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COMPARATIVE EVALUATION OF SALL4 IMMUNOHISTOCHEMICAL EXPRESSION IN AMELOBLASTOMA, ODONTOGENIC KERATOCYST & DENTAL FOLLICLE – A QUALITATIVE STUDY.

Oral Pathology

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ABSTRACT

Background: SALL4 (Spalt like transcription factor 4) is a transcription factor that plays a key role in maintaining pluripotency and self-renewal of embryonic stem cells. Its expression is downregulated after birth and is absent in most adult human tissues. Specifically, its expression in adult tissues is restricted to germ cells, except for human CD34+ hematopoietic stem cells. However, its high expression and dysregulation have been demonstrated in several types of cancer. It has been reported that ameloblastic cells originate from odontogenic stem cells located in the dental lamina and that tumors are likely initiated in normal stem cells that contain a perpetual minority of cancer stem cells. Despite being a benign tumor, the biological behaviour of ameloblastoma is aggressive & recent development in management aspect couldn't lower the rate of recurrence. The purpose of the study is to investigate the immunohistochemical expression of SALL4, its role in biological behavior of ameloblastoma, OKC and Dental follicle. The role of stem cells in the genesis and progression of the odontogenic lesions. **Method:** All formalin-fixed paraffin-embedded blocks (n = 02) for each studied group were SALL4 immuno-histostained and then assessed under compound microscope. **Results:** IHC staining for SALL4 was predominantly observed in the epithelial cells of the tumor islands. **Conclusions:** Stem cell may be related to the origin and progression of odontogenic tumors and cysts as SALL4 play a significant role in the biologic behavior of ameloblastoma, OKC and other lesions of odontogenic origin.

KEYWORDS

SALL4 (Spalt like transcription factor 4), Ameloblastoma, Odontogenic Keratocyst, Dental Follicle etc.

INTRODUCTION

Odontogenic lesions comprise of odontogenic cyst & odontogenic tumors. Odontogenic tumors form diverse spectrum of lesion with less than 1% among head & neck tumors ranging from benign to hamartomatous to malignant tumors.[1] Globally & within the country they show variations in incidences due to dynamic geographical, socioeconomic, cultural, ethnic diversity & cultural factors.[2],[3] Ameloblastoma is the second most common benign, slow-growing, but locally invasive odontogenic tumor after odontome originating from the dental lamina remnants, developing enamel organ, epithelial lining of odontogenic cysts, or basal cells of the oral mucosa. It occurs more frequently in the mandible than in the maxilla & there is no significant sex predilection.[4], [5] Ameloblastoma is categorized into three types as conventional, unicystic, and extraosseous/peripheral according to the most recent classification by the World Health Organization (WHO) 2022.[6] The present management options of ameloblastoma involves conservative which include enucleation and curettage and radical approaches more invasive treatments involve marginal resection, which is the method of choice, considering the high recurrence rates reported in ameloblastoma despite that this method can result in relapse rates of up to 15% in more aggressive ameloblastoma types as well as significant facial deformities[7] why this benign entity has aggressive biological behavior and high recurrence rate is not fully understood. stem cells and their possible relationship with the etiopathogenesis of neoplasms have been relevant in elucidating this behavior.[8]

It is well established fact that the Stem cells have property of self-perpetuation through self-renewal mechanisms and differentiate into cells in specific tissues. This mechanism is similar to the process which occurs in tumor cells involving similar pathways. Therefore, normal stem cells and tumorigenic cells shares two things in common first proliferative potential & the ability to give rise to new tissues.[9]

Cancer stem cells proliferation is uncontrollable which drives it to the formation and growth of tumors result in generating heterogeneous malignant cells associated with recurrence and metastasis. It is believed that cancer cells can appropriate the self-renewal machinery that is normally expressed in normal stem cells.[8]

The role of cancer stem cell in tumor progression & prognosis has been studied very extensively by Immunohistochemical technique using markers like ALDH, BCL11B, BMI-1, CD133, CD24, CD44 & found its correlation with lymph node metastasis, Clinical TNM staging, tumor differentiation, tumor size & overall survival rate.[10] [11]

Many studies evaluating the role of cancer stem cells in ameloblastoma have been conducted & found stem cell's role in aggressiveness & recurrence, thus predicting the biological behavior & growth potential of ameloblastoma. One such study conducted by Dr. Manjushri Vanje et al. (2019)[12] where the found positive correlation between staining intensity of CD44 marker and the known biological behavior of the lesion (Ameloblastoma) & concluded that cancer stem cells may be responsible for its aggressiveness & local recurrence.

Ameloblastoma originates from odontogenic stem cells located in the dental lamina & that tumors are likely initiated in normal stem cells that contain a perpetual minority of cancer stem cells.[13]

In this context, the SALL4 (Spalt-Like Transcription Factor 4) acts as essential regulator of pluripotency and embryonic self-renewal and can mediate tumor progression and differentiation, is relevant biomarker for the analysis of stem cells.

SALL4 is an essential transcription factor for the maintenance of self-renewal and pluripotency of embryonic stem cells that occurs in early embryonic development[14] Its expression is downregulated after birth and is absent in most adult human tissues. Specifically, its expression in adult tissues is restricted to germ cells.[15] except for human CD34+ hematopoietic stem cells.[16] However, its high expression and dysregulation have been demonstrated in several types of cancer.[14],[16] such as leukemia, germ cell tumors, hepatocellular carcinoma, and lung cancer,[17] where it is acting as an oncogene and participates in the processes of initiation, development, and progression of cancer.[14]

It can be helpful to elucidate the role of these cells in the etiopathogenesis of ameloblastoma by understanding the molecular mechanisms underlying the tumor through the expression of stem cell biomarkers.

Very few studies are available elucidating the expression of SALL4 in odontogenic lesion.

So, the aim & objective of this study was to evaluate the immunohistochemical expression of SALL4, comparative evaluation in ameloblastoma, OKC & dental follicle as well as to understand their biological behavior.

METHODS

Ethical Approval

This study was conducted in accordance with the ethical guidelines & standard protocols laid down by institutional ethical committee.

Sample Collection

Histologically confirmed cases of Ameloblastoma, Odontogenic Keratocyst & Dental follicle with adequate tissue were selected for each group n=2. formalin-fixed paraffin-embedded blocks were selected retrospectively from the block cabinet of the institution.

IHC Procedure

The standard IHC protocol was followed as per the manufacture's guidelines as follows.

The immunohistochemistry technique used in the present study was performed according to the following protocol. First, deparaffinization of the slides in xylene and hydration in decreasing alcohol concentrations (100%, 90%, 80% and 70%) was conducted. Ten endogenous peroxidases were blocked by immersing the slides in 3% H2O2 to methanol at a 1:1 ratio for 30 min. Antigenic recovery was conducted in a Pascal pressure chamber (Dako Cytomation, Carpinteria, CA, USA) in a citrate buffer (pH 6.0) for 30 s with a temperature of 123°C and pressure of 13.5 psi. Finally, non-specific antibodies were blocked with 1% bovine serum albumin (BSA; SigmaAldrich) in a phosphate-buffered saline solution for 1 h. Incubation of primary antibodies for Anti-Sall4 (1:25, mouse, Santa Cruz Biotechnology, Santa Cruz, CA, USA) was conducted for 12–14 h (overnight) and for 1 h for the Anti-LIN-28 (1:30, mouse, Santa Cruz Biotechnology ®) and Anti-GKLF (1:100, mouse, Santa Cruz Biotechnology®) in the Ameloblastoma, Odontogenic Keratocyst, and Dental Follicle samples. Secondary antibodies were incubated with an Immunoprobe Plus detection system (Advanced Biosystems, San Francisco, CA, USA) for 30 min. Diaminobenzidine chromogen (ScyTek, Logan, UT, USA) was used. The slides were counterstained with hematoxylin (Sigma-Aldrich) and mounted using Permount (Fisher Scientific, Fair Lawn, NJ, USA). Testicular seminoma samples were used as positive controls. As a negative control, the primary antibody was replaced with BSA (Sigma-Aldrich) or nonimmune serum.

IHC Evaluation

The immunohistochemical (IHC) evaluation was performed by measuring the area (µm) and fraction (%) of SALL4 in Ameloblastoma, Odontogenic Keratocyst & Dental follicle. Images were analyzed under the compound microscope for intensity & localization of SALL4. Five areas in each sample were selected based on the quantity and morphological preservation of the tissue. Images were acquired using a 40x objective. Immunostaining differences found in Ameloblastoma, Odontogenic Keratocyst, and Dental Follicle were analyzed.

RESULTS

Table 01. Epidemiological, Clinical Data & Histopathological Diagnosis of Study Group.

Case	Gender	Age	location	Histopathological diagnosis
1	M	16	Mandible posterior	Dental follicle
2	M	26	Mandible posterior	Granular cell Ameloblastoma
3	M	23	Posterior Maxilla	Odontogenic keratocyst
4	M	17	Posterior mandible	Plexiform ameloblastoma
5	F	26	Posterior mandible	Odontogenic keratocyst
6	F	14	Anterior maxilla	Dental follicle

Mean age was 20 years with male representing 66% of sample. Histopathologically two case for each group comprising of two cases od ameloblastoma which involves one granular cell ameloblastoma & one plexiform amelolobla stoma, two cases of Odontogenic keratocyst & two developing dental follicles were selected. (Table 01).

Immunohistochemical Staining for SALL4

Table 02: IHC Findings (+++ intense staining, ++ moderate staining, + mild staining, - Negative staining)

Histolopathogical diagnosis	Localization	Intensity
Granular Cell Ameloblastoma	Nuclear & cytoplasmic	+++
Plexiform Ameloblastoma	Nuclear & cytoplasmic	+++
Odontogenic Keratocyst	Nuclear	+
Dental Follicle	Negative	-

IHC staining was observed in epithelial islands of ameloblastoma consisting of centrally located tall to low columnar cells & peripherally located interconnecting stellate reticulum like cells. (See fig. 01). Intense staining was evident in both plexiform & granular cell ameloblastoma. localization of staining was evident in nucleus & cytoplasm of tall columnar & stellate reticulum-like cells for plexiform ameloblastoma & for granular cell ameloblastoma staining was evident in granular cells also apart from peripheral low columnar cells.

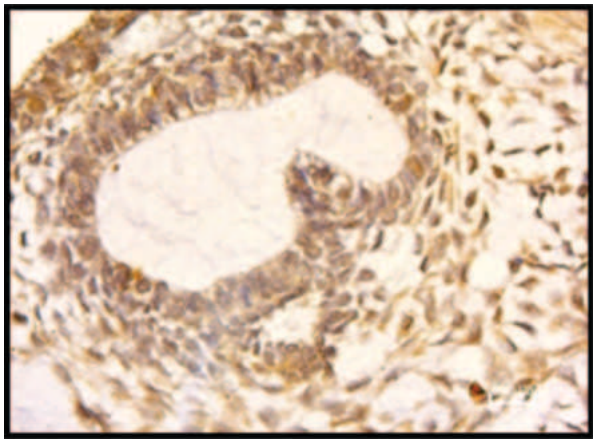

Fig 01: 40x Plexiform Ameloblastoma Showing Intense Immuno-expression for SALL4 Localization in Membrane & Cytoplasm of Epithelial Cells & Interconnecting Stellate Reticulum Like Cells.

Mild nuclear staining was evident in basal & suprabasal layer of odontogenic keratocyst. (Fig 02)

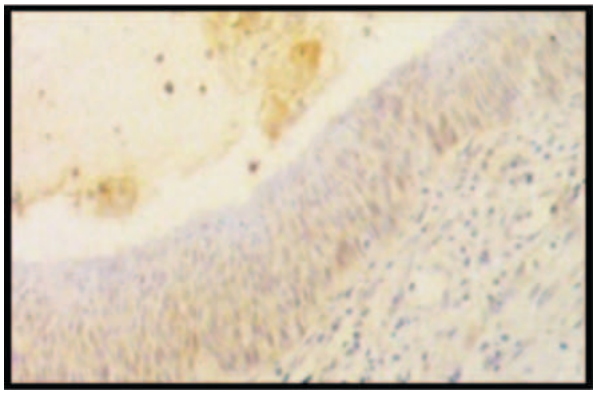

Fig 02: 40x Odontogenic Keratocyst Showing Mild Nuclear Staining for SALL4 in Basal & Suprabasal Cells of Lining Epithelium.

Dental follicles didn't show any evidences of staining (Fig. 03)

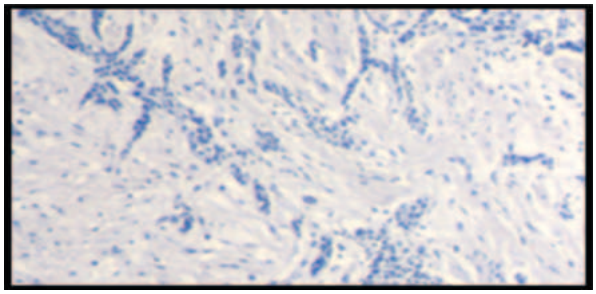

Fig 03: 40x Dental Follicles Showing Negative SALL4 Immuno-expression.

DISCUSSION

This Qualitative study showed significantly higher level of immunopositivity in Ameloblastoma compared to Odontogenic Keratocyst & dental follicle. SALL4 expression was noted in ameloblastic islands showing intense nuclear & cytoplasmic staining for low to tall columnar cells, stellate reticulum-like cells & granular cells. Mild staining was observed in Odontogenic keratocyst. & negative staining with dental follicle.

This study has positive findings similar to IHC study done by Rafaela de Albuquerque Dias et al. (2023)[18] where they found SALL4 was predominantly observed in the epithelial cells of the tumour islands of ameloblastoma with intense nuclear & cytoplasmic staining for peripherally located low to tall columnar cells & centrally located stellate reticulum like cells & the expression was significantly higher for ameloblastoma compared to dentigerous cyst & dental follicle.

Spoorti Kulkarni al. (2024)[19] in their study found moderate to weak staining in in the cytoplasm of Ameloblastoma which was in contrast with our results. The SALL4 positivity was heterogeneous with varied intensity and staining pattern. In most of the histopathological variant of ameloblastoma, the immunopositivity observed, was diffuse in the cytoplasm and less localized to the nucleus. Regarding Odontogenic Keratocyst mild nuclear positivity was observed in basal layer which was in line with our finding.

Nuclear labelling indicated the transcriptional activity of SALL4. Such activity is associated with transcriptional repression mechanisms that prevent stem cell differentiation and increase the proliferation of undifferentiated cells[20]

Studies in the past have shown that SALL4 protein expression is negatively regulated by miRNAs (miRNAs) belonging to the Let-7 family, particularly by miR-98, which leads to a reduction in tumor cell proliferation, indicating that miR-98 acts as a tumor suppressor that inhibits SALL4 protein expression[21]

CONCLUSION

This study verifies the expression of SALL4 as stem cell marker in Ameloblastoma by IHC suggesting the possible participation of stem cells in origin of the tumour. The intense staining in ameloblastoma compared to mid staining in Odontogenic Keratocyst highlighting their biological behaviour. The possible outcome of the study is that in Odontogenic Keratocyst the basal cell & suprabasal cells showed nuclear positivity for the marker but the intensity and the no. of cells per unit area are less as compared to ameloblastoma suggesting that stem cells have less role in biological behaviour of odontogenic Keratocyst. further studies are needed to be carried out using the marker by increasing the no. of sample size to correlate the relation of aggressiveness and expression of the marker.

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