Prognostic Significance of Reck in Ameloblastoma and Ameloblastic Carcinoma

Mofoluwaso Abimbola OLAJIDE[1*], Bukola Folasade ADEYEMI[2], Akinyele ADISA[2], Bamidele KOLUDE[2]

[1] Department of Oral Pathology& Oral Medicine, Faculty of Dentistry, Lagos State University College of Medicine, Ikeja, Nigeria.

[2] Department of Oral Pathology /Oral Medicine, Faculty of Dentistry, University of Ibadan/ University College Hospital, Ibadan, Nigeria.

*Corresponding author

ABSTRACT

Background- Ameloblastoma is a benign aggressive neoplasm of odontogenic epithelium. It has a high rate of recurrence, occasional metastasis and malignant transformation. Reversion-inducing cysteine rich protein with kazal motifs (RECK) gene is a tumour suppressor gene expressed in various normal organs and has been found to be important in suppressing tumour invasion, metastasis and angiogenesis. RECK levels are significantly down regulated in many tumours compared with the adjacent normal tissue and detection of normal or elevated RECK levels in tumour samples has been associated with decreased invasiveness and metastatic potential, with improved prognosis. Poor clinical prognosis in ameloblastoma may be associated with decreased RECK expressions. The aim of this study was to assess the prognostic significance of RECK in ameloblastoma and ameloblastic carcinoma, by determining expression of RECK protein.

Methods- Selected Paraffin blocks of 50 ameloblastoma cases, 8 ameloblastic carcinoma cases and 50 control cases (pyogenic granuloma) were sectioned and stained with commercial antibodies for RECK. Immunohistochemical staining of stromal and tumour cells in individual cases and study controls was assessed at X100 magnification to obtain a quantified combined score for each one. Proportion of cases with positive expression of RECK were compared between ameloblastoma and ameloblastic carcinoma, using chi square statistics. Mean scores for RECK expression in ameloblastoma and ameloblastic carcinoma cases were compared with that of the control group using the ANOVA test.

Results- RECK was positive in a greater proportion of ameloblastoma (96%) than ameloblastic carcinoma (87.5%) than in control cases (80%) (p = 0.049). The mean score of RECK expression in Ameloblastoma was higher than Ameloblastic carcinomas. The mean score of RECK expression was also higher in unicystic ameloblastoma than solid multicystic ameloblastoma.

Conclusion-   RECK may be a promising prognostic marker in ameloblastoma and ameloblastic carcinoma.

INTRODUCTION

Ameloblastoma is a benign aggressive neoplasm of odontogenic epithelium.[1] Odontogenic tumours are generally said to be rare and mostly benign, with odontomas having the highest frequency in western populations.[2-4] A review of odontogenic tumours in Nigeria however shows ameloblastoma as the most common accounting for 63%.[5] The predominance of ameloblastoma is consistent with other African and Asian studies.[6-8]

Ameloblastoma is characterized by local invasiveness, with propensity for facial deformity, tooth displacement and malocclusion; a high rate of recurrence, occasional metastasis and malignant transformation. Although

classified as benign, its biologic aggression poses a significant therapeutic challenge.[1,3, 10]

Various molecular alterations responsible for development and progression of odontogenic tumours have been identified.[10, 11,12] Reversion-inducing cysteine rich protein with kazal motifs (RECK) was initially discovered due to its ability to induce reversion in ras-activated fibroblasts. RECK gene is therefore a tumour suppressor gene. However, another prominent function of RECK is inhibition of Matrix Metalloproteinases (MMPs), especially MMP-2 and MMP-9 thereby significantly limiting invasion.[13, 1] Several odontogenic tumours, including ameloblastoma, keratocystic odontogenic tumours (KOT) and odontogenic myxoma have been found to express MMPs -1,2,7,9,26 and inhibitors- like reversion inducing cysteine-rich protein with kazal motifs (RECK).[15-19] . In ameloblastoma, inhibition of MMP-2 expression and activity by RECK is thought to be at the post-transcriptional level.[19]

Several studies have shown that RECK levels are significantly down regulated in many tumours including human pancreatic, mammary, lung, prostate, liver, colorectal cancers and neuroblastoma, compared with the adjacent normal tissue.[20-23] In the same vein, detection of normal or elevated RECK levels in tumour samples is associated with decreased invasiveness and metastatic potential and improved prognosis.

Treatment recommendation for ameloblastoma based on current knowledge is mainly surgical, and even where conservative surgical intervention is indicated (such as in unicystic ameloblastoma) there are still recurrence in up to 60% of cases.[24, 25] Hence, better understanding of the molecular mechanisms involved in the pathogenesis and progression of ameloblastoma may provide direction for targeted therapy.[13]

The aim of this study is to determine the prognostic utility of RECK by assessing differential expression in biologic sub-types of ameloblastoma, ameloblastic carcinoma and pyogenic granuloma.

MATERIALS AND METHODS

This was a laboratory-based study to demonstrate the expression RECK in ameloblastoma and ameloblastic carcinoma and compare to a control group of pyogenic granuloma using immunohistochemistry. The study was carried out at the Department of Oral Pathology, University College Hospital (UCH), Ibadan. Immunohistochemistry was done at the Breast Cancer Research Project Laboratory, Institute for Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, using formalin-fixed paraffin embedded (FFPE) tissues of ameloblastoma and ameloblastic carcinoma histologically diagnosed between January 2000 and December 2011.

Cases of ameloblastoma were histologically sub-typed as plexiform, follicular, acanthomatous, granular, cystic, haemangiomatous and mixed variants. The ameloblastomas were also classified according to the 2022 WHO classification of odontogenic tumours into Conventional, unicystic and peripheral biological types.[4]

Fifty (50) cases of ameloblastoma were used along with eight (8) ameloblastic carcinomas. Selection of cases was done with a deliberate attempt to ensure that biological types of Ameloblastoma according 2022 WHO classification of Odontogenic Tumours was included, by selecting representative cases for each biological group. For the control group, FFPE of fifty (50) cases of pyogenic granuloma were also consecutively selected for immunohistochemistry. Test and control group slides were stained for RECK.

Immunohistochemical Staining Procedure:

 Deparaffinisation: One slide per FFPE was produced for immunohistochemical staining for the cases and controls. They were deparaffinised by passing them through changes of xylene twice for 5 minutes each, then rehydrated in decreasing grades of alcohol. This was followed by rinsing in phosphate buffered saline (PBS).

Antigen unmasking and retrieval: The slides were immersed in heat induced epitope retrieval citrate buffer (10 mM sodium citrate buffer at pH 6.0) and pre-treated in a microwave oven set at 90^0C for 1 hour. They were removed and placed in fresh citrate to cool for 20 minutes. This was followed by a 10 minute rinse in PBS.

Blocking of endogenous peroxidase: The slides were placed in a humidity chamber, the tissue periphery marked with a hydrophobic pen and blocking of endogenous peroxidase was done with 3% hydrogen peroxide for 10 minutes at room temperature after which the slides were rinsed in PBS.

Immunoperoxidase staining: The slides were incubated for an hour with appropriately characterized and diluted primary antibodies (40 – 130 µl, depending on the surface area of the tissue): anti-RECK (1:50 mouse monoclonal antibody, by Santa Cruz biotechnology). Excess reagent was wiped dry from each slide with laboratory tissue paper. This was followed by incubation of the slide sections with the appropriately labelled polymer horse radish peroxidase (HRP) conjugated anti-mouse secondary antibody for 30 minutes, after which they were rinsed in PBS.

The areas surrounding the paraffin section were wiped dry with laboratory tissue paper and 1ml of diaminobenzidene (DAB) chromogen was added to cover the specimen. After visualization of the reaction with the diaminobenzidine chromogen, incubation in humidity chamber for 15 minutes was done. The slides were immersed in a bath of aqueous haematoxylin about 10 times for 1 minute each and then rinsed in distilled water for 3 minutes after 10 immersions for counterstaining. The slides were then passed by dipping through a series of graded alcohol to dehydrate the sections and then also rinsed with xylene. A mounting fluid (glycerine gel) was applied and a cover slip placed.

Laboratory positive and negative controls for each of the antibodies were used as recommended by the manufacturer to validate the procedure. Normal colon specimen was used for RECK.[20] Negative controls were slide sections from each control FFPE tissue, but incubated with PBS only, instead of the indicated primary antibody. Slides of each of the positive and negative controls followed the same protocol for immunohistochemical staining as the other slides for the study.

Evaluation of Immunohistochemical Staining:

The indicated positive control slide was used as a reference guide. Both stromal and tumour cells were assessed for staining in cases of ameloblastoma and ameloblastic carcinoma while the stroma was evaluated in the case of pyogenic granuloma. The manufacturer's guide was used to evaluate staining colour quality for positive staining of cells. Cytoplasmic staining was used for positive staining in each of the antibodies, as documented by manufacturers.

Immunohistochemical staining of cells was assessed using both stain intensity (depth of colour or intensity of staining) and proportion of cells stained. Stain intensity was graded using a semi quantitative 4-point scale: 0 = no staining; 1= mild staining; 2= moderate staining; and 3= intense staining. The proportion of cells stained was assessed at X100 magnification using a semi quantitative 4-point scale: 0 = no cell staining in any microscopic fields; 1= <25% staining; 2= 25-50% staining; and 3= >50% staining. The combined score (proportion plus intensity) was determined. The final combined score in the case of ameloblastomas and ameloblastic carcinomas was determined by finding the average of combined score in the stromal and tumour epithelial cells. The combined score was categorised as follows: <2, negative staining or low staining (-); 2 and 3, moderate staining (+); and ≥4, strong staining (++). A combined score equalling or exceeding (+) was defined as positive for RECK.[26]

Data Analysis:

Data was analysed with SPSS version 20.0. Data was presented as tables and graphs using summary statistics such as mean and standard deviation for quantitative data like age and scores for intensity and proportion of antibody staining for RECK. Qualitative data such as age group, sex, histopathological variants and biological types were expressed as proportions/percentages. Immunohistochemical staining were compared between ameloblastoma and ameloblastic carcinoma and the pyogenic granuloma using Pearson's Chi square statistics. Mean scores for RECK staining intensity and proportion in ameloblastoma and ameloblastic carcinoma cases were compared were compared with that of the control group (pyogenic granuloma) using the ANOVA test. Mean scores of these proteins were compared within biological types, as well as between the sex groups using the student t test. The level of significance was set at $p < 0.05$.

RESULTS

Table 1- Categories of RECK expression in the cases and control lesions

Staining category	Pyogenic granuloma (50)	Ameloblastoma (50)	Ameloblastic Carcinoma (8)
-	10 (20%)	2 (4%)	1 (12.5%)
+	11 (22%)	8 (16%)	3 (37.5%)
++	29 (58%)	40 (80%)	4 (50%)

KEY- (Negative), + (Positive), ++ (Strongly positive)

Table 1 shows RECK was positive in a greater proportion of ameloblastoma (96%) than ameloblastic carcinoma (87.5%) and least in pyogenic granuloma (80%). This distribution was statistically significant (Pearson's $\chi2$ = 6.05, df = 2, p = 0.049).

Table 2- Semi quantitative expression of RECK protein in study controls (pyogenic granuloma) and ameloblastic neoplasms

Pyogenic granuloma (n = 50)	Ameloblastoma (n = 50)	Ameloblastic Carcinoma (n = 8)	p value
3.50 (SD±1.61)	4.00 (SD±1.12)	3.00 (SD ±2.03)	0.09

The mean values of RECK demonstrated by immunohistochemical staining in pyogenic granuloma, ameloblastoma and ameloblastic carcinoma are shown in Table 2. The mean RECK value was lower in ameloblastic carcinoma than benign ameloblastoma. The mean value of RECK was also lower in pyogenic granuloma than ameloblastoma but higher than ameloblastic carcinoma. This difference was however not statistically significant.

Table 3- Semi-quantitative expression of RECK in Ameloblastoma and Ameloblastic carcinoma in the sex groups.

SEX (n=50)	AMELOBLASTOMA	SEX (n=8)	AMELOBLASTIC CARCINOMA
MALE (n=27)	3.94 (SD±1.09)	MALE (n=3)	2.83 (SD±2.57)
FEMALE (n=23)	4.06 (SD±1.16)	FEMALE (n=5)	3.10 (SD± 1.98)
p value	0.71	p value	0.87

Table 3 shows the semi-quantitative expression of RECK was not significantly different in both genders.

Table 4- Semi-quantitative expression of RECK within age groups of Ameloblastoma and Ameloblastic Carcinoma

Age Group (years)	Number of Cases	Mean Score (SD)
0–29	27	3.69 (SD ± 1.21)
30–49	15	4.27 (SD ± 0.96)
≥ 50	8	4.56 (SD ± 0.73)
p-value	—	**0.08**
Age Group (years)	Number of Cases	Mean Score (SD)
10–19	1	5.00
20–29	2	4.25 (SD ± 1.06)
30–39	3	3.00 (SD ± 2.18)

40–49	1	0.00
50–59	1	1.50
p-value	—	**0.43**

Table 5- Semi-quantitative expression of proteins within the biological types of Ameloblastoma

BIOLOGICAL TYPES	RECK
Conventional (n = 30)	3.95 (SD±1.15)
Unicystic (n = 20)	4.08 (SD±1.00)

Conventional type showed a lower score of RECK protein than Unicystic Ameloblastoma.

Table 9 Semi-quantitative expression of proteins within histological variants of Ameloblastoma

HISTOLOGICAL VARIANTS	NO. OF CASES	RECK
Plexiform	14	3.93(SD±1.07)
Follicular	5	4.20(SD±0.97)
Cystic mural	15	4.23(SD±0.84)
Cystic luminal	2	2.25(SD±1.06)
Cystic intraluminal	3	4.50(SD±0.50)
Hybrid (Desmoplastic + Follicular)	1	6.00
Haemangiomatous	1	1.00
Mixed follicular + plexiform	1	4.50
Mixed plexiform + basaloid	2	4.25(SD±0.35)
Mixed plexiform + haemangiomatous	4	3.50(SD±1.29)
Mixed-plexiform + granular	1	5.50
Mixed-plexiform +acanthomatous	1	3.00

There was statistically significant difference in the expression of RECK, with the lowest values being observed in the haemangiomatous and cystic luminal subtypes, while the highest values were observed the hybrid and mixed (plexiform and granular) subtypes (F= 2.50, df = 11, p = 0.02).
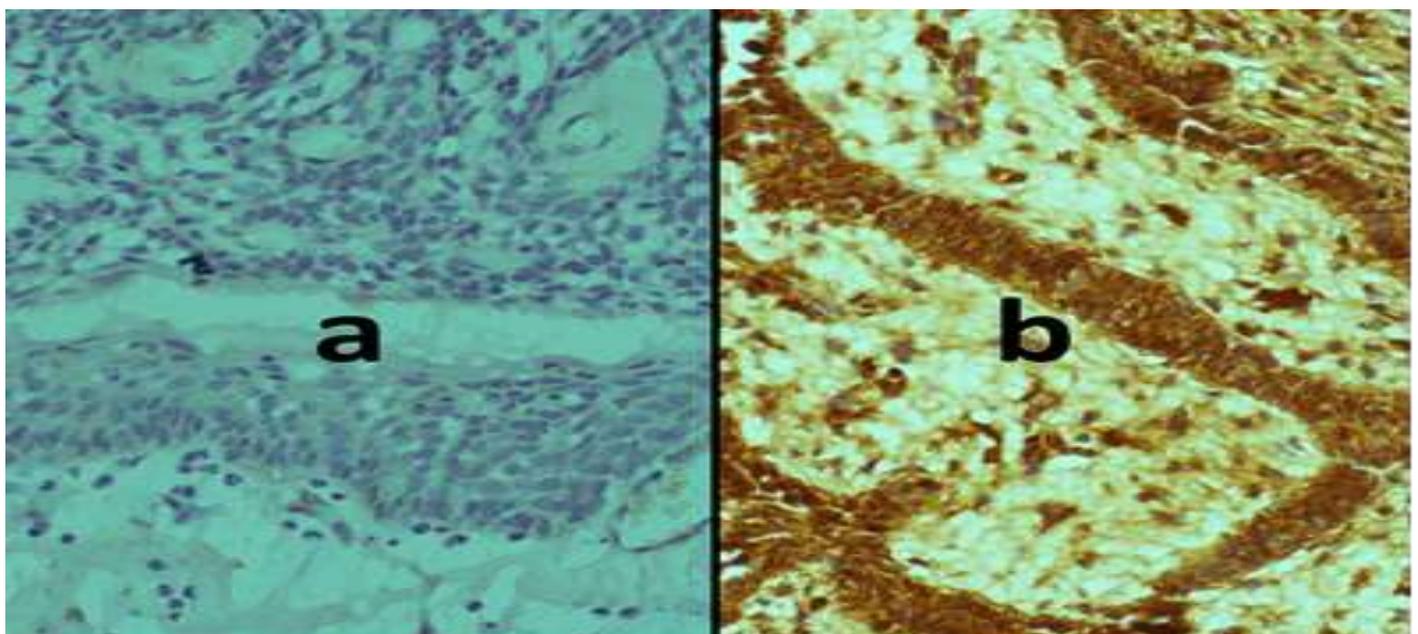
Figure 8: a. Photomicrograph showing negative staining for RECK in a case of ameloblastoma (X40); b. Photomicrograph showing strongly positive staining for RECK in a case of ameloblastoma (X40)
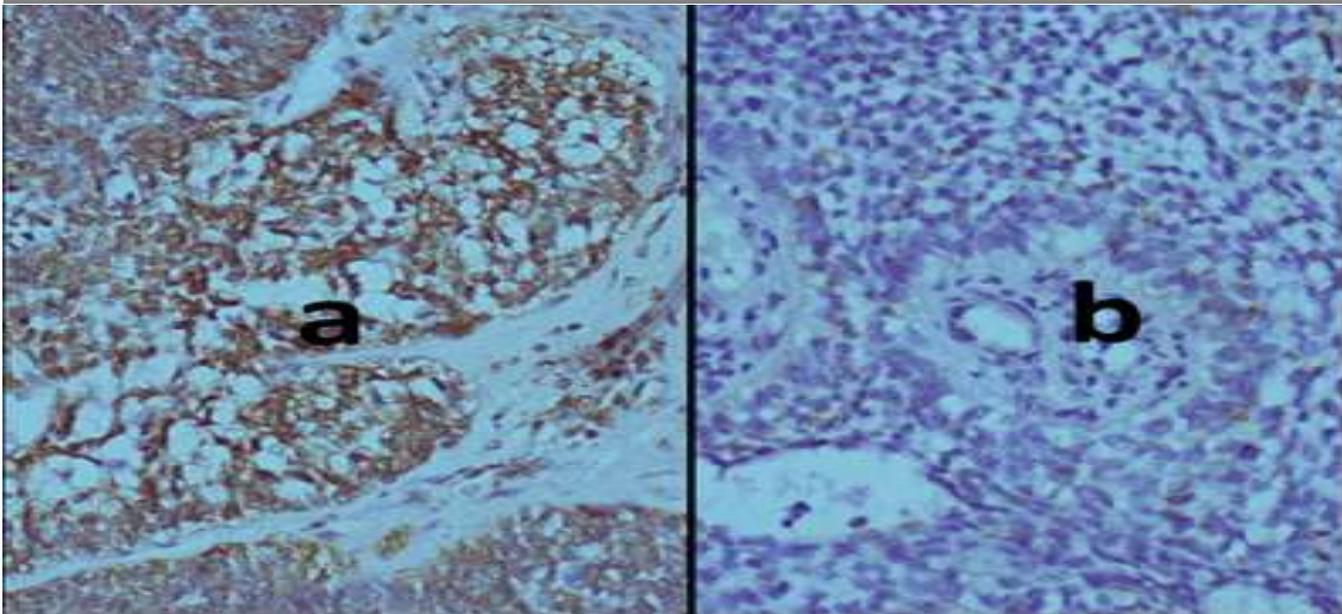
Figure 9: a. Photomicrograph showing strongly positive staining for RECK in a case of ameloblastic carcinoma (X40); b. Photomicrograph showing weakly positive staining for RECK in a case of ameloblastoma (X40)

DISCUSSION

In this study, RECK was significantly positive in more cases of ameloblastoma than ameloblastic carcinoma cases. This may not be too far-fetched, since RECK was first discovered as a tumour suppressor gene and has been found to be down regulated in many human cancers.[27, 28] The difference in mean RECK expression of pyogenic granuloma being lower than that of ameloblastoma but higher than in ameloblastic carcinoma, though not statistically significant, does not explain the role of RECK in reactive lesions. Paucity of studies on RECK expression in pyogenic granuloma make it difficult to explain its role in this lesion.

Among the biologic types studied, the unicystic types had a higher mean RECK expression than the conventional types. This reflects on the tumor suppressor role of RECK being of less effect, in the more aggressive conventional type, though there is paucity of comparable studies on this protein among the biologic types of ameloblastoma.

This study noted a statistically significant difference in the expression of RECK among the histological variants. On the contrary, the study by Bin Zhang et al[16] reported that the expression of RECK did not differ by histological type of ameloblastoma. The differences in number of cases, detection methodology and study power may account for the differences, however, histological variants of ameloblastoma are not known to have any clinical significance[28], so findings in our study should be interpreted with caution.

For ameloblastic carcinoma, this study observed that mean RECK expression decreased with higher age with no expression seen in the single case represented in the fifth decade. Generally, it is known that older ages have worse prognosis in malignancies compared to younger ages and a lower RECK score may support this. In benign ameloblastoma, the reverse pattern was observed, where the mean RECK expression increased with increasing age and the highest score was seen in the age group > 50. This may suggest that conventional ameloblastoma in first to third decade may be more locally aggressive. In a study by Yue Zhang et al, which investigated RECK in invasive breast cancer, they reported not finding any significant difference in RECK expression and age, and age had no prognostic significance for RECK expression by cox regression analysis.[29] The limited number of ameloblastic carcinomas in our study restricts generalizability of our findings regarding age and RECK expression.

In conclusion, results from our study suggest that ameloblastoma and ameloblastic carcinoma with lower RECK expression may display more aggressive clinical behaviour.

Limitations in this study include the restricted number of samples and the semi- quantitative method of evaluating immunohistochemical staining. A larger cohort of samples and direct quantification of proteins or mRNA studies using Polymerase Chain Reaction may give more precise data.

REFERENCES

1. Regezi JA Scuibba J, Jordan C. (Eds.). Odontogenic Tumors. Oral Pathology:Clinico pathologic correlations. 5th edition, Saunders Elsevier; 2003: 265-270.
2. Guerrisi M, Piloni MJ, Keszler A. Odontogenic tumors in children and adolescents. A 15-year retrospective study in Argentina. Medicina Oral, Patologia Oral y Cirugia Bucal. 2007;12(3):E180-185.
3. Fletcher DM. (Ed.). Odontogenic Tumors. Diagnostic Histopathology of Tumors. 2nd edition, Churchill livingstone; 2000: 217-220.
4. WHO Classification of Tumours Editorial Board. Head and neck tumours [Internet; beta version ahead of print]. Lyon (France): International Agency for Research on Cancer; 2022. (WHO classification of tumours series, 5th ed.; vol. 9). Available from: https://tumourclassification.iarc.who.int/chapters/52.
5. Ladeinde AL, Ajayi OF, Ogunlewe MO, Adeyemo WL, Arotiba GT, Bamgbose BO, *et al*. Odontogenic tumors: a review of 319 cases in a Nigerian teaching hospital. Oral Surgery, Oral Medicine, Oral Pathology, Oral adiology, and Endodontics. 2005;99(2):191-195.
6. Hendra FN, Van Cann EM, Helder MN, et al. Global incidence and profile of ameloblastoma: A systematic review and meta-analysis. Oral Dis. 2020; 26: 12–21.
7. Akinshipo AW, Sivaramakrishnan G, Enwuchola J, Effiom O, Adeoye J, Ramanathan A, Chaurasia A. Unraveling the Mysteries of Ameloblastoma in African Population: A Comprehensive Analysis of 371 Cases from Clinical, Radiological, and Histopathological Perspectives. Head Neck Pathol. 2025; Jan 7; 19(1):2.
8. Intapa C. Analysis of Prevalence and Clinical Features of Ameloblastoma and its Histopathological Subtypes in Southeast Myanmar and Lower Northern Thailand Populations: A 13-Year Retrospective Study. J Clin Diagn Res. 2017 Jan;11(1):ZC102-ZC106.
9. Mendenhall WM, Werning JW, Fernandes R, Malyapa RS, Mendenhall NP. Ameloblastoma. American Journal of Clinical Oncology. 2007;30(6):645-648.
10. Kumamoto H. Molecular pathology of odontogenic tumors. Journal of Oral Pathology & Medicine. 2006;35(2):65-74.
11. Heikinheimo K, Jee KJ, Niini T, Aalto Y, Happonen RP, Leivo I, *et al*. Gene expression profiling of ameloblastoma and human tooth germ by means of a cDNA microarray. Journal of Dental Research. 2002;81(8):525-530.
12. .Owosho AA, Ladeji AM, Adebiyi KE, Olajide MA, Okoye ISI, Kehinde T, Nwizu NN, Summersgill KF. BRAF V600E mutation-specific immunohistochemical analysis in ameloblastomas: a 44-patient cohort study from a single institution. Eur Arch Otorhinolaryngol. 2021 Aug; 278(8):3065-3071.
13. Zhang B, Zhang J, Xu ZY, Xie HL. Expression of RECK and matrix metalloproteinase-2 in ameloblastoma. BMC Cancer. 2009;9:427.
14. Birkedal-Hansen H, Moore WG, Bodden MK, Windsor LJ, Birkedal-Hansen B, DeCarlo A, *et al*. Matrix metalloproteinases: a review. Critical reviews in Oral Biology and Medicine. 1993;4(2):197-250.
15. Kumamoto H, Yamauchi K, Yoshida M, Ooya K. Immunohistochemical detection of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) in ameloblastomas. Journal of Oral Pathology & Medicine. 2003;32(2):114-120.
16. Nonaka CF, Augusto Vianna Goulart Filho J, Cristina da Costa Miguel M, Batista de Souza L, Pereira Pinto L. Immunohistochemical expression of matrix metalloproteinases 1, 2, and 9 in odontogenic myxoma and dental germ papilla. Pathology, Research and Practice. 2009;205(7):458-465.
17. Cavalcante RB, Pereira KM, Nonaka CF, Nogueira RL, de Souza LB. Immunohistochemical expression of MMPs 1, 7, and 26 in syndrome and non-syndrome odontogenic keratocysts. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics. 2008;106(1):99-105.
18. Kumamoto H, Ooya K. Immunohistochemical detection of MT1-MMP, RECK, and EMMPRIN in ameloblastic tumors. Journal of Oral Pathology & Medicine. 2006;35(6):345-351.
19. Bast BT, Pogrel MA, Regezi JA. The expression of apoptotic proteins and matrix metalloproteinases in odontogenic myxomas. Journal of Oral and Maxillofacial Surgery. 2003;61(12):1463-1466.

20. Takeuchi T, Hisanaga M, Nagao M, Ikeda N, Fujii H, Koyama F, *et al*. The membrane-anchored matrix metalloproteinase (MMP) regulator RECK in combination with MMP-9 serves as an informative prognostic indicator for colorectal cancer. Clinical Cancer Research. 2004;10(16):5572-5579.

21. Furumoto K, Arii S, Mori A, Furuyama H, Gorrin Rivas MJ, Nakao T, *et al*. RECK gene expression in hepatocellular carcinoma: correlation with invasion-related clinicopathological factors and its clinical significance. Reverse-inducing--cysteine-rich protein with Kazal motifs. Hepatology. 2001;33(1):189-195.

22. Span PN, Sweep CG, Manders P, Beex LV, Leppert D, Lindberg RL. Matrix metalloproteinase inhibitor reversion-inducing cysteine-rich protein with Kazal motifs: a prognostic marker for good clinical outcome in human breast carcinoma. Cancer. 2003;97(11):2710-2715.

23. Masui T, Doi R, Koshiba T, Fujimoto K, Tsuji S, Nakajima S, *et al*. RECK expression in pancreatic cancer: its correlation with lower invasiveness and better prognosis. Clinical Cancer Research. 2003;9(5):1779-1784.

24. Lau SL, Samman N. Recurrence related to treatment modalities of unicystic ameloblastoma: a systematic review. International Journal of Oral and Maxillofacial Surgery. 2006;35(8):681-690.

25. Olaitan AA, Adekeye EO. Unicystic ameloblastoma of the mandible: a long-term follow-up. Journal of Oral and Maxillofacial Surgery. 1997;55(4):345-348; discussion 349-350.

26. Zhang B, Zhang J, Xu ZY, Xie HL. Expression of RECK and matrix metalloproteinase-2 in ameloblastoma. BMC Cancer. 2009;9:427.

27. Cardeal LB, Boccardo E, Termini L, Rabachini T, Andreoli MA, di Loreto C, *et al*. HPV16 oncoproteins induce MMPs/RECK-TIMP-2 imbalance in primary keratinocytes: possible implications in cervical carcinogenesis. PloS One. 2012;7(3):e33585.

28. Figueira RC, Gomes LR, Neto JS, Silva FC, Silva ID, Sogayar MC. Correlation between MMPs and their inhibitors in breast cancer tumour tissue specimens and in cell lines with different metastatic potential. BMC Cancer. 2009;9:20.

29. Zhang, Y., Cheng, S., Zhang, G., Ma, W., Liu, Y., Zhao, R., Zhang, Q. and Pang, D. Low expression of RECK indicates a shorter survival for patients with invasive breast cancer. Cancer Sci. 2012; 103: 1084-1089.