate (2 follicular, 1 basaloid, 1 plexiform)	3 moderate
	5 Strong	11 strong
Mean area fraction		
Epithelial cells	5.961 (±1.49)	10.803 (±1.86)
Stromal cells	1.23 (±0.035)	1.593 (±0.035)

Table (2): Results of post-hoc Tukey HSD test

Category	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inference
AMB (EP) vs AMB (ST)	6.7356	0.0010053	**P< 0.01
AMB (EP) vs KCOT (EP)	7.1224	0.0010053	* P< 0.01
AMB (EP) vs KCOT (ST)	6.4311	0.0010053	* P< 0.01
AMB (ST) vs KCOT) (EP)	14.1258	0.0010053	** P< 0.01
AMB (ST) vs KCOT (ST)	0.5722	0.8999947	Insignificant
KCOT (EP) vs KCOT (ST)	14.1389	0.0010053	* P< 0.01

4. Discussion

Given that both AM and KCOT are characterized by a locally invasive behavior with a high risk of recurrence, the activated pathways of osteoclastogenesis are much considered a common ground between AM and KCOT [12]. Accordingly, this study recruited a versatile monoclonal antibody, CD44, to compare between these two lesions. Xu et al [13] have concluded that CSCs are present not only in cancers but also in benign lesions. This study supports this finding by reporting strong immunoreactivity of AM and KCOT for CD44. It also reproves the allegation of considering the KCOT a cyst, with no neoplasticity, because monoclonal antibodies are specific for tumors, unlike polyclonal antibodies.

Consistent with Wang and Liu [14] and Salehinejad et al [15], this study reported a strong expression of KCOT for CD44. The mean area fraction (MAF) of KCOT immunoreactivity was almost twice that of the MAF of AM (p-value<0.01). Furthermore, this may account for the higher recurrence rate of OKCT than AM.

Either CD44+ cells are responsible for tumor initiation and progression [11] or are considered able to initiate tumor recurrence upon completion of treatment [16], the least MAF of unicystic AM can be suggestive of the non-aggressive nature of this type of AM.

By the same token, the weak immunoreactivity of stromal cells in AM and KCOT criticizes the claim of Wang and Liu [14] and Sathi et al [11] which attributed an active role of these cells in the clinicopathological aggression of KCOT and of AM respectively.

However, our study goes hand in hand with Sathi et al [11] as regards the strongest immunoexpression of the plexiform patterns among the variant of solid/multicystic AM.

5. Conclusion

CD44, the cancer stem cell marker, is highly expressed in AM and in KCOT. The strong expression of KCOT for the monoclonal antibody of CD44 defies the cystic nature and supports the neuroplasticity of KCOT. Since CD44 is also known as homing for cellular adhesion, the breakdown in the cellular adhesion, as a clue to osteoclastogenesis, is stronger in KCOT than in AM.

- **Conflict of Interest**

The author declares no conflict of interest.

5. References

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