Stem Cell Marker as a Factor for The Different biological behavior of Ameloblastoma and Odontogenic Keratocyst

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Abstract:

Objective: The aim of the study was to investigate the Immunohistochemical expression of SOX2 among the studied cases of ameloblastoma and odontogenic keratocyst in order to show if it correlates to the biological behavior of those odontogenic lesions. **Materials and Methods:** The study was carried out on 30 histo-pathologically confirmed cases of (15 ameloblastomas and 15 odontogenic keratocysts). These blocks were collected from archived files of the Department of Oral Pathology, Faculty of Dentistry, Mansoura University. Sections were subjected to Immunohistochemical staining by SOX2 according to a standard protocol. **Results:** OKCs showed higher expression of SOX2 than Ab in all epithelial layers not confined to the basal layer only with a p-value <0.001. The reaction in recurrent ameloblastomas was significantly higher than in non-recurrent cases (p= 0.036). **Conclusions:** SOX2 is a reliable marker for identifying the stem cell population in odontogenic lesions. Targeted therapies may be designed against these SC populations to render more effective treatment and prevent recurrence.

Introduction:

Odontogenic cysts and tumors are heterogeneous group of osteo-destructive lesions which represent a wide spectrum of clinical and biological behavior although they arise from the tooth germ epithelial cells.[1] The majority of odontogenic tumors are benign.[2] Ameloblastoma (Ab) is the most common form of odontogenic tumors after odontoma.[3] it is a slowly-growing, infiltrative benign but locally invasive neoplasm which may grow to massive proportions and cause significant morbidity.[4] It has a propensity for local recurrence, this high recurrence rate, suggests that tumor progenitors have stem-cell properties.[5] Odontogenic keratocyst (OKC) is a benign intraosseous lesion of the jaws, of odontogenic origin, representing about 11.7% of odontogenic cysts.[6] World health organization in its third edition in 2005, has classified OKC as a benign odontogenic tumor (KCOT) depending on its growth potential, the putatively high recurrence rate, and the presence of mutations in OKC specimens.[7] In 2017, the WHO reclassified it as a developmental odontogenic cyst depending on its regression following decompression procedures, presence of molecular alterations, and lack of evidence supporting the neoplastic nature of OKC.[8] Stem cells are unspecialized cells that have the capacity for self-renewal and differentiation.[9] They support histogenesis and organogenesis during Development, maintaining a balance in the cell turnover process. [10] Stem cell markers such as sex-determining region Y (SRY)-box 2 (SOX2) are capable of identifying these stem cells expressed during the early stages of tooth development. [11] SOX2 is a member of the SOX family Of transcription factors that share homology with the High Mobility Group (HMG) domain of the Sex determining Region Y (SRY) protein.[12] It exerts an important influence on maintaining pluripotency. It is significantly expressed in the dental lamina.[13] It is also a specific and sensitive marker for high-grade lesions so it can differentiate easily between malignant, aggressive benign, and benign lesions.[14] Its expression could imply that a particular group of cells has stem cell properties.[15] Few studies investigated SOX2 in odontogenic cysts and tumors.[16-18] This study is an attempt to shed light on SOX2 expression in Ameloblastoma and odontogenic keratocyst to assess the possible correlation of its expression with the biological behavior of these lesions.

Materials and Methods:

Tissue samples: This study was retrospectively applied on 30 formalin-fixed and paraffin-embedded tissue blocks (15 Ab and 15 OKCS), collected from archival files of Oral and General Pathology Departments Faculties of Dentistry, and Medicine, Mansoura University. Ethical approval was obtained from the Research Ethics Committee of the Faculty of Dentistry, Mansoura University (Code number: A081602321).

Immunohistochemical markers: Monoclonal antibody for Sex-determining region Y (SRY)-box 2 (SOX2) which is a mouse monoclonal antibody, was obtained from Bio-care Medical, USA (Catalog No. AM441-5M).

Methods: Four serial tissue sections were cut at 4 µ thickness. Sections were deparaffinized in xylene and rehydrated in alcohol with descending concentrations. Antigen Retrieval was performed. Slides were immersed in citrate buffer PH6 (10 minutes), heated, blocked (30 minutes) with 1.5% horse serum "Santa Cruz Biotechnology" and finally were diluted in phosphate buffered solution (PBS). Incubation of monoclonal primary antibodies was done at room temperature (45 minutes). Two drops of the antibody (anti-SOX2) were used. Slides were washed with PBS (3 minutes) twice, treated with 4-5 drops of "Ultra

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Vision biotinylated goat anti-polyvalent secondary antibody" (10 minutes), and then washed in PBS (3 minutes). Slides were treated with streptavidin-biotin enzyme reagent "DAKO, Denmark" (10 minutes) and rewashed in PBS (3 minutes). Drops of "3.3-Diaminobenzidine tetrahydrochloride" (DAB) were applied as a chromogen for color development. Slides then; were incubated (10 minutes) and then washed with PBS (3 minutes). Sections were counterstained with Mayers hematoxylin and were fixed using A xylene-based mounting medium (3 minutes).

Evaluation of Immunohistochemical staining: The immunoreactivity for SOX-2 was semi-quantitatively assessed based on the percentage and intensity of staining of the cells of interest according to Khan et al. [19] Five fields were selected that were rich in lesional cells at 400x magnification. Expression percentages were graded from 0 to 4 according to the following levels: 0%, 1%-25%, 26%-50%, 51%-75% and 76%-100%. The Intensity was recorded from 0 to 3 representing: Negative, Weak, Moderate, and Strong respectively. The percentage and intensity scores were then added to obtain a total score. The final score ranged from 0 to 7. Expression was categorized into 1 of 3 groups: (1) Negative = 0 point, (2) Low = 1-3 points, and (3) High = 4–7 points.

Statistical analysis: The results were statistically analyzed using the computer program SPSS software for windows version 26.0 (Statistical Package for Social Science, Armonk, NY: IBM Corp).

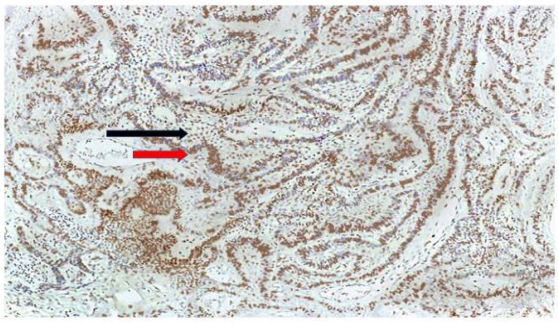
Results:

SOX2 expression in Ab was detected in all cases where it was highly positive in six cases (40%) and low positive in nine cases (60%) cases. Out of these six high positive cases, there were 3 recurrent cases. SOX2 expression in Ab mostly is nuclear that is confined to the outer peripheral cells, decreasing or absent in the central cells. It was observed that in a few cases, there was a nuclear and cytoplasmic reaction of SOX2 (Fig. 1). In OKCs, the expression of SOX2 appeared in all cases where it was highly positive in 14 cases and low positive in only one case.

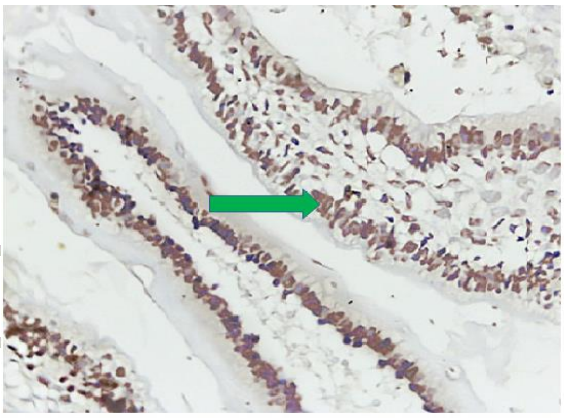
The expression of SOX2 was observed in the basal and superficial layer (Fig. 2).Statistical analysis of SOX2 expressions revealed that there was a significant difference between groups for the score with the highest score recorded in the OKC group, while in the Ab group the low score was the most frequent, (Table).

Discussion:

SOX2 was presented in all the currently studied Ab cases, these findings are in accordance with Pagella et al.,[20] They observed a widespread expression of the dental epithelial stem cell markers SOX2 within Ameloblastoma, this suggests that a major proportion of the cells composing these tumors present CSC-like properties. On the other hand, the absent expression of SOX2 in Ab specimens was reported by the study by Silva et al., [21] This could imply that this particular Odontogenic tumo presents another molecular



(A)



(B)

Figure 1: (A) Photomicrograph showing Immunohistochemical expression of in SOX2 plexiform ameloblastoma in the peripheral cells (Red arrow), decreasing in the center cells (Black arrow) (PAP- DAB x 200). (B) Higher magnification showing nuclear with little cytoplasmic reaction more in the ameloblast like cells (green arrow) (SOX2 x400).

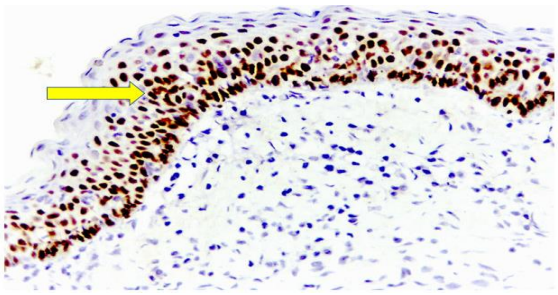


Figure 2: Photomicrograph showing Immunohistochemical expression of SOX2 in OKC with high positive nuclear reaction in the basal and supra-basal layers (yellow arrow) (SOX2 x400).

pathway responsible for conserving its neoplastic behavior. [20] According to a recent study by Tseng et al,[22] recurrence of Ab has been linked to the presence of SOX2 expressing stem cells, this might explain the strong SOX2 overexpression in the cases of recurrent Ab in the present study, and also they suggested that BRAF(V600E) mutation may contribute to the expansion of SOX2-positive cell compartment. Cytoplasmic expression of SOX2 in Ab was seen in some cases. According to Van Schaijik et al.[23] this might be related to differentiating potential so the cytoplasmic expression of SOX2 could be an indicator

Table: SOX2 percentage area, intensity, point and score in Ab, OKCs

	Group A (Ab)	Group B (OKC)	
SOX2 percentage area	N (%)	N (%)	P value
1:25%	8 (53.3)	0 (0.0)	<0.001**
26:50%	3 (20.0)	2 (13.3)	
51:75%	4 (26.7)	0 (0.0)	
76:100%	0 (0.0)	13 (86.7)	
SOX2 Intensity			
Weak	8(53.3)	1 (6.7)	<0.001**
Moderate	6(40.0)	5(33.3)	
Strong	1(6.7)	9(60.0)	
Point			
Mean± SD	3.27b	6.27±1.22	<0.001**
Min-max	2-6	3-7	
Score			
Low	9(60.0)	1(6.7)	<0.001**
High	6(40.0)	14(93.3)	

of more tumor aggressiveness. In the present work, there was a high expression of SOX2 in the basal and parabasal layers of the studied cases of OKCS. This finding is in consistent with other previous studies. [21, 24] This high expression of SOX2 could denote that OKC cells have significant self-renewal and proliferative properties suggesting that there is an imbalance between cell growth and cell death in OKC, which could be a sign of neoplastic behavior. High SOX 2 expression in OKC may explain the high mitotic index and aggressive nature of the lesion. [21, 25]
This high proliferative ability and mitotic index were confirmed by Li TJ., [26] who reported that OKCS have High expression of PCNA and Ki-67, indicating its inherently increased proliferative potential. So finally, SOX2 is differentially expressed in odontogenic cysts and tumors and it is somehow associated with the biological behavior of the studied lesions, moreover, it could serve as a reliable marker for identifying stem cell population in Ab and OKCS, thereby supporting the recurrent formation in some of these lesions, however, the presence of a single marker is not sufficient to prove that. Targeted therapies may be designed against these SC populations to render more effective treatment.

Conclusions:

1- SOX2 Immunohistochemical staining could serve as a useful marker to highlight unsure areas in Ab. It was also positive for recurrent cases of Ab and can be used to monitor the patients.

2-SOX2 is a more reliable marker for identifying stem cell population in OKCs. The high expression Pattern of SOX 2 in OKCs may also explain the aggressive nature of the lesion and account for the presence of numerous daughter cysts responsible for its high recurrence rate.
Further investigations are needed to clarify the role of SOX2 in the biological behavior in a large sample size of odontogenic lesions.

References:

1. Avelar RL, Antunes AA, Carvalho RWF, Bezerra PGCF, Oliveira Neto PJ, Andrade ESS. Odontogenic cysts: a clinicopathological study of 507 cases. J Oral sci. 2009;51(4):581-587.
2. Wright JM, Soluk Tekkesin M. Odontogenic tumors: where are we in 2017? J Istanb Univ Fac Dent. 2017;51(3):10-30.
3. Agbaje JO, Olumuyiwa Adisa A, Ivanova Petrova M, Adenike Olusanya A, Osayomi T, Ajibola Effiom O. Biological profile of ameloblastoma and its location in the jaw in 1246 Nigerians. J Oral Surg Oral Med Oral Pathol Oral Radiol. 2018;126(5):424-431.
4. Shi HA, Ng CWB, Kwa CT, Sim QXC. Ameloblastoma: A succinct review of the classification, genetic understanding and novel molecular targeted therapies. J Surg. 2021;19(4):238-243.
5. Pandolfis P, Montagnaniv, Olivitob. Hedgehog-GLI signaling drives self-renewal and tumorigenicity of human melanoma-initiating cells. J SCs. 2012;30 (2):1808-1818.
6. Johnson NR, Gannon OM, Savage NW, Batstone MD. Frequency of odontogenic cysts and tumors: a systematic review. J Investig Clin Dent. 2014;5(1):9-14.
7. Soluk TM, Wright JM. The World Health Organization Classification of Odontogenic Lesions: A Summary of the Changes of the 2017 (4th) Edition. J Turk Patolo Derg. 2018;34 (3):1-18.
8. Wright JM, Vered M. Update from the 4th edition of the world health organization classification of head and neck tumours: Odontogenic and maxillofacial bone tumors. J Head Neck Pathol. 2017;11(1):68-77.
9. Clarke MF. Clinical and therapeutic implications of cancer stem cells. J N Engl Med. 2019;380(23):2237-2245.
10. Sarkar A, Hochedlinger K. The sox family of transcription factors: versatile regulators of stem and progenitor cell fate. J Cell. 2013;12(1):15-30.
11. Kim BW, Cho H, Choi CH, Ylaya K, Chung JY, Kim JH. Clinical significance of OCT4 and SOX2 protein expression in cervical cancer. J BMC Canc. 2015; 15(1):1015-1020.
12. Hall JM, Weathers DR, Unni KK. Ameloblastic carcinoma: an analysis of 14 cases. J Oral Surg Oral Med Oral Pathol. 2007;103(6):799-807.
13. Juuri E, Isaksson S, Jussila M, Heikinheimo K, Thesleff I. Expression of the stem cell marker, SOX2, in ameloblastoma and dental epithelium. J Oral Sci. 2013;121(6):509-516.

14. Talebi A, Kianersi K, Beiraghdar M. Comparison of gene expression of SOX2 and OCT4 in normal tissue, polyps, and colon adenocarcinoma using Immunohistochemical staining. J Adv Biomed Res. 2015; 4(3):234-238.
15. Liu K, Lin B, Zhao M, Yang X, Chen M, Gao A. The multiple roles for Sox2 in stem cell maintenance and tumorigenesis. J Cell Sig. 2013;25(5):1264-1271.
16. Sanjai K, Sangappa SB, Shivalingaiah D, Kumar HM, Baker A. Expression of SOX2 and EGFR in Ameloblastoma, Odontoameloblastoma and Ameloblastic Carcinoma. J Clin & Diag Res. 2018;4(7): 235-240.
17. Sa B, Lorena RS, Kaique L, Amanda CF, Co A, Cd A. SOX2 and BCL-2 Expressions in and Ameloblastoma. J Oral Med, Oral Patho. 2020;25(2):149-160.
18. Abhishek B, Venkatesh VK, Lavanya S, Shruthi S. OCT4 and SOX2 are reliable markers in detecting stem cells in odontogenic lesions. J Orofaci Sci. 2016;8(1): 124-135.
19. Khan W, Augustine D, Rao RS, Sowmya SV, Haragannavar VC, Nambiar S. Stem cell markers SOX-2 and OCT-4 enable to resolve the diagnostic dilemma between ameloblastic carcinoma and aggressive solid multicystic ameloblastoma. J Adv Biomed Res. 2018;7(1):149-155.
20. Pagella P, Catón J, Meisel CT, Mitsiadis TA. Ameloblastomas exhibit stem cell potential, possess neurotrophic properties, and establish connections with trigeminal neurons. J Cells. 2020;9(3):644-659.
21. Silva BS, Silva R, Lima KL, Dos Santos AC, Oliveira AC, Dezzen B. SOX2 and BCL-2 expressions in Odontogenic Keratocyst and Ameloblastoma. J Med Oral Patol Oral Cir. 2020;25(2): 283-290.
22. Tseng CH, Lu PH, Wang YP, Chang JYF. Enrichment of SOX2-positive cells in BRAF V600E mutated and recurrent ameloblastoma. J Pers Med. 2022;12(1):77-100.
23. Van Schaijik B, Davis PF, Wickremesekera AC. Subcellular localization of the stem cell markers OCT4, SOX2, NANOG, KLF4 and c-MYC in cancer: A review. J of Clini Patho. 2018;71(1):88-91.
24. Phattarataratip E, Panitkul T, Khodkaew W, Anupuntanun P, Jaroonvechatam J, Pitarangsikul S. Expression of SOX2 and OCT4 in odontogenic cysts and tumors. J Head Face Med. 2021;17(1):29-45.
25. Behura S, Bandyopadhyay A, Nishat R, Panda A, Ramachandra S, Mohiddin G. Cancer stem cell markers, SOX 2 and OCT 4 in ameloblastoma and keratocystic odontogenic tumor: An immunohistochemical study. J Int Oral Health. 2017;9(1):28-35.
26. Li T-J. The odontogenic keratocyst: a cyst, or a cystic neoplasm?: A cyst, or a cystic neoplasm? J Dent Res. 2011;90(2):133-142.