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ORIGINAL ARTICLE

Cyclooxygenase-2 protein expression modulates cell proliferation and apoptosis in solid ameloblastoma and odontogenic keratocyst. An immunohistochemical study

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Abstract

Background: Cyclooxygenase-2 protein is a critically important mediator in inflammation that influences proliferation, apoptosis, angiogenesis and metastasis. Previous works showed a relationship between cyclooxygenase-2 and tumourigenesis in humans and animal models. In epithelial odontogenic tumours and cysts, increased cell proliferation and survival have been linked to its pathogenesis and tumour development. The aim of the present study was to analyse the immunohistochemical expression of cyclooxygenase-2 in solid ameloblastoma and odontogenic keratocyst and its association with proteins related to cell proliferation and apoptosis.

Methods: This study was conducted on 40 cases from the Pathological Anatomy Service, University of Chile. The cases were diagnosed as solid ameloblastoma ($n = 21$) and odontogenic keratocyst ($n = 19$) according to WHO 2017. Slides prepared from paraffin-embedded sections were immunohistochemically stained for cyclooxygenase-2, cyclin D1, Ki-67, p63 and Bcl-2. Statistical evaluation was performed by the Shapiro–Wilk test, ANOVA Mann–Whitney test and Spearman's correlation coefficient ($p < 0.05$).

Results: There were significant differences in the immunoexpression of cyclin D1, Ki-67 and Bcl-2 between solid ameloblastoma and odontogenic keratocyst. Likewise, there was a significant difference in the immunoexpression of p63 between follicular and plexiform histological types/subtypes of solid ameloblastoma. Lastly, there were statistical associations between cyclooxygenase-2 and Ki-67 for solid ameloblastoma and between cyclooxygenase-2 and p63 for odontogenic keratocyst.

Conclusion: A high level of cyclooxygenase-2 is related to increased cell survival and proliferative activity in solid ameloblastoma and odontogenic keratocyst. This event might contribute to tumoural progression and local invasiveness in these lesions.

KEYWORDS
apoptosis, cell proliferation, cyclooxygenase-2, odontogenic tumours

1 | INTRODUCTION

Odontogenic tumours (OT) and cysts are lesions that arise from the tooth forming apparatus-derived tissues. OT show a slow-growing without the potential for metastasis. However, benign OT, such as ameloblastoma, invade the surrounding structures to be locally aggressive and a tendency for local recurrence.[1] There are three variants of ameloblastoma: solid (SA), unicystic and peripheral.[1] In 2005, the World Health Organization (WHO) defined odontogenic keratocyst as an 'epithelial odontogenic tumor' and reclassified it as keratocystic odontogenic tumour (KCOT).[2] Interestingly, the last 2017 WHO classification reclassified KCOT as an odontogenic cyst (OKC), accepting KCOT as a synonym for OKC.[3] This terminology change has aroused controversy due to its locally aggressive behaviour described in various reports.[4]

Cyclooxygenase-2 (COX-2) is the inducible isoform of cyclooxygenases. The COX-2 overexpression has been evidenced during carcinogenesis.[5] Previous works evidenced COX-2 modulates biological processes like cell division and proliferation, and apoptosis.[6–8] Moreover, high levels of COX-2 are involved in tumour growth by increased cell proliferation and cell survival.[5] Thereby, COX-2 expression has been related to the up-regulation of cyclin D1 (CCD1), Ki-67, p63 and Bcl-2 proteins.[5] The overexpression of CCD1 has been linked to the progression of various types of neoplasms.[7] The Ki-67 protein expression is commonly used as a marker of proliferative activity of odontogenic cell populations.[9,10] P63 is the most ancient p53 gene family member.[11] The p63 gene encodes three isoforms with a transactivation domain at the N terminus (TA) and three negative isoforms without the transactivation N-terminal domain (ΔN).[11] TAp63 induces cell cycle arrest and apoptosis. Besides, ΔNP63 is related to p53 and TAp63 isoforms inhibition.[11] Also, P63 is commonly overexpressed as the ΔNp63 isoform.[11] Bcl-2 protein is an inhibitor of apoptosis and interferes with programmed cell death, independently of its ability to promote cell division.[12] The overexpression of Bcl-2 is associated with genesis and development of ameloblastoma[13] and with a locally aggressive nature of KCOT/OKC.[13,14]

This study aimed to analyse the COX-2 protein expression and its association with proteins related to cell cycle and proliferation, and apoptosis in SA and KCOT/OKC.

2 | MATERIAL AND METHODS

2.1 | Sample selection

A sample of 40 cases were collected. All cases and demographic data were retrieved from the Pathological Anatomy Service, University of Chile, from 1994 to 2015. Haematoxylin–eosin (H&E)-stained slides of the cases were studied by two pathologists and re-evaluated and diagnosed according to the WHO 2017 classification. Previously, a Cohen's Kappa coefficient was performed between two calibrated pathologists (κ-value = 0.8). These cases were distributed into categories: SA (n = 21), according to histopathological types/subtypes (follicular SA [FSA; n = 8], plexiform SA [PSA; n = 9] and acanthomatous SA [ASA; n = 4]) and KCOT/OKC (n = 19).

The cases with moderate to heavy inflammatory infiltration were excluded.[15] Furthermore, we excluded cases from recurrent lesions and cases of KCOT/OKC associated with the nevoid basal cell carcinoma syndrome (NBCCS) or obtained after decompression therapy. Positive external controls were colon adenocarcinoma (COX-2), lymphoma (CCD1 and Bcl-2), squamous cell carcinoma (Ki-67) and prostate (p63). Infiltration lymphocytes positive for Bcl-2 and COX-2 immunostaining served as an internal positive control. Negative external controls were the same samples of external positive controls incubated with phosphate-buffered saline (PBS; PBS-9990, BION) instead of the primary antibodies.

This study was approved by the Ethics Committee (No.: 2013/11) and the Institutional Biosecurity Committee (FDO N°15) of the Faculty of Dentistry, University of Chile, Santiago, Chile.

2.2 | Immunostaining method

Four-μm thick sections, from paraffin-embedded blocks in a Reichert-Jung® Biocut Microtome (Mod.1130/Biocut), were placed on positively charged slides (CellPath Ltd.,). Cases were deparaffinized in xylene for 20 min and hydrated in graded ethanol for 10 min each, and later, they were placed on distilled water for 10 min. Deparaffinized slides were placed in PBS (pH = 7.4 ± 0.2) for 10 min. The slides were immersed in 10 mM citrate buffer solution (pH 6.0) for antigen retrieval and were transferred to Oster® Food Steamer until the boiling point. The cases were maintained for 20 min and left for cooling until 21°C. Internal peroxidase activity was blocked using 3% hydrogen peroxide and Mouse/Rabbit Immunodetector Peroxidase Blocker Solution (BSB 0003, Bio SB) for 20 min each at room temperature. Primary monoclonal antibodies were incubated for 1 h at room temperature to rabbit anti-human Ki-67 (BSB 5712, clone: EP5, 1:50) and for overnight at 4°C to rabbit anti-human COX-2 (BSB 5362, clone: RBT-COX2, 1:100); rabbit anti-human CCD1 (BSB 5366, clone: RBT14, Ready-To-Use); mouse anti-human p63 (BSB 5852, clone 4A4, 1:100); and mouse anti-human Bcl-2 (BSB 5074, clone: BCL2/A4, 1:50). Next, the cases were incubated with Mouse/Rabbit Immuno-Detector Biotin-Link and Mouse/Rabbit Immuno-Detector HRP Label (BSB 0003) for 20 min each at room temperature. PBS was used as a wash buffer for 30 min when required between each stage. All were antibodies from Bio SB. The peroxidase reaction was performed with chromogen 3,3′-diaminobenzidine reagent. The cases were slightly counterstained with Harris's haematoxylin solution.

2.3 | Immunohistochemical scoring

Five micrographs from each case containing areas rich in odontogenic epithelial cells (OEC) were taken. Each microphotography

was equivalent to a high-power field (HPF) with a magnification of 400×. The photomicrographs were captured with a digital camera in a light microscope (Olympus BX41) with the software program Micrometrics SE Premium 4.4. All images were stored in JPGE format.

Odontogenic epithelial cells were counted by two independent observers, using the ImageJ free software package. Ki-67, CCD1 and p63 immunoexpression were assessed for the nuclear staining. COX-2 and Bcl-2 immunoexpression were assessed for the cytoplasmic staining. Stained OEC (positive cells) and total number of OEC were counted in five HPF. The number of positive cells was counted and calculated as a percentage relative to the total number of cells. Finally, we performed the Bland–Altman test (a bias of 0.04 ± 2.15 with a 95% confidence interval [CI 95%] of −4.27 to 4.19) to analyze the interobserver agreement of the protein immunoexpression count.

2.4 | Statistical analysis

Statistical analysis was performed using GraphPad Prism v8.01 (GraphPad). Comparisons with Gaussian distributions were made using the Shapiro–Wilk test. Statistics were performed using the Mann–Whitney test and unpaired *t*-test with Welch's correction. Spearman correlation analysis was performed to check the possible correlation between the variables. Statistical significance was defined as $p < 0.05$.

3 | RESULTS

3.1 | Clinical features

The clinical features are summarized in Table 1. For SA, 10 cases were males (47.62%) and 11 were females (52.38%). The age range was 16–75 years, with a mean of 43.71 years and a median of 42 years. The posterior region of the mandible was the most common involvement (14 cases). Maxilla was involved in five cases. For KCOT/OKC, a total of eight cases were males (42.11%), and 11 were females (57.89%). The ages of patients ranged from 9 to 67 years, with a mean of 31.4 years and a median of 23 years. All the cases were found in the mandible, mainly in the posterior region (73.69%). Furthermore, SA showed a higher frequency of expansion of the jaws than KCOT/OKC, but KCOT/OKC revealed a greater relationship with included teeth.

3.2 | COX-2 expression

There was no statistically significant difference between SA and KCOT/OKC immunostaining ($p = 0.29$; Table 2A). Likewise, there was no statistical difference ($p = 0.165$) between PSA, FSA and ASA (Table 2B). Positive yellowish-brown COX-2 immunostaining in SA was cytoplasmic and located in peripheral ameloblast-like cells and stellate reticulum-like cells (Figure 1A–C). In KCOT/OKC, positive yellowish-brown cytoplasmic COX-2 immunostaining was observed both in the suprabasal and basal epithelial compartments (Figure 1D).

3.3 | CCD1 expression

There was a statistical difference between SA and KCOT/OKC immunoexpression ($p = 0.0145$) (Table 2A). However, there was no statistical difference ($p = 0.1886$) between FSA, PSA and ASA (Table 2B). In SA, positive yellowish-brown nuclear CCD1 immunostaining was observed in peripheral ameloblast-like cells and stellate reticulum-like cells (Figure 1E–G). In KCOT/OKC, positive yellowish-brown nuclear CCD1 immunostaining was expressed principally in the suprabasal epithelial compartment (Figure 1H).

3.4 | Ki-67 expression

There was a statistical difference between SA and KCOT/OKC immunostaining ($p = 0.0013$; Table 2A). However, there was no statistical difference ($p = 0.5892$) between FSA, PSA and ASA (Table 2B). Positive yellowish-brown Ki-67 nuclear immunostaining was observed mainly in peripheral ameloblast-like cells (Figure 1I–K). In KCOT/OKC, positive nuclear Ki-67 immunostaining was observed both in basal and suprabasal layers of the epithelial lining (Figure 1L).

3.5 | P63 expression

There was no statistical difference between SA and KCOT/OKC immunoexpression ($p = 0.14$; Table 2A). However, there was a statistical difference ($p = 0.016$) between FSA and PSA (Table 2B). Peripheral ameloblast-like cells and stellate reticulum-like cells showed positive yellowish-brown nuclear p63 immunostaining (Figure 1M–O). In KCOT/OKC, positive yellowish-brown nuclear p63 immunostaining was detected both in basal and parabasal layers of the epithelial lining (Figure 1P).

3.6 | Bcl-2 expression

There was a statistical difference between SA and KCOT/OKC immunoexpression ($p = 0.0033$; Table 2A). However, there was no statistical difference ($p = 0.3694$) between FSA, PSA and ASA (Table 2B). Positive yellowish-brown cytoplasmic Bcl-2 immunostaining was observed mainly in peripheral ameloblast-like cells of SA (Figure 1Q–S) and basal layer in the stratified squamous epithelium of KCOT/OKC (Figure 1T).

TABLE 1 Clinicopathological characteristics of the involved patients and distribution of the lesions by anatomic location

Variable	Total	Histological types/subtypes of SA			All types/subtypes of SA	KCOT/OKC
		FSA	PSA	ASA		
Cases (*n*)	40	8	9	4	21	19
Age (years)						
Range	9–75	31–75	16–68	24–50	16–75	9–67
Mean	37.85	54.63	37.56	35.75	43.71	31.37
Median	36.50	53.50	31.00	34.50	42	23
± SD	19.04	16.25	19.76	12.34	18.68	17.71
Sex: *n* (%[a])						
Male	18	2 (25.00)	6 (66.67)	2 (50.00)	10 (47.62)	8 (42.11)
Female	22	6 (75.00)	3 (33.33)	2 (50.00)	11 (52.38)	11 (57.89)
Anatomic location: *n* (%[a])						
Maxilla	5	3	2	0	5	–
Anterior	1	1 (12.50)	–	–	1 (4.76)	–
Posterior	4	2 (25.00)	2 (22.22)	–	4 (19.05)	–
Mandible	35	5	7	4	16	19
Anterior	7	–	–	2 (50.00)	2 (9.52)	5 (26.32)
Posterior	28	5 (62.50)	7 (77.78)	2 (50.00)	14 (66.67)	14 (73.68)
Swelling or expansion of the jaws: *n* (%[a])						
Cases	28 (70.00)	8 (100)	8 (88.89)	2 (50.00)	18 (85.71)	10 (52.63)
Cortical perforation: *n* (%[a])						
Cases	9 (22.50)	3 (37.50)	3 (33.33)	–	6 (28.57)	3 (15.79)
Symptomatology: *n* (%[a])						
Cases	17 (42.50)	3 (37.50)[b]	3 (33.33)[c]	1 (25.00)	7 (33.33)	10 (52.63)
Relationship with teeth: mobility *n* (%[a])						
Cases	9 (22.50)	1 (12.50)	4 (44.44)	1 (25.00)	6 (28.57)	3 (15.79)
Relationship with teeth: included tooth: *n* (%[a])						
Cases	7 (17.50)	1 (12.50)	–	–	1 (4.76)	6 (31.58)
Average range of evolution referred by patient (months)						
Cases	21	31	20	24[d]	25	12[e]

[a]Calculated as a percentage for each lesion.

[b]One case with paraesthesia.

[c]Five cases without information.

[d]Two cases without information.

[e]Eight cases without information and three cases were radiographic finding.

3.7 | Correlation analysis

Spearman correlation test showed a statistical association between COX-2 and Ki-67 for SA ($p = 0.0437$; Table 3A) and between COX-2 and p63 for KCOT/OKC ($p = 0.044$; Table 3B).

4 | DISCUSSION

Several genetic and molecular alterations are described for SA and KCOT/OKC.[1,2] BRAF gene mutations have been identified in ameloblastomas.[1] PTCH1 gene mutation has been detected in NBCCS-related KCOT/OKC and KCOT/OKC.[3] It has been suggested that a high expression of COX-2 might induce tumour promotion in SA and KCOT/OKC.[7–9,16] COX-2 protein is a relevant mediator of inflammation that valuably influences cell proliferation, apoptosis, angiogenesis, metastasis and inhibiting immune surveillance.[17] Also, it can be rapidly upregulated in response to various proinflammatory agents, including cytokines, mitogens and tumour promoters.[17] The expression of COX-2 leads to the transition from acute to chronic inflammation.[5] Chronic inflammation has been depicted to enhance the risk of cancer.[5] Accordingly, COX-2 overexpression has been reported in several cancers, including oral squamous cell carcinoma.[5,17] Likewise, the activation and cell proliferation of odontogenic epithelium are related to higher levels of COX-2 protein.[6,8,9] Therefore, an increased COX-2 expression plays a critical role in multiple genetic

TABLE 2 (A) Comparison of *n* (%) positive cells immunostaining for SA and KCOT/OKC (mean ± SD and ± SEM), (B) Comparison of *n* (%) positive cells immunostaining by histological types/subtypes of SA (mean ± SD and ± SEM)

A.			
	SA	**KCOT/OKC**	**Statistical significance**
COX-2	51.33	62.55	No, $p > 0.05$
SD	(± 31.80)	(± 24.89)	
SEM	(± 6.94)	(± 5.71)	
CCD1	14.57	19.58	Yes, $p = 0.0145$
SD	(± 24.63)	(± 12.15)	
SEM	(± 5.38)	(± 2.79)	
Ki-67	5.12	9.22	Yes, $p = 0.0013$
SD	(± 4.62)	(± 4.31)	
SEM	(± 1.01)	(± 0.99)	
p63	72.17	59.72	No, $p > 0.05$
SD	(± 21.58)	(± 24.90)	
SEM	(± 4.71)	(± 5.71)	
Bcl-2	53.11	28.83	Yes, $p = 0.0033$
SD	(± 31.42)	(± 12.72)	
SEM	(± 6.86)	(± 2.92)	

B.				
	FSA	**PSA**	**ASA**	**Statistical significance**
COX-2	36.96	66.04	46.98	No, $p > 0.05$
SD	(± 26.77)	(± 30.25)	(± 37.47)	
SEM	(± 9.465)	(± 10.08)	(± 18.74)	
CCD1	4.951	17.89	26.34	No, $p > 0.05$
SD	(± 4.228)	(± 23.38)	(± 45.63)	
SEM	(± 1.495)	(± 7.793)	(± 22.81)	
Ki-67	4.324	5.15	6.658	No, $p > 0.05$
SD	(± 2.251)	(± 2.927)	(± 10.15)	
SEM	(± 0.7959)	(± 0.9757)	(± 5.075)	
p63	88.45	61.79	62.97	Yes, $p = 0.016$ between FSA vs PSA
SD	(± 10.79)	(± 21.19)	(± 22.18)	
SEM	(± 3.815)	(± 7.063)	(± 11.09)	
Bcl-2	63.16	52.03	35.44	No, $p > 0.05$
SD	(± 26.82)	(± 31.18)	(± 40.26)	
SEM	(± 9.481)	(± 10.39)	(± 20.13)	

Abbreviations: ASA, acanthomatous solid ameloblastoma; FSA, follicular solid ameloblastoma; PSA, plexiform solid ameloblastoma; SD, standard deviation; SEM, standard error of mean.

and epigenetic functional pathways of biological events, leading to a locally aggressive behaviour of odontogenic epithelial cells.[7,8]

This study demonstrated a high expression of COX-2 in the epithelium of SA and KCOT/OKC. In previous studies, COX-2 levels have also elevated in ameloblastoma[7,18,19] and KCOT/OKC.[18] Seyedmajidi et al.[18] evidenced that COX-2 promotes locally aggressive behaviour in SA and KCOT/OKC. KCOT/OKC shows increased infiltrative potential and a higher recurrence rate than any other odontogenic cysts.[15] Thus, the COX-2 overexpression might promote the tumour progression and their surrounding tissues' invasion by increasing cell growth.[7,8] Moreover, the COX-2 overexpression in SA was associated with recurrence and reduced disease-free survival.[19] In contrast, the levels of COX-2 significantly decreased post-decompression in KCOT/OKC.[20]In the analysis of the immunohistochemical expression for COX-2, no statistical differences were observed between histological types/subtypes for SA. Similar results were found by Sánchez-Romero et al.[16] and Montezuma et al.[19] Despite the differences in proteins expression, such as Ki-67, between histological variants of SA,[21] there is no evidence that histological variants differ in their response to treatment or prognostic.[1,22]

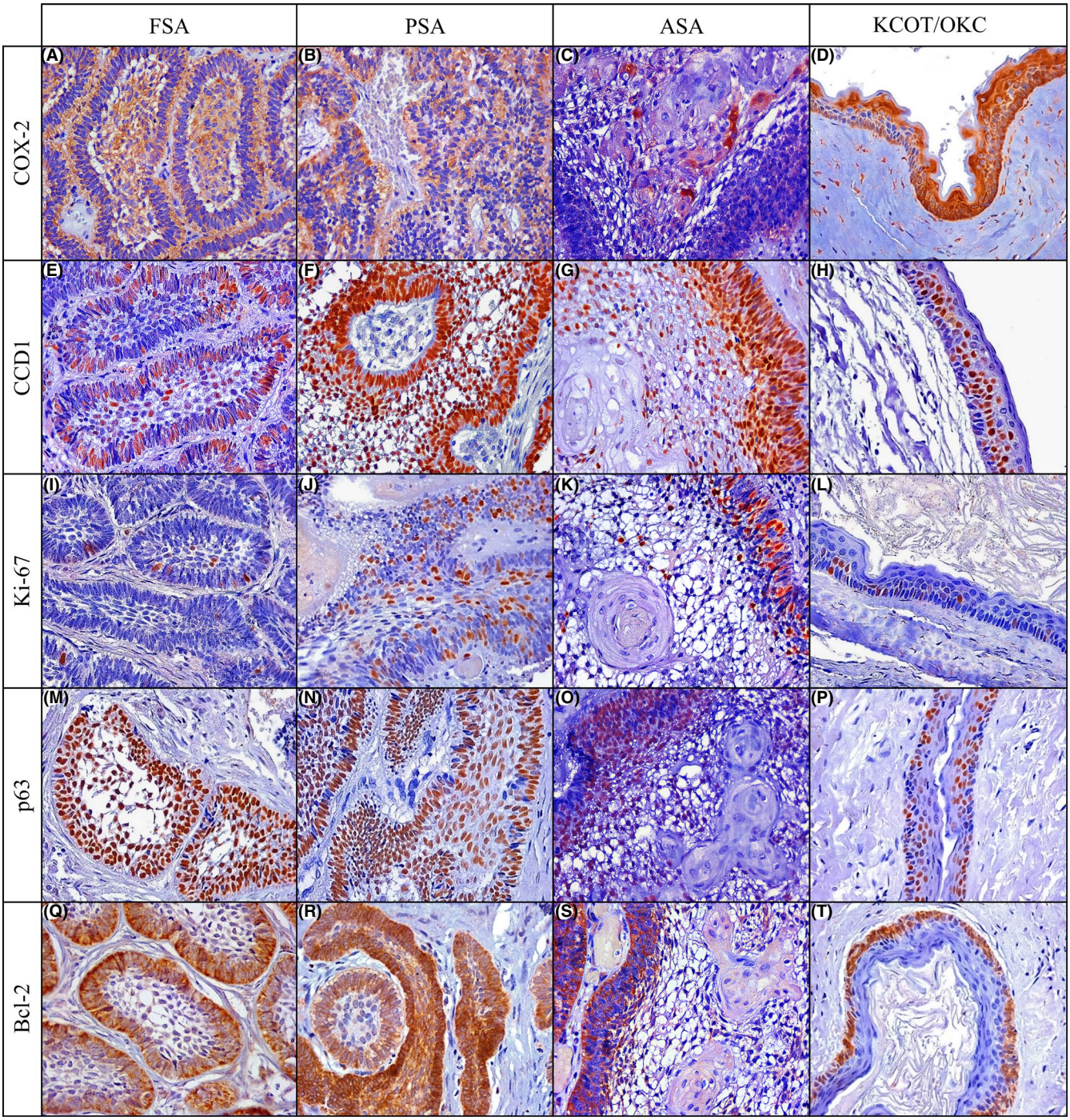

FIGURE 1 Immunohistochemical protein expression of COX-2, Ki-67, CCD1, p63 and Bcl-2 (400×) COX-2 protein expression: (A) Follicular solid ameloblastoma (FSA), (B) Plexiform solid ameloblastoma (PSA), (C) Acanthomatous solid ameloblastoma (ASA), (D) Keratocystic odontogenic tumour/odontogenic keratocyst (KCOT/OKC); CCD1 protein expression: (E) FSA, (F) PSA, (G) ASA, (H) KCOT/OKC; Ki–67 protein expression: (I) FSA, (J) PSA, (K) ASA, (L) KCOT/OKC; p63 protein expression: (M) FSA, (N) PSA, (O) ASA, (P) KCOT/OKC; Bcl-2 protein expression: (Q) FSA, (R) PSA, (S) ASA, (T) KCOT/OKC

Ameloblastoma and KCOT/OKC local invasiveness have been associated with the PI3K-Akt pathway, trough kappa-light-chain-enhancer of activated B cells nuclear factor (NF-κB), β-catenin, CCD1 and COX-2.[7,23,24] Cecim et al.[7] showed a correlation between NF-κB and COX-2 in stromal cells of ameloblastoma, suggesting that activation of NF-κB might regulate COX-2 activity in ameloblastomas. Concordantly, increased expression of β-catenin leads to significant up-regulation of COX-2, suggesting that β-catenin may regulate COX-2 expression. Likewise, an abnormal cytoplasmic and nuclear β-catenin expression was described in KCOT/OKC.[7] As a result, the β-catenin expression could be related to the up-regulation of CCD1 protein, promoting

TABLE 3 Spearman correlation in SA (A) and KCOT/OKC (B) for COX-2, CCD1, p63 y Bcl-2

A. Spearman Test SA	COX-2 vs CCD1	COX-2 vs Ki-67	COX-2 vs p63	COX-2 vs Bcl-2
R	0.2293	0.4442	−0.2468	−0.2195
95% confidence interval	−0.2377 to 0.6102	0.001653 to 0.7412	−0.6217 to 0.2201	−0.6036 to 0.2474
p-value				
p (two-tailed)	0.3174	0.0437[a]	0.2809	0.3391
Significant? (alpha = 0.05)	No	Yes	No	No
B. Spearman Test KCOT/OKC	**COX-2 vs CCD1**	**COX-2 vs Ki-67**	**COX-2 vs p63**	**COX-2 vs Bcl-2**
R	0.02456	0.107	0.4667	−0.08596
95% confidence interval	−0.4463 to 0.4847	−0.3775 to 0.5456	0.001199 to 0.7659	−0.5305 to 0.3956
p-value				
p (two-tailed)	0.9205	0.6628	0.044[a]	0.7264
Significant? (alpha = 0.05)	No	No	Yes	No

[a]Spearman correlation test showed statistical association between COX-2 and Ki-67 for SA ($p = 0.0437$) and between COX-2 and p63 for KCOT ($p = 0.044$).

cell proliferation and growth regulation, DNA repair and cell migration control.[23]

Interestingly, we found a higher expression of CCD1 in OKC (19.58%) than SA (14.57%) ($p = 0.00145$). According to our study, de Vicente et al.[24] found statistical differences in CCD1 expression in KCOT/KOC vs ameloblastoma. These findings suggest that CCD1 may regulate the cell division cycle odontogenic cysts such as KCOT/OKC. We evidenced CCD1 expression principally in the suprabasal compartment of KCOT/OKC. High levels of CCD1 in suprabasal layers suggest that dysregulation of cell cycle progression, from G1 to the S phase, contributes to the different aggressiveness of KCOT/OKC.[25] In SA, we observed that the peripheral ameloblast-like cells express more CCD1 than central stellate reticulum-like cells, suggesting the peripheral compartment of SA presents an increased intrinsic proliferative potential.

The regulation of cell proliferation in solid tumours and haematological malignancies has been related to increase in COX-2 protein expression.[5] Previous studies evidenced high levels of COX-2 and Ki-67 in ameloblastoma and KCOT/OKC.[9,10] These results suggest that COX-2 may contribute to the biological regulation epithelial lining in KCOT/OKC, and to the local extension in ameloblastoma, promoting epithelial proliferation.[9,10]

In our study, a significant positive correlation ($p = 0.0437$) was found between COX-2 and Ki-67 expression in SA. This result was similar to that reported by Alsaegh et al.,[10] whose study showed a positive correlation ($p = 0.004$) between COX-2 and Ki-67 expression in ameloblastoma. Thereby, we hypothesized that the COX-2 pathway could activate tumourigenic mechanisms through interactions with Ki-67 protein and could promote cell proliferation in lesions with odontogenic epithelial origin.

Diverse studies have demonstrated that the overexpression of COX-2 inhibits apoptotic events and the attendant cellular survival.[11,26] P63 regulates epithelial proliferation and differentiation of normal odontogenic epithelial cells. Likewise, it could have a tumourigenic effect in the odontogenic epithelium.[27] P63 has the least six protein isoforms divided into two groups with opposing functions: TA and ΔN isoforms.[11] TA isoforms induce cell cycle arrest, cell differentiation and apoptosis, and ΔNP63 is involved in cell proliferation.[11] COX-2 has been identified as a novel p63 target, particularly for the ΔNP63β isoform, promoting tumourigenesis.[26] It has been shown that ΔNP63β has inhibitory effects on p53, leading to apoptosis and anti-tumourigenic effects of COX-2 inhibitors.[26] This event might be due to the apoptosis-induced p53 promotion under genotoxic stresses. In this context, p53 might mediate ΔNp63 degradation and balances the capacity of ΔNp63 to accelerate tumourigenesis or induce epithelial proliferation.[26]

Our results showed COX-2 expression was positively correlated with a high p63 expression in KCOT/OKC ($p = 0.044$). P63 and COX-2 may control cell proliferation mechanisms during KCOT/OKC development. Thus, the increased expression of COX-2 and p63 protein might synergistically promote combined tumour progression and enhance KCOT/OKC growth.[27] Likewise, a high level of p63 would be a molecular event required for cell infiltration capacity by the tumourigenic role of ΔNp63 isoforms.

COX-2 is known to increase Bcl-2 expression, suppressing apoptosis by preventing activation of caspases responsible for cell death.[12] Bcl-2 protein is associated with developing tooth germ and plays an essential role in tissue differentiation of the odontogenic epithelium.[28] Hence, Bcl-2 expression in odontogenic tissues could help identify cell populations from which OT may arise.[13] In KCOT/OKC, a high expression of Bcl-2 in the lining epithelium promotes continuous cell proliferation and resistance to apoptosis.[13,14,29] Interestingly, we evidenced that Bcl-2 was observed mainly in the peripheral ameloblast-like cells of SA and the basal layer of KCOT/OKC. Previous studies showed that Bcl-2 positive cells were detected mainly in ameloblast-like cells of SA and exclusively in the basal layer for KCOT/OKC.[27,29] Therefore, morphological features of ameloblast-like cells reflect growth activity in ameloblastomas[13] and high level of Bcl-2 in basal layer indicates abnormal control of cell cycle in KCOT/OKC.[13] These results

suggested an increased cell proliferation potential modulated by the antiapoptotic role of Bcl-2 in ameloblast-like cells of SA and the basal layer of KCOT/OKC. Thus, COX-2 overexpression affects the biological behaviour for both lesions by the up-regulation of this antiapoptotic protein, ensuring the overproliferating cells survive to further mutations and promoting tumourigenesis.[6] In this context, Bcl-2 performed a relevant role in cell proliferation, leading to cell-cycle abnormal control. Hence, Bcl-2 must be relevant in the early phase of epithelial tumourigenesis and cell survival maintenance in SA and KCOT/OKC.

Although the overexpression of the COX-2 protein occurs in odontogenic epithelial cells,[7,8] other studies detect this protein's expression in tumour stromal cells, such as fibroblasts, macrophages and lymphocytes.[5] Thus, in inflamed KCOT/OKC, inflammatory microenvironment promoted by infiltrating lymphocytes might maintain the antiapoptotic activity of Bcl-2 on these inflammatory cells. Consequently, Bcl-2 promotes cell survival in odontogenic epithelial cells and also lymphocytes.[5,29]

An interesting model to study the role of COX-2 in the pathogenesis of these lesions is to analyse its expression in human tooth germ. This is because human tooth development involves precisely coordinated processes of cell proliferation, differentiation and cell death.[30] However, there is only one study evaluating COX-2 expression in early human tooth germ.[16] Moreover, immunohistochemical studies in developing human tooth are very scarce.[30] Another experimental approach would be to evaluate the use of COX-2 selective inhibitors, as adjuvant in the therapeutic management of SA and KCOT/OKC, affecting the regulation of COX-2 protein.

We propose COX-2 protein overexpression acts synergistically by triggering different canonical pathways to up-regulate proteins crucial that participate in the development and progression of epithelial odontogenic lesions. Concordantly, Mendes et al.[8] described the overexpression of various markers in KCOT/OKC that act as a 'network addition' pattern rather than a pathological mechanism dependent on the activation or suppression of a specific gene, which explains its aggressive behaviour and may influence the local invasiveness.

However, some limitations should be noted. First, our work lacks follow-up data. Second, we did not obtain clinical information about the treatments performed for each lesion or its radiological description. Third, the categorization of the different types/subtypes of SA reduces the total number of each group, which may be detrimental to the statistical interpretation and comparison. Lastly, we were unable to maturate our results with other experimental techniques.

Our study suggests that COX-2 overexpression plays an important role in the biological profile and behaviour of SA and KCOT/OKC, promoting cell proliferation and survival. Further studies are necessary to understand the mechanisms by which COX-2 modulates apoptosis and cell cycle, especially in odontogenic cysts and tumours. Besides, future studies could analyse the potential role of COX-2 as a marker in these lesions. Moreover, in such a way that selective COX-2 inhibitors should be considered to the treatment of SA and KOCT/OKC.

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CONFLICT OF INTEREST

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

AUTHOR CONTRIBUTIONS

Enrico Escobar: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Supervision; Writing-original draft; Writing-review & editing. **Cristian Peñafiel:** Data curation; Methodology; Resources. **Fernán Gómez-Valenzuela:** Formal analysis; Software; Supervision; Writing-original draft. **Eduardo Chimenos-Küstner:** Formal analysis; Supervision; Writing-original draft; Writing-review & editing. **Ricardo Pérez-Tomás:** Conceptualization; Formal analysis; Investigation; Supervision; Validation; Writing-original draft; Writing-review & editing.

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