Pathol. Oncol. Res. (2015) 21:65–71
DOI 10.1007/s12253-014-9789-3

RESEARCH

Expression of Human Papillomavirus is Correlated with Ki-67 and COX-2 Expressions in Keratocystic Odontogenic Tumor

Mohammed Amjed Alsaegh · Hitoshi Miyashita · Sheng Rong Zhu

Received: 11 November 2013 / Accepted: 20 April 2014 / Published online: 15 May 2014

Abstract The aim of the current study was to investigate the presence of human papillomavirus (HPV) and evaluate its association with Ki-67 and cyclooxygenase-2 (COX-2) expressions in keratocystic odontogenic tumor (KCOT). Nineteen cases were included in the present study. Conventional PCR method and immunohistochemical analysis were performed for the detection of HPV-DNA and HPV-L1 capsid protein. Moreover, the expressions of Ki-67 and COX-2 proteins were analyzed immunohistochemically. HPV-DNA was detected in 36.8 % (7/19) of tumor samples, whilst HPV-L1 protein was identified in 68.4 % (13/19) of them. The Kappa coefficient statistical test showed a moderate agreement (κ0.424) between PCR and IHC assays for HPV detection. Expression of HPV-DNA was positively correlated with Ki-67 and COX-2 expressions ($p<0.05$), whereas HPV-L1 positive staining was positively correlated with COX-2 ($p<0.05$) and highly associated with those of Ki-67 ($p<0.01$). There was no significant correlation between the presence of HPV and the recurrence of the studied lesions. The results of the current study showed that active HPV infection was present in the odontogenic epithelium of KCOT, and it was associated with increased proliferation rate and COX-2 expression. These findings suggest that HPV may have a role in the pathogenesis and aggressiveness of KCOT. Based on these conclusions, we recommend further investigations of HPV vaccine or antiviral therapy and COX-2 inhibitors as nonsurgical options in the prevention and management of KCOT.

Keywords Human papillomavirus (HPV) · Keratocyst · Odontogenic · Ki-67 · Cyclooxygenase-2 (COX-2)

M. A. Alsaegh · H. Miyashita · S. R. Zhu (✉)
Department of Stomatology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China
e-mail: tongji36@yahoo.com

M. A. Alsaegh
e-mail: mo_am_77@yahoo.com

M. A. Alsaegh
Department of Oral and Maxillofacial Surgery, College of Dentistry, University of Mosul, Mosul, Iraq

H. Miyashita
Division of Oral and Maxillofacial Surgery, Department of Oral Medicine and Surgery, Graduate School of Dentistry, Tohoku University, Tohoku, Japan

Introduction

Odontogenic keratocyst has been known for its rapid growth [1], and tendency to invade the adjacent tissues, including bone [2, 3]. In 2005, the World Health Organization (WHO) reclassified 'odontogenic keratocyst' to 'keratocystic odontogenic tumor' (KCOT) [1]. KCOT has a high recurrence rate, and it is associated with mutation of tumor suppressor PTCH gene on chromosome 9q22.3–q31 [4]. It can occur at any age group. However, data suggest a bimodal age distribution around the third and sixth decades of life [5]. KCOT may occur as solitary or multiple lesions. The latter is usually one of the components of the inherited nevoid basal cell carcinoma syndrome (NBCCS) [1].

The Ki-67 antigen is a protein expressed in all phases of the cell cycle, except in G0. Thus, Ki-67 expression informs about the number of proliferating cells in a tissue [6]. Ki-67 is a widely used proliferation marker for assessing cellular proliferation of KCOT [7–9].

Cyclooxygenase enzymes catalyze the formation of the prostaglandin endoperoxide from arachidonic acid to prostaglandin H2 (PGH2). Cyclooxygenase-2 (COX-2) isoform expression induced in response to inflammatory and mitogenic stimuli [10, 11]. Increased COX-2 expression in tumors is known to be correlated with tumor invasion, angiogenesis,

resistance to apoptosis, and suppression of host immunity [12].

Human papillomavirus (HPV) is a small non-enveloped double strand DNA virus with about 7900 nucleotide bases long [13]. It is nearly ubiquitously present in humans, but ultimately only a small proportion of infected individuals develops cancer [14]. The HPV genome contains eight open reading frames, which are potential coding sites of six early (E1, E2, E4, E5, E6, and E7) proteins and two late (L1 and L2) proteins [13].

HPV was a possible factor or a cofactor in the development of ameloblastoma [15–17]. Studies investigated the presence of HPV in KCOT are very rare [18, 19]. The aim of the current study was to investigate the presence of HPV and evaluate its association with Ki-67 and COX-2 expressions in KCOT.

Materials and Methods

A total of 19 odontogenic lesions were used; 13 samples of them were formalin fixed paraffin embedded (FFPE) samples, retrieved from the archive of oral pathology laboratory, Tongji Hospital, Huazhong University of Science and Technology, over the years (2009–2011). The remaining six samples were frozen tissue samples that were prospectively collected from patients intended maxillofacial surgery at Tongji Hospital, Huazhong University of Science and Technology during the research time. All patients were free from the acquired immune deficiency syndrome (AIDS) and did not take the HPV vaccine, or any antivirus or immunosuppressive therapies. All lesions were solitary and not associated with the NBCC syndrome. This study was approved by our Institutional Review Board. The Declaration of Helsinki protocols were followed during the whole study.

Immunohistochemistry Immunohistochemical study was performed using 1:100 dilutions of the following primary polyclonal antibodies: (a) rabbit anti-HPV (Beijing Biosynthesis Biotechnology Co, China (b) rabbit anti-human Ki-67 antigen and (c) rabbit anti-human COX-2 (Boster Biological Technology, China). A standard Streptavidin-biotin peroxidase complex method (Boster Biological Technology, China) was used for immunostaining. FFPE samples were cut into 5 μm thickness tissue sections and dewaxed, rehydrated then endogenous peroxidase was quenched in 3 % H_2O_2 followed by the antigen unmasking in 0.01 M citrate buffer that heated to the boiling point in a microwave oven. Whilst the frozen samples were cut into 5 μm thickness sections and fixed in 4 % paraformaldehyde. The endogenous peroxidase was blocked using 0.03 % H_2O_2 in absolute methanol. After that, both FFPE and frozen samples were treated with normal goat serum for 50 min at room temperature. Subsequently, the sections were incubated with primary antibodies at 4 °C overnight and then treated with 10 μg/ ml biotinylated secondary antibody for two hours at room temperature. After that, the tissue sections were incubated with SABC- peroxidase complex. Finally, the sections were developed with 3,3'-Diaminobenzidine substrate and slightly counterstained with Mayer's hematoxylin. Negative controls were incubated by PBS instead of the primary antibodies. Standard light microscope was used to score the staining. For HPV-L1 staining, a positive result was interpreted as a yellowish-brown precipitate, predominantly intranuclear, in a focal or diffuse pattern, whereas a negative result was indicated by a lack of precipitate intracellularly as described by Kahn [15], whilst the scoring for Ki-67 and COX-2 staining was confirmed by counting the percentage of positive cells in 10 high power (400×) representative fields as follows: 0, when no identified staining of the odontogenic epithelium or when the staining was questionable; 1+ ≤ 25 %; 2+ for 26 %-50 %, and 3+ for >50 % positivity rate of the odontogenic epithelium. The different intensities for COX-2 staining was regarded the same in the used criteria.

PCR Analysis Each FFPE sample was cut into five pieces of 10 μm thick sections and deparaffinized using xylene and rehydrated by subsequent descending concentrations of ethanol and distilled water, then DNA was extracted using DN32 DNA extraction kit (Aidlab. Co. LTD, Beijing, China). Nevertheless, several represented pieces for a total weight of 30 mg were used for DNA extraction from each frozen tissue sample using DN08 DNA extraction kit (Aidlab, Co. LTD, Beijing, China). Each sample was amplified with primers recognizing the albumin gene. Consequently, all samples were suitable for molecular analysis. The prevalence of general HPV-DNA was analyzed with the broad-spectrum SPF1/2 HPV primers using conventional PCR method as described previously [20]. The PCR reaction was performed in a final reaction volume of 25 μl containing 3 μL of the isolated DNA solution, 12.5 μl of 2 × Taq MasterMix (Aidlab Co. LTD, Beijing, China), 200 mmol/L each deoxynucleoside triphosphate, and 15 pmol of forward and reversed primers. The PCR condition was as follows: one cycle of activation for nine min at 94ºC followed by 60 cycles of amplification, each cycle consisted of 30 sec at 94ºC, 45 sec at 52ºC, and 45 sec at 72ºC. The final extension step was five min at 72ºC. Each PCR experiment was performed with positive and negative PCR controls. The PCR products were run through 3 % agarose gel and the 65-bp product was visualized with ethidium bromide staining.

Statistical Analysis The statistical package for the social sciences (SPSS) 19.0 software was used for the statistical analyses. Correlations of HPV-DNA and HPV-L1 protein with Ki-67 and COX-2 proteins were analyzed with Spearman's rank correlation coefficient test. Kappa statistics were used to

assess the degree of agreement between IHC and PCR analyses for HPV detection. Differences were deemed statistically significant if ($P<0.05$).

Results

The most relevant clinical features are summarized in Table 1. HPV-DNA was detected in 36.8 % (7/19) of samples, while HPV-L1 protein was identified in 68.4 % (13/19) of them (Table 2). Kappa coefficient showed moderate agreements ($\kappa=0.424$) between PCR and IHC results. HPV-L1 protein was immunohistochemically detected as a yellowish- brown stain that was mainly expressed in the nucleus of odontogenic epithelial cells. The expression level was higher in the suprabasal epithelial layers than that of the basal one (Fig. 1).

Ki-67 expression was mild in 26.3 % (5/19) of samples, moderate in 21.1 % (4/19), and high in 52.6 % (10/19) (Table 2). Ki-67 staining was detected as yellowish-brown nuclear stain. Positive cells were located throughout the cystic wall of KCOT. However, more staining positivity was found in the suprabasal layers than that of the basal epithelium (Fig. 1).

COX-2 expression was absent in 15.8 % (3/19) of samples, mild in 15.8 % (3/19), moderate in 21.1 % (4/19), and high in 47.4 % (9/19) of the samples (Table 2). COX-2 staining was demonstrated as a yellowish-brown granular stain of different intensities that expressed in the cytoplasm and nuclear membrane of odontogenic epithelium, the staining of the suprabasal epithelial layers was more positive and intense than that of basal layers (Fig. 1). Intensely stained lymphocytes were noted throughout the connective tissue of KCOT.

Expression of HPV-DNA was positively correlated with Ki-67 and COX-2 expressions ($p<0.05$), whereas HPV-L1 positive staining was positively correlated with COX-2 ($p<0.05$) and highly associated with those of Ki-67 ($p<0.01$). There was no significant correlation between the presence of HPV and the recurrence of the studied lesions. (Table 3).

Table 1 Clinical findings in patients with KCOT (n=19)

Variable	No	%
Total	19	
Age (year)		
Mean	39	
Range	(15–68)	
Gender		
Male	11	58
Female	8	42
Location of tumor		
Maxilla	6	32
Mandible	13	68
Recurrence		
Primary lesions	10	52.6
Recurrent lesions	9	47.4

Discussion

Several previous reviews exist on HPV prevalence in normal, benign, premalignant, and malignant oral lesions [21]. HPV was 2–3 times and 4.7 times more likely detected in oral precancerous lesions and oral carcinomas, respectively, compared with normal mucosa [22]. HPV was discovered in the saliva of healthy individuals [23, 24]. However, the prevalence of HPV in saliva was higher in patients with oral carcinoma than that of normal individuals [24]. The specific role of HPV in the development of premalignant and oral squamous cell carcinoma continues to be a debated topic [25].

In the present study, genomic HPV-DNA responsible for the expression of HPV-L1 capsid protein was investigated by conventional PCR analysis using SPF 1/2 consensus primers, and The HPV-L1 capsid protein was examined by IHC analysis. The result of PCR analysis is comparable with that of IHC, and they showed positive correlations with Ki-67 and COX-2 expressions.

HPV-L1 capsid protein is expressed in the active phase of the viral infection and it is necessary for viral cellular cycle completion. Consequently, viral protein detection by immunohistochemical reaction is an evidence of active HPV infection in the examined tissues [26].

HPV-L1 and L2 capsid proteins are expressed during the late stage of the viral life cycle in highly differentiated suprabasal cells [27]. This fact may support our results of this study that showed the predominant pattern of HPV-L1staining intensity in suprabasal layers of KCOT.

Previous studies demonstrated the presence of HPV in various ameloblastomas using different investigation methods [15–17, 28–30]. HPV was considered to be a possible

Table 2 Expressions of HPV-DNA, HPV-L1, Ki-67 and COX-2 in KCOT (n=19)

	No	%
HPV-DNA*	7	36.8
HPV-L1**	13	68.4
Ki-67**	19	100
Mild	5	26.3
Moderate	4	21.1
High	10	52.6
COX-2**	16	84.2
Mild	3	15.8
Moderate	4	21.1
High	9	47.4

*Conventional PCR method

**Immunohistochemical analysis

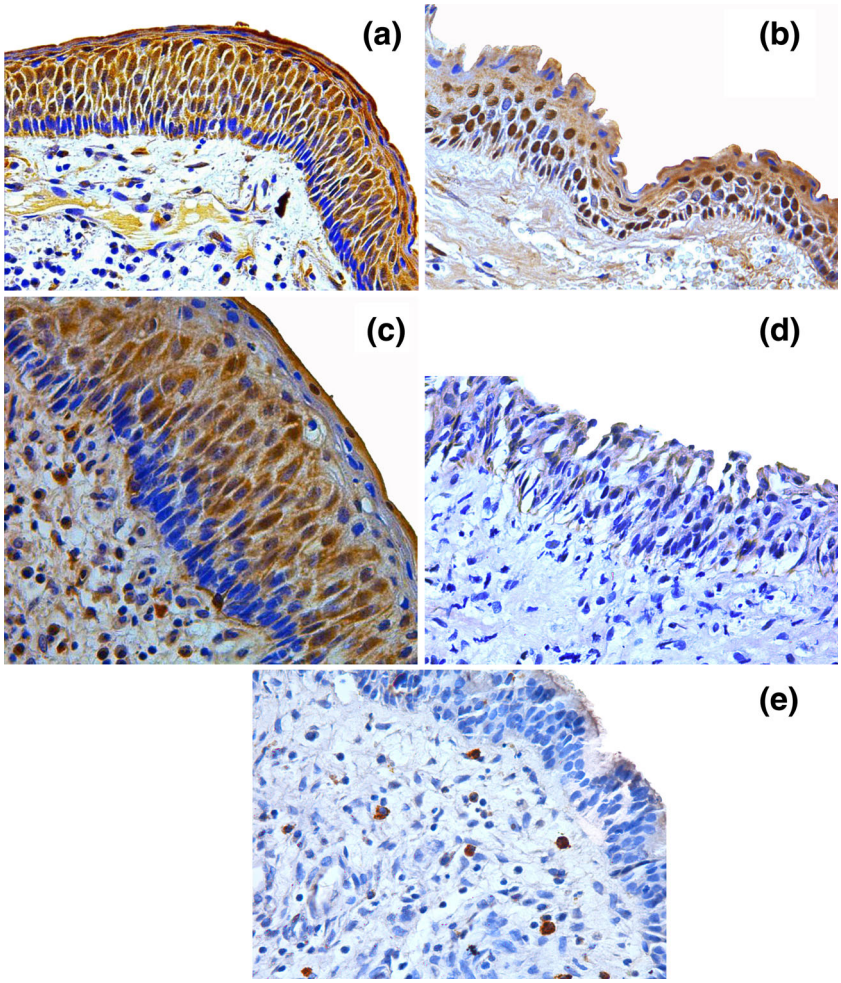

Fig. 1 Immunohistochemical staining of keratocystic odontogenic tumor (KCOT) (400×). A predominant suprabasal yellowish-brown Immunohistochemical staining in the odontogenic epithelium of HPV-L1 (A), Ki-67 (B), and COX-2 (C). HPV-L1 and Ki-67 staining are nuclear, while COX-2 staining is in the nuclear membrane and the cytoplasm of the positive cells. (D) HPV-L1 negative case. HPV-L1 is not detectable in the nucleus. (E) Cox-2 negative case. Cox-2 is not detectable in the nuclear membrane and the cytoplasm of the odontogenic epithelium.

etiological factor or a cofactor in the development of ameloblastoma [15–17]. van Heerden *et al.* regarded the presence of HPV in their studied ameloblastoma samples as a secondary infection, thereby they did not consider HPV as a causative factor in the pathogenesis of ameloblastoma [29]. Sand *et al.* concluded that the presence of HPV in ameloblastoma was a result of contamination from the surface mucosal epithelium induced by the surgical manipulation [30]. However, another study did not find HPV in 4 μm thick hematoxylin and eosin- stained ameloblastoma sections using immunohistochemistry, in situ hybridization, and PCR analysis [31]. There are only two previous reports that investigated the presence of HPV in KCOT. The first one was a case report that demonstrated the presence of HPV type 16 in KCOT using PCR analysis [18], whereas, the other one did not detect HPV-DNA in the investigated samples by using PCR analysis [19]. It appeared that the studies on the presence of HPV in odontogenic tumors are highly inconsistent both in detecting the presence of the virus and in the subsequent conclusions regarding the role of HPV in the pathogenesis of the involved lesions. The completely negative results may be attributed to the methodology used and / or the quality of the available samples, like choosing PCR primers that targets a large genomic area in FFPE samples. Another possibility is the differences in the geographic distribution of the patients involved in the previous studies, which regarded as a property of a virus related neoplastic disorder [32]. In this concern, all the samples used in the current study were taken from the Chinese patients.

Table 3 Spearman's rank correlation coefficient for HPV-DNA and HPV-L1 protein with Ki-67, COX-2 proteins and recurrence in KCOT.

	Ki-67	COX-2	Recurrence
HPV-DNA	0.536	0.467	−0.069
	p=0.018	p=0.044	p= .779
HPV-L1	0.590	0.562	−0.263
	p=0.008	p=0.012	P=0.277

The proliferating marker, Ki-67 is a widely used one. It is expressed in all stages of the cell cycle except for G0 phase [6]. The current study showed profuse Ki-67 protein expression in the odontogenic epithelium of KCOT. This indicates increasing the proliferation rate of odontogenic epithelium

that corresponds with its clinical behavior like aggressiveness. The distribution of Ki-67 protein expression was mostly in the suprabasal layers of the lining epithelium. This result was previously attributed to the alteration in the cell cycle that indicating the presence of suprabasal proliferative compartments [1, 7–9]. The current results could give another explanation for this commonly noted finding which associated with the existence of HPV infection in odontogenic epithelium of KCOT. During HPV infection and in order to allow for the production of viral progeny, viral proteins override the normal cell cycle exit that occurs in differentiating cells [33]. Since HPV replication is dependent mainly on the host cell machinery, the capacity for DNA synthesis must remain intact throughout the upper layers of the epithelial strata. For this reason, and by using a process mediated primarily by the HPV E6 and E7 proteins, HPV-infected cells do not exit the cell cycle [34], and it is suspected to continue proliferation and express more Ki-67 proteins. This fact supports our results of both location and positive correlation between HPV-L1 and Ki-67 proteins in KCOT. In accordance with the present study, Samir *et al.* showed that Ki-67 was correlated positively with high risk-HPV infection in cervical lesions [35].

Previous study demonstrated that ki-67 were significantly more prevalent in odontogenic keratocysts than in dentigerous cysts, radicular cysts and ameloblastomas [36]. Furthermore, KCOT linings seem to have great potential than other odontogenic cysts to evolve into squamous cell carcinoma, and although rare, it has been reported [37].

COX-2 is an enzyme that involved in the conversion of arachidonic acid into prostaglandin (PG) in different physiopathological conditions affecting the tissues, such as inflammatory stimuli, cytokines, growth factors, tumor promoters, and viral transformation [10, 11].

Regulation of COX-2 expression is a complex process mediated by numerous factors [38]. This regulatory process is altered by the presence of HPV [39]. The correlation between HPV and COX-2 expression in various locations and lesion types was studied before. Subbaramaiah and Dannenberg showed that HPV16 E6 and E7 oncoproteins stimulated COX-2 transcription by activating the epidermal growth factor receptor→Ras→mitogen-activated protein kinase→activator protein-1 pathway [40]. Moreover, Kim *et al.* showed that HPV E5 expression induced COX-2 expression through the effect of EGFR-signaling pathway [38]. COX-2 levels were also upregulated in esophageal epithelial cells immortalized with E6E7 and hTERT [41]. The present investigation showed a positive correlation of HPV and COX-2 expression in the odontogenic epithelium of KCOT. Interestingly, the location of both HPV-L1 protein and COX-2 expressions was more predominant in the suprabasal layers of the odontogenic epithelium.

COX-2 levels have been found to be elevated in various tumors. However, the mechanism of COX-2 overexpression for the induction of tumorigenesis remains poorly understood [42]. Some explanations are based on the fact that COX-2 is an oxygenase that producing highly reactive intermediates which cause genomic instability [43]. In addition, COX-2 has been proven to increase the level of the anti-apoptotic protein, bcl-2, that causes resistance to apoptosis [44]. Furthermore, COX-2 increases the synthesis of PGs in transformed cells and tumors. PGs are believed to be important in the pathogenesis of cancer because of its effects on cell proliferation, angiogenesis, immune surveillance, and apoptosis [45]. COX-2-dependent PGs have been implicated in the induction of matrix metalloproteinases in different cell types [46–48]. metalloproteinases are important in matrix degradation during tumor growth, invasion, and induction of angiogenesis [49]. The primary requirement for any lesion to expand within the bone is the ability to resorb the dense crystalline environment. KCOT has been shown to release PGEs that resorbing the surrounding bone [50]. PGs, mainly PGE2, can stimulate bone resorption by increasing the amount and functional activity of osteoclasts. However, it has been shown that hormones and cytokines involved in bone resorption can stimulate COX-2 expression and PG synthesis [51]. Gao and Li, concluded that odontogenic lesions could promote bone resorption in vitro and it's likely to be related to some of the cytokines secreted by the lesions. The concentrations of PGE2 in the culture media of the odontogenic lesions are significantly higher than that of the black controls [52].

In accordance with our study, Mendes *et al.* showed a distinct over expression of COX-2 in KCOT [53]. Moreover, a previous report demonstrated the expression of COX-2 in the radicular cyst, suggesting that COX-2 may play an important role in the pathogenesis of the radicular cyst [54].

Hedgehog signaling pathway and PTCH mutations are well understood in the pathogenesis of both NBCC related [55] and sporadic KCOT [56]. This fact could further explain the current study results for the association of HPV with Ki-67 and COX-2 in KCOT. PTCH and SMO form a receptor complex in the cell membrane which has a suppressor effect on growth-signal transduction. In cases of mutated PTCH, this inhibitory effect is lost, and proliferative and stimulating effects of SMO outweigh the inhibition [57]. The binding of SHH to PTCH also releases this inhibition and facilitates the growth signal transduction resulting in proliferation and stimulating effects of SMO [4]. Xuan *et al.* demonstrated a relation between the expression of SHH and HPV16 infection in cervical cancer [58]. This relation is not induced directly by HPV-encoded proteins but rather that hedgehog-activating mutations are selected in cells initially immortalized by HPV [59]. Yagyuu *et al.* showed that the cases of KCOT with strong SMO expression presented a higher Ki-67 labeling than SMO-negative cases [60]. Further concordance between PTCH mutation and the induction of COX-2 expression was concluded through the relief of SMO inhibition that

upregulates the proliferative markers like p53, leading to the activation of Ras/Raf/ERK cascade which in turn induces the expression of COX-2 [53]. The authors strongly suggest further investigation of coexpression of HPV and these molecules (such as p53), and correlating the current study findings with the clinical implications including tumor aggressiveness and recurrence.

The results of the present study showed that active HPV infection was existent and associated with increased proliferation rate and COX-2 expression in the odontogenic epithelium of KCOT. These findings suggest that HPV may have a role in the pathogenesis and aggressiveness of KCOT.

Surgery alone could result in a great morbidity and a high recurrence rate in the management of KCOT, it seems worth to investigate the effect of different antiviral vaccine and therapies in managing KCOT especially in the cases that shows the presence of HPV. In addition, the study represents COX-2 as a possible target in the management of KCOT.

Acknowledgments We would like to thank the Noble Prize winner, Prof. Harald zur Hausen for supplying us with the HPV positive PCR control. Also we thank Prof. Wei Min Chen, Prof. Xue Jin Tao, As. Prof. Le Nan Shao, As. Prof. Xin Yu Ji, and Dr. Guo Xiong Tang for their help in clinical samples collection.

References

1. Barnes L, Eveson JW, Reichart P, Sidransky D (2005) World Health Organization classification of tumours. Pathology and genetics of head and neck tumours. IARC, Lyon
2. Stoelinga PJ, Bronkhorst FB (1988) The incidence, multiple presentation and recurrence of aggressive cysts of the jaws. J Craniomaxillofac Surg 16:184–195
3. Lund VJ (1985) Odontogenic keratocyst of the maxilla: a case report. Br J Oral Maxillofac Surg 23:210–215
4. Madras J, Lapointe H (2008) Keratocystic odontogenic tumour: reclassification of the odontogenic keratocyst from cyst to tumour. J Can Dent Assoc 74:165–165h
5. Shear M, Speight PM (2007) Odontogenic keratocyst. In: Shear M, Speight PM (eds) Cysts of the Oral and Maxillofacial Regions, 4th edn. Blackwell Munksgaard, Oxford, pp 6–58
6. Scholzen T, Gerdes J (2000) The Ki-67 protein: from the known and the unknown. J Cell Physiol 182:311–322
7. Gurgel CA, Ramos EA, Azevedo RA, Sarmento VA, da Silva Carvalho AM, dos Santos JN (2008) Expression of Ki-67, p53 and p63 proteins in keratocyst odontogenic tumours: an immunohistochemistry study. J Mol Histol 39:311–316
8. Gadbail AR, Chaudhary M, Patil S, Gawande M (2009) Actual Proliferating Index and p53 protein expression as prognostic marker in odontogenic cysts. Oral Dis 15:490–498
9. de Oliveira MG, Lauxen Ida S, Chaves AC, Rados PV, Sant'Ana Filho M (2011) Odontogenic epithelium: immunolabeling of Ki-67, EGFR and survivin in pericoronal follicles, dentigerous cysts and keratocystic odontogenic tumors. Head Neck Pathol 5:1–7
10. Smith WL, DeWitt DL, Garavito RM (2000) Cyclooxygenases: structural, cellular, and molecular biology. Annu Rev Biochem 69: 145–182
11. Marnett LJ, DuBois RN (2002) COX-2: a target for colon cancer prevention. Annu Rev Pharmacol Toxicol 42:55–80
12. Kurihara Y, Hatori M, Ando Y, Ito D, Toyoshima T, Tanaka M, Shintani S (2009) Inhibition of cyclooxygenase-2 suppresses the invasiveness of oral squamous cell carcinoma cell lines via down-regulation of matrix metalloproteinase-2 production and activation. Clin Exp Metastasis 26:425–432
13. Chang F, Syrjänen S, Kellokoski J, Syrjänen K (1991) Human papillomavirus (HPV) infections and their associations with oral disease. J Oral Pathol Med 20:305–317
14. Psyrri A, Boutati E, Karageorgopoulou S (2011) Human papillomavirus in head and neck cancers: biology, prognosis, hope of treatment, and vaccines. Anticancer Drugs 22:586–590
15. Kahn MA (1989) Ameloblastoma in young persons: a clinicopathologic analysis and etiologic investigation. Oral Surg Oral Med Oral Pathol 67:706–715
16. Kahn MA (1992) Demonstration of human papillomavirus DNA in a peripheral ameloblastoma by in situ hybridization. Hum Pathol 23: 188–191
17. Namin AK, Azad TM, Eslami B, Sarkarat F, Shahrokhi M, Kashanian F (2003) A study of the relationship between ameloblastoma and human papilloma virus. J Oral Maxillofac Surg 61:467–470
18. Cox M, Eveson J, Scully C (1991) Human papillomavirus type 16 DNA in an odontogenic keratocyst. J Oral Pathol Med 20: 143–145
19. González-Moles MA, Mosqueda-Taylor A, Delgado-Rodríguez M et al (2006) Analysis of p53 protein by PAb240, Ki-67 expression and human papillomavirus DNA detection in different types of odontogenic keratocyst. Anticancer Res 26:175–181
20. Kleter B, van Doorn LJ, ter Schegget J et al (1998) Novel short-fragment PCR assay for highly sensitive broad-spectrum detection of anogenital human papillomaviruses. Am J Pathol 153: 1731–1739
21. Syrjänen S, Lodi G, von Bültzingslöwen I et al (2011) Human papillomaviruses in oral carcinoma and oral potentially malignant disorders: a systematic review. Oral Dis 17(Suppl 1):58–72
22. Miller CS, Johnstone BM (2001) Human papillomavirus as a risk factor for oral squamous cell carcinoma: a meta-analysis, 1982–1997. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 91:622–635
23. Montaldo C, Mastinu A, Quartuccio M et al (2007) Detection and genotyping of human papillomavirus DNA in samples from healthy Sardinian patients: a preliminary study. J Oral Pathol Med 36:482–487
24. SahebJamee M, Boorghani M, Ghaffari SR, AtarbashiMoghadam F, Keyhani A (2009) Human papillomavirus in saliva of patients with oral squamous cell carcinoma. Med Oral Patol Oral Cir Bucal 14: e525–528
25. Adelstein DJ, Ridge JA, Gillison ML et al (2009) Head and neck squamous cell cancer and the human papillomavirus: summary of a National Cancer Institute State of the Science Meeting, November 9–10, 2008, 31. Head Neck, Washington DC, pp 1393–1422
26. Balan R, Simion N, Giuşcă SE et al (2011) Immunohistochemical assessment of p16, COX-2 and EGFR in HPV-positive cervical squamous intraepithelial lesions. Rom J Morphol Embryol 52: 1187–1194
27. Ozbun MA, Meyers C (1997) Characterization of late gene transcripts expressed during vegetative replication of human papillomavirus type 31b. J Virol 71:5161–5172
28. Correnti M, Rossi M, Avila M, Perrone M, Rivera H (2010) Human papillomavirus in ameloblastoma. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 110:e20–24
29. van Heerden WF, van Rensburg EJ, Raubenheimer EJ, Venter EH (1993) Detection of human papillomavirus DNA in an ameloblastoma using the in situ hybridization technique. J Oral Pathol Med 22:109–112

30. Sand L, Jalouli J, Larsson PA, Magnusson B, Hirsch JM (2000) Presence of human papilloma viruses in intraosseous ameloblastoma. J Oral Maxillofac Surg 58:1129–1134
31. Migaldi M, Pecorari M, Rossi G et al (2005) Does HPV play a role in the etiopathogenesis of ameloblastoma? An immunohistochemical, in situ hybridization and polymerase chain reaction study of 18 cases using laser capture microdissection. Mod Pathol 18:283–289
32. Dalgleish AG (1991) Viruses and cancer. Br Med Bull 47:21–46
33. Stubenrauch F, Laimins LA (1999) Human papillomavirus life cycle: active and latent phases. Semin Cancer Biol 9:379–386
34. Garner-Hamrick PA, Fostel JM, Chien WM et al (2004) Global effects of human papillomavirus type 18 E6/E7 in an organotypic keratinocyte culture system. J Virol 78:9041–9050
35. Samir R, Asplund A, Tot T, Pekar G, Hellberg D (2011) High-risk HPV infection and CIN grade correlates to the expression of c-myc, CD4+, FHIT, E-cadherin, Ki-67, and p16INK4a. J Low Genit Tract Dis 15:280–286
36. de Vicente JC, Torre-Iturraspe A, Gutiérrez AM, Lequerica-Fernández P (2010) Immunohistochemical comparative study of the odontogenic keratocysts and other odontogenic lesions. Med Oral Patol Oral Cir Bucal 15:e709–715
37. González-Alva P, Tanaka A, Oku Y, Yoshizawa D, Itoh S, Sakashita H, Ide F, Tajima Y, Kusama K (2008) Keratocystic odontogenic tumor: a retrospective study of 183 cases. J Oral Sci 50:205–212
38. Kim SH, Oh JM, No JH, Bang YJ, Juhnn YS, Song YS (2009) Involvement of NF-kappaB and AP-1 in COX-2 upregulation by human papillomavirus 16 E5 oncoprotein. Carcinogenesis 30:753–757
39. Wu R, Coniglio SJ, Chan A, Symons MH, Steinberg BM (2007) Up-regulation of Rac1 by epidermal growth factor mediates COX-2 expression in recurrent respiratory papillomas. Mol Med 13:143–150
40. Subbaramaiah K, Dannenberg AJ (2007) Cyclooxygenase-2 transcription is regulated by human papillomavirus 16 E6 and E7 oncoproteins: evidence of a corepressor/coactivator exchange. Cancer Res 67:3976–3985
41. Zhuang ZH, Tsao SW, Deng W et al (2008) Early upregulation of cyclooxygenase-2 in human papillomavirus type 16 and telomerase-induced immortalization of human esophageal epithelial cells. J Gastroenterol Hepatol 23:1613–1620
42. Prescott SM, Fitzpatrick FA (2000) Cyclooxygenase-2 and carcinogenesis. Biochim Biophys Acta 1470:M69–78
43. Mohan S, Epstein JB (2003) Carcinogenesis and cyclooxygenase: the potential role of COX-2 inhibition in upper aerodigestive tract cancer. Oral Oncol 39:537–546
44. Mestre JR, Subbaramaiah K, Sacks PG et al (1997) Retinoids suppress epidermal growth factor-induced transcription of cyclooxygenase-2 in human oral squamous carcinoma cells. Cancer Res 57:2890–2895
45. Mendes RA, Carvalho JF, Waal I (2009) An overview on the expression of cyclooxygenase-2 in tumors of the head and neck. Oral Oncol 45:e124–128
46. Zhang Y, McCluskey K, Fujii K, Wahl LM (1998) Differential regulation of monocyte matrix metalloproteinase and TIMP-1 production by TNF-α, granulocyte-macrophage CSF and IL-1β through prostaglandin-dependent and -independent mechanisms. J Immunol 161:3071–3076
47. Callejas NA, Casado M, Diaz-Guerra MJ, Bosca L, Martin-Sanz P (2001) Expression of cyclooxygenase-2 promotes the release of matrix metalloproteinase-2 and −9 in fetal rat hepatocytes. Hepatology 33:860–867
48. Ottino P, Bazan HE (2001) Corneal stimulation of MMP-1, −9 and uPA by platelet-activating factor is mediated by cyclooxygenase-2 metabolites. Curr Eye Res 23:77–85
49. Gomes CC, Duarte AP, Diniz MG, Gomez RS (2010) Review article: Current concepts of ameloblastoma pathogenesis. J Oral Pathol Med 39:585–591
50. Harris M (1978) Odontogenic cyst growth and prostaglandin-induced bone resorption. Ann R Coll Surg Engl 60:85–91
51. Fracon RN, Teófilo JM, Satin RB, Lamano T (2008) Prostaglandins and bone: potential risks and benefits related to the use of nonsteroidal anti-inflammatory drugs in clinical dentistry. J Oral Sci 50:247–252
52. Gao L, Li TJ (2005) In vitro study on bone resorption of odontogenic cysts and ameloblastomas. Zhonghua Kou Qiang Yi Xue Za Zhi 40: 233–236
53. Mendes RA, Carvalho JF, van der Waal I (2011) Potential relevance of cyclooxygenase-2 expression in keratocystic odontogenic tumours - an immunohistochemical study. J Oral Pathol Med 40:497–503
54. Tsai CH, Huang FM, Yang LC, Chou MY, Chang YC (2002) Immunohistochemical localization of cyclooxygenase-2 in radicular cysts. Int Endod J 35:854–858
55. Johnson RL, Rothman AL, Xie J et al (1996) Human homolog of patched, a candidate gene for the basal cell nevus syndrome. Science 272:1668–1671
56. Ren C, Amm HM, DeVilliers P et al (2012) Targeting the sonic hedgehog pathway in keratocystic odontogenic tumor. J Biol Chem 287:27117–27125
57. Bhargava D, Deshpande A, Pogrel MA (2012) Keratocystic odontogenic tumour (KCOT)–a cyst to a tumour. Oral Maxillofac Surg 16:163–170
58. Xuan YH, Li GL, Jiang HY, Lin ZH (2009) Relationship between hedgehog signaling pathway molecules and HPV16 infection in uterine cervical cancers. Zhonghua Bing Li Xue Za Zhi 38:178–182
59. Samarzija I, Beard P (2012) Hedgehog pathway regulators influence cervical cancer cell proliferation, survival and migration. Biochem Biophys Res Commun 425:64–69
60. Yagyuu T, Kirita T, Sasahira T, Moriwaka Y, Yamamoto K, Kuniyasu H (2008) Recurrence of keratocystic odontogenic tumor: clinicopathological features and immunohistochemical study of the Hedgehog signaling pathway. Pathobiology 75:171–176