Histol Histopathol (2013) 28: 1175-1184
DOI: 10.14670/HH-28.1175

http://www.hh.um.es

Histology and Histopathology
Cellular and Molecular Biology

Beta-catenin and survivin expression in keratocystic odontogenic tumor (KCOT). A comparative immunohistochemical study in primary, recurrent and nevoid basal cell carcinoma syndrome (NBCCS)-associated lesions

R. Leonardi[1], J.B. Matthews[2], C. Loreto[3], G. Musumeci[3], G. Campisi[4], L. Lo Muzio[5], J.N. dos Santos[6], L. Pastorino[7], P. Bufo[8] and G. Pannone[8]

[1]Department of Medical and Surgical Science. II Dental Unit - University of Catania, Catania, Italy, [2]Unit of Oral Pathology, School of Dentistry, University of Birmingham, Birmingham, United Kingdom, [3]Department of Anatomy, Diagnostic Pathology, Forensic Medicine, Hygiene and Public Health, University of Catania, Italy, [4]Department of Oral Sciences, University of Palermo, Palermo, Italy, [5]Section of Oral Pathology, [8]Section of Pathological Anatomy, Department of Clinical and Experimental Medicine, University of Foggia, Foggia, Italy, [6]Universidade Federal Da Bahia Faculdade De Odontologia Laboratório De Patologia Cirúrgica, Brazil and [7]Department of Genetics - DOBIG- University of Genova, Genova, Italy

Summary. Aim: To determine the epithelial expression of ß-catenin and survivin in sporadic (primary, and recurrent) and nevoid basal cell carcinoma syndrome (NBCCS) keratocystic odontogenic tumour (KCOT) in order to assess activation of the ß-catenin pathway and evidence of apoptotic inhibition, processes that may contribute to the known differences in their biological behaviour.

Materials and Methods: Sections from 40 cases of KCOT (19 sporadic/primary; 9 sporadic/recurrent and 12 NBCCS-associated) were immunohistochemically stained for ß-catenin and survivin. The extent and intensity of immunoreactivity within the lining epithelium was assessed, using semi-quantitative scales, independently by two pathologists who were blinded to the clinical-pathological data. Data were analysed using Kruskal-Wallis test and, for pair-wise comparisons, Mann-Whitney test with Bonferroni correction.

Results: All cystic epithelial linings stained for ß-catenin and survivin but there were differences in the pattern and intensity of staining among KCOT types. Sporadic primary KCOT showed weaker staining for ß-catenin (P=0.0003) and survivin (P<0.0048) that was restricted to the basal and para-basal layers only, compared to sporadic recurrent and NBCCS-associated KCOT, which showed expression throughout all epithelial layers. There were no differences in ß-catenin expression among recurrent and NBCCS-associated KCOT, whereas the intensity of survivin staining was higher in NBCCS-KCOT (P=0.0003). Nuclear staining for ß-catenin was found exclusively in recurrent (5/9 cases) and NBCCS-associated (4/12 cases) KCOT.

Conclusion: The data demonstrate ß-catenin delocalization and survivin over-expression in recurrent sporadic and NBCCS-associated KCOT suggesting that these pathways are related to apoptotic inhibition have a role in KCOT growth and recurrence.

Key words: NBCCS, ß-catenin, Survivin, KCOT

Offprint requests to: Dr. Rosalia Leonardi, Department of Orthodontics, University of Catania, via S. Sofia n 78. Catania, Italy. e-mail: rleonard@unict.it

Introduction

Keratocystic odontogenic tumor (KCOT), previously called odontogenic keratocyst (OCK), is a benign odontogenic neoplasm that has a high recurrence rate. In the new WHO classification revised in 2005 (Barnes et al., 2005), KCOT is defined as benign intraosseous neoplasm of odontogenic origin with a characteristic lining of parakeratinized squamous epithelium. It is generally agreed that the origin of KCOT is odontogenic epithelium, particularly the dental lamina and its

remnants and extensions of basal cells from overlying oral epithelium (Kramer, 1992; Shear, 2002; Qi et al., 2010).

KCOTs are normally sporadic lesions but they can be associated with nevoid basal cell carcinoma syndrome syndrome (NBCCS), when multiple lesions can be present. Interestingly, these two varieties of KOCTs (NBCCS-KCOTs and sporadic KCOTs) are known to differ in their biological behaviour (see (Shear, 2002) for review). Thus, differences in cellular proliferation rates and/or in the expression of oncoproteins and tumour suppressor genes have been described, with positivity for PCNA (de Paula et al., 2000; Lin et al., 2005), Ki-67 and p53 (Lombardi et al., 1995; Ogden et al., 1992) being more frequently observed in NBCCS-KCOTs than in sporadic KCOTs.

It has been hypothesized (Lo Muzio et al., 1999) that underlying genetic alterations could be responsible for the different biological behaviour. In fact, the loss of patched (PTCH) function could contribute to the transformation of certain cell types via the wingless/wingless-type (WG/Wnt) signalling pathway. Aberrant activation of the Wnt/ß-catenin signalling pathway has been frequently reported in a broad spectrum of human malignancies (Lo Muzio et al., 2002; Uraguchi et al., 2004; Pannone et al., 2010) and altered expression of ß-catenin has been suggested to play an important role in oral squamous cell carcinoma (OSCC) progression (Tanaka et al., 2003). ß-catenin, a key signaling factor involved in the canonical pathway, is exclusively localized to the membrane in normal oral mucosal epithelium. Abnormal activation of Wnt/-catenin signaling pathway is linked to the accumulation of ß-catenin in the nucleus; this in turn can contribute to neoplastic transformation through transcriptional activation of target genes involved in tumor cell proliferation and progression (Vidya Priyadarsini et al., 2012) including cyclin D1, VEGF (vascular endothelial growth factor) and survivin, all of which are known as contributors to cancer progression.

Survivin is a member of the inhibitor of apoptosis (IAP) family of proteins that is prominently up-regulated in many human cancers (Altieri, 2010), yet virtually undetectable in normal adult tissues. The survivin protein functions to inhibit caspase activation, thereby leading to negative regulation of apoptosis or programmed cell death. Survivin in tumors has been detected in both cytoplasmic and nuclear compartments. Cytoplasmic and nuclear localization of survivin is regulated by its nuclear export signal (NES) (Engels et al., 2007; Lippert et al., 2007). Nuclear survivin is mainly related to the control of cell division, whereas cytoplasmic survivin is considered cytoprotective functioning against apoptosis machinery. The intracellular localization of survivin in tumours has been suggested as a prognostic marker in malignant tumors of the Head and Neck (Lo Muzio et al., 2001, 2003) however the sub-cellular localization of survivin has not been studied in benign cysts and tumours of the oral cavity.

Some authors have described an abnormal expression of tumour-suppressor genes and oncogenes in the cystic epithelium of KCOT but relatively few studies have focused on apoptotic mechanisms and the published data are contradictory, (Lombardi et al., 1995; Li et al., 1996; Lo Muzio et al., 1999, 2005; Clark et al., 2006; de Oliveira et al., 2008; Gurgel et al., 2008; Malcic et al., 2008; Moreira et al., 2009; Gabdail et al., 2011; Mendes et al., 2011; Sharifi-Sistani et al., 2011). In fact, different findings have been reported on P53 and Bcl-2 expression in sporadic primary KCOTs, sporadic recurrent KCOTs, and NBCCS-associated KCOTs. In this respect, in some studies both sporadic (primary and recurrent) and NBCCS-associated KCOTs were positive for P53, whereas in contrast some other investigations showed that only the NBCCS-associated KCOTs were immunolabelled by P53. Also Bcl-2 positivity in NBCCS-associated KCOTs was significantly higher when compared to sporatic KCOTs in some studies, while in some others no significant difference on Bcl-2 was presented.

Here we hypothesized that the behaviour of KCOTs will differ depending upon aberrant activation of the ß-catenin signalling pathway and to subsequently impaired apoptosis. According to this premise we carried out an immunohistochemical study on ß-catenin and survivin expression in sporadic primary KCOTs (sp-KCOT), sporadic recurrent KCOTs (sr-KCOT), and NBCCS-associated KCOTs (NBCCS-KCOT).

Materials and methods

Specimens

Cases of KOCT were retrieved from the files of the Oral Pathology Unit, School of Dentistry, University of Birmingham U.K, the Faculdade De Odontologia Laboratório De Patologia Cirúrgica Universidade Federal Da Bahia, Brazil and from Pathological Anatomy, University of Foggia, Italy. The KOCTs were surgically removed from 23 men and 17 women with a mean age of 32 years (SD ± 8.7; minimum 16 and maximum 49); 36 KOCTs were in the mandible and 4 in the maxilla. All the lesions were treated with the same Partch II surgical approach. The lesions (Leonardi et al., 2010) were classified as sporadic primary KCOTs (sp-KCOT, n=19) if they were not treated previously, sporadic recurrent KCOTs (sr-KCOT, n=9) if the lesions recurred more than 1 year after the first surgical intervention (follow up period of 2-5 years) and NBCCS-associated KCOTs (NBCCS-KCOT, n=12) if the patients fulfilled two of the four major criteria or one major and two minor criteria required for NBCCS diagnosis (High and Zedan, 2005; Evans and Farndon, 1993). Clinicopathological diagnosis of NBCCS has been further confirmed by molecular analysis showing PTCH gene mutations as evaluated by previously described methods (Pastorino et al., 2005). A

representative elettropherogram showing PTCH mutation is shown in Fig. 2. All pathological diagnoses were made comparing the clinical, radiological and histological data and conformed to the parameters recommended by Kimonis et al. (1997) and Kramer and to World Health Organization classification of odontogenic cysts and tumors (Kramer, 1992; Barnes et al., 2005). All the cases selected for the study demonstrated well preserved histopathological features and dentigerous non inflamed follicular cysts (DC, n=7), served as controls.

Immunohistochemistry

Four-micron serial sections from formalin-fixed, paraffin-embedded blocks of representative areas of cysts were cut for each case. Only sections containing sufficient epithelium to assess the antibody reactivity of 200 cells were considered for this study.

Sections were mounted on poly-L-lysine-coated glass slides. Deparaffined and rehydrated sections were incubated for 30 min in 3% H_2O_2/methanol to quench endogenous peroxidase activity and then rinsed for 20 min with phosphate-buffered saline (PBS) (Bio-Optica M107, Milan, Italy). The sections were irradiated (3x5 min) in capped polypropylene slide-holders with citrate buffer (pH 6), using a microwave oven (750 W) to unmask antigenic sites. Non-specific protein binding sites were blocked by incubation for 30 min with 5% horse serum in PBS prior to overlaying with primary antibodies and incubation at 4°C in a moist chamber. Monoclonal antibody clone 6B3 (1:150 dilution; Cell Signaling Technology, Inc., 3 Trask Lane, Danvers, MA 01923 USA) was used to detect ß-catenin, as it is known to react with nuclear, cytoplasmatic and membranous protein expression. Survivin was detected using a rabbit polyclonal antibody (Ab469 AbCam, Cambridge UK) diluted 1:400 in normal goat serum/PBS. The sections were washed three times with PBS at room temperature prior to overlaying with biotinylated secondary antibody (30 minutes). After further washing, sections were incubated with streptavidin peroxidase (30 minutes; Vectastain ABC kit, Vector Laboratories, Burlingame, CA, USA). After rinsing with three changes of PBS the immunoreactivity was visualized by development for 2 min with 0.1% 3,3'-diaminobenzidine and 0.02% hydrogen peroxide (DAB substrate kit, Vector Laboratories). All incubations were performed at room temperature. Sections were counterstained with type-II-Gill's haematoxylin, mounted with permanent mounting medium and examined by light microscopy.

Positive controls consisted of tissue sections of colon-adenocarcinoma. A negative control was performed in all cases by substituting the primary antibody with normal mouse serum.

Evaluation of immunohistochemistry

Immunoreactivity was evaluated as previously described (Kawahara et al., 2011; Nikitakis et al., 2009), by two expert pathologists who were blinded to the clinical-pathological data and scored by a semi-quantitative scale assigning cases to one of the three following categories (extent score=ES): (a) score 0, 0 to ≤5%; (b) score 1, 6 to ≤40%; (c) score 2, 41 to ≤70% and (d) score 3, >71% of total cells stained. The percentage of positive cells was determined from the analysis of 100 cells in 10 random areas at 40x magnification. The intensity of staining (IS) was evaluated and graded on a scale of 0-3, according to the following assessment: 0=no detectable staining, 1=weak staining, 2=moderate staining, 3=strong staining-very strong staining.

Staining of the lining epithelium of the cystic lesions was assessed separately in the basal cell layer, the para-basal layer, which included one-to-two cell layers above the basal layer, and the remaining epithelial layers up to the lumen surface (collectively termed the luminal layer). For each case, the individual score of each layer was recorded and the total score (TS) was calculated as the mean of all the individual scores. Results are presented as the mean score per epithelial layer, and as the mean total score per type of lesion and per antibody. The localization of staining (membrane, cytoplasm, nucleus) was also evaluated for both ß-catenin and survivin.

Statistical analysis

Means and standard deviations were obtained for the extent (ES) and intensity (IS) of staining for the three linings of cystic epithelium (basal, para-basal, luminal) in each sample, and for each antibody. Total epithelial scores for extent and intensity of staining were also calculated as the mean of the three individual layer scores. The data were analyzed using the Kruskal-Wallis test, which allowed comparison of ß-catenin and survivin protein expression scores among epithelial linings of the four cyst types. Mann-Whitney test was used for pair-wise comparisons using $p \leq 0.05$ as the level of significance after Bonferroni correction for multiple comparisons among the cyst types Statistical computation was conducted using SPSS (SPSS® release 16.0, Chicago, IL, USA).

Results

Histologically, the tumors showed a cystic space lined with a uniform parakeratinized squamous epithelium 5 to 10 cell layers thick. The basal cells were aligned with vertically elongated nuclei at right angles to the basement membrane. Some cases showed subepithelial splits and epithelial buds and satellite cysts. In addition, discrete focal mononuclear inflammatory infiltrates were seen in 14 lesions. In particular, inflammatory infiltrate was detected in NBCCS-KCOTs especially in areas showing daughter cysts with keratins shedding into the connective cystic wall.

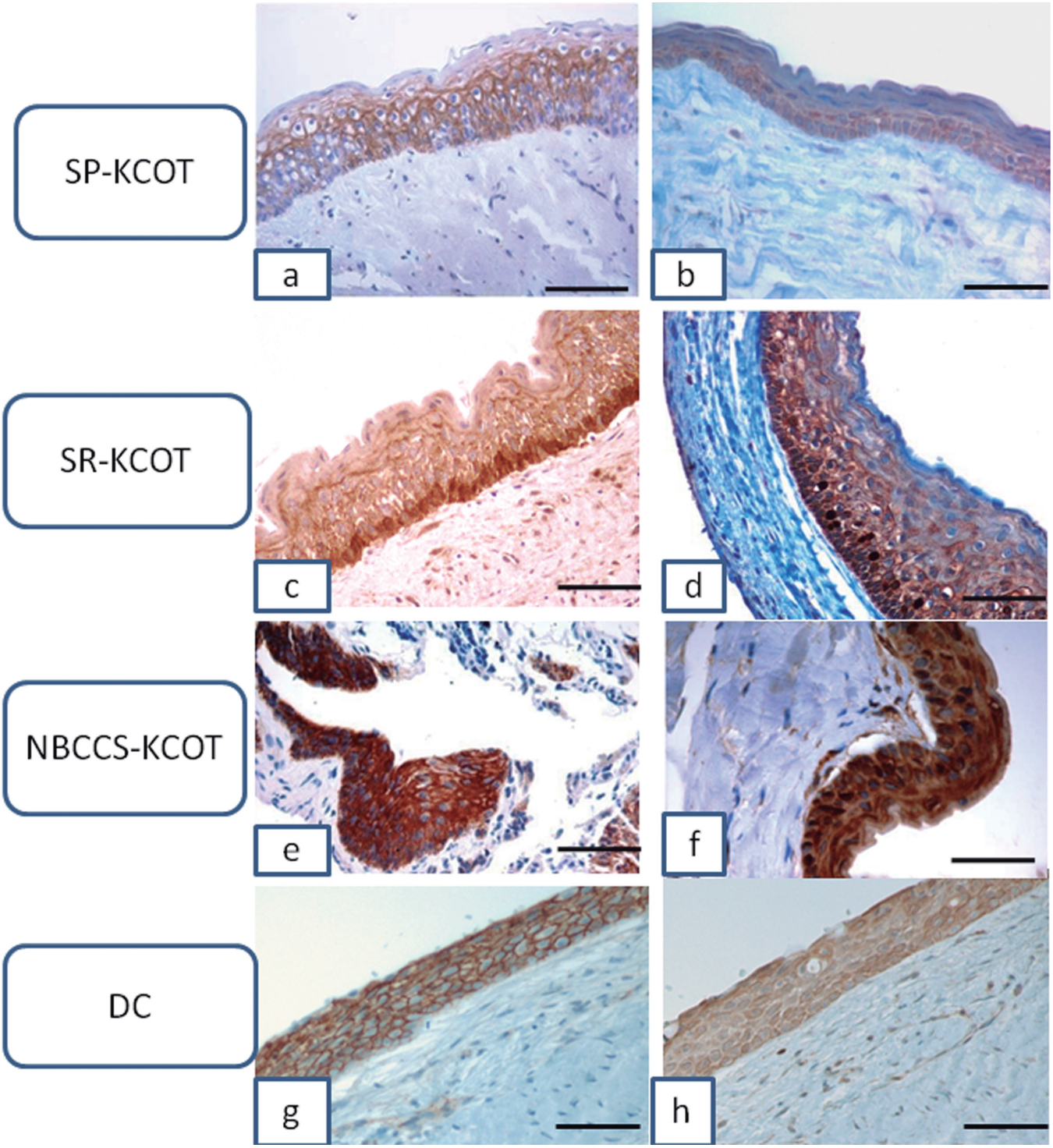
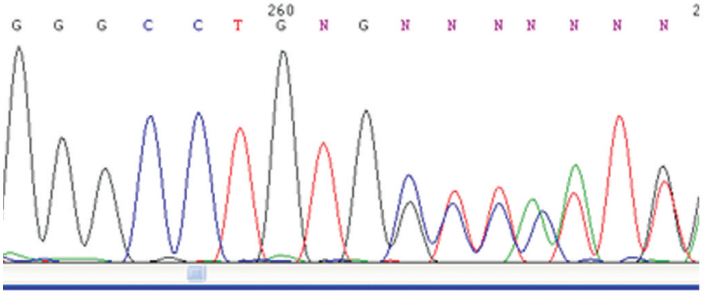


Fig. 1. Expression of ß-catenin and survivin in different histotypes of dentigerous cyst. ß-catenin (left column) and survivin (right column) expression in sporadic-primary (SP-KCOT), sporadic-recurrent (SR-KCOT) and NBCCS-associated KCOT (NBCCS-KCOT) and in dentigerous follicular cyst (DC). ß-catenin is located on sub-membranous cell compartment in DC **(g)** and SP-KCOT **(a)**, whereas this protein is delocalized/hyper-expressed in SR-KCOT **(c)** and NBCCS-KCOT **(e)** respectively. In parallel survivin expression stained slightly cytoplasmic in DC **(h)**, moderately cytoplasmic in SP-KCOT **(b)**, and strongly nucleo-cytoplasmic in SR-KCOT **(d)** and NBCCS-KCOT **(f)**; LSAB-HRP with type-II-Gill's haematoxylin. Bar: 100 μm.

The pattern of ß-catenin immunoreactivity showed extensive variation among lesions (Fig. 1). ps-KCOT cases were positive, with the staining concentrated in the basal and para-basal layers and weak or no staining of the luminal layers. Total staining scores were heterogeneous from low to intermediate as regards intensity (ES=1.0±0.8; IS=1.2±0.9) and not delocalized from the sub-membranous cell compartment. In sr-KCOTs, both basal and parabasal layers showed strong cytoplasmic and nuclear staining for ß-catenin with weak, mainly cytoplasmic, staining of the luminal layers; therefore, ß-catenin showed a delocalized pattern



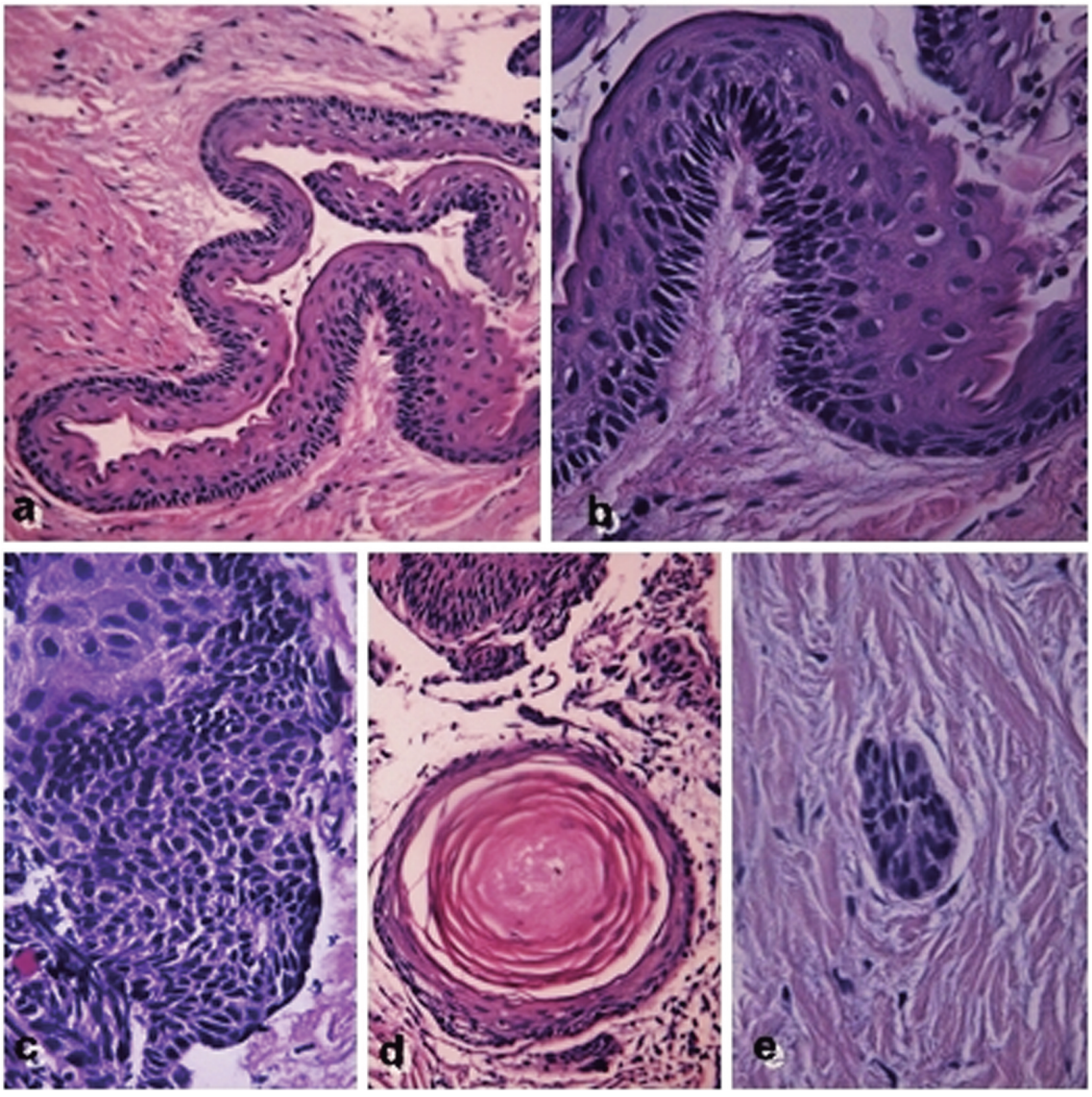


Fig. 2. Histological finding of NBCCS and molecular detection of PTCH mutation. Representative case of NBCCS-KCOT stained with haematoxylin and eosin showing some details of lining epithelium **(a, b, c)**, daughter cyst **(d)**, and epithelial droplet in the connective wall. The case is biologically proved by PTCH mutation as shown in elettropherogram (top picture).

in rs-KCOTs. In the NBCCS-KCOTs, all three epithelial layers showed strong reactivity for ß-catenin with total epithelial staining scores being high (ES=2.4±0.6 and IS=2.7±0.6). ß-catenin staining in dentigerous non inflamed cysts was observed in all epithelial layers but without protein delocalization from the submembranous cell compartment.

Although both cytoplasmic and membrane staining for ß-catenin was detected in all lesions, qualitatively, cytoplasmic staining was most pronounced in sr-KCOT and NBCCS-KCOT linings. Furthermore, nuclear staining was also observed in some srKCOT and NBCCS-KCOT linings but not in those of spKCOT and DC. Nuclear staining was apparent in the basal layer of 55% (5/9) of sr-KCOT linings and in the basal and para-basal layers of 33% (4/12) of NBCCS-KCOTs, indicating activation of ß-catenin in these lesions (Fig. 2). It is also interesting to note that nuclear staining for ß-catenin was not evenly distributed throughout positive epithelial linings (Fig. 2).

Survivin expression varied among lesions with immunostaining being generally more extensive (ES=2.3) and intense (IS=2.5) in NBCCS-KCOT epithelial linings than linings of other types of cyst (Fig. 1). In pKCOT, cytoplasmic survivin was detected weakly in the basal and para-basal layers only, whereas all epithelial layers of rKCOT and NBCCS-KCOT linings showed intermediate to strong staining. In addition, NBCCS-KCOT linings contained scattered cells within the basal and para-basal layers that showed strong nuclear reactivity for survivin (Fig. 3).

Overall there were statistically significant differences in the expression of both ß-catenin and survivin among NBCCS-KOCTs, rKOCTs, pKOCTs and RCs using the total extent (ES; $p<0.001$) and intensity (IS; $p<0.001$) of staining scores. Pair-wise comparisons demonstrated that the total expression of ß-catenin and survivin by primary KCOT linings was significantly lower than that detected in the linings of recurrent and NBCCS-associated KCOT (P=0.0003). ß-catenin expression did not differ between recurrent and NBCCS-KCOT but survivin appeared to be more intensely expressed in NBCCS-associated lesions (IS=2.5±0.8 v 1.5±0.5; P=0.0003). Similarly, whereas the intensity of expression of both ß-catenin and survivin was higher in NBCCS-KCOT compared to DC (P=0.0003), this was only true for ß-catenin in rs-KCOT (P=0.0003).

Details of ß-catenin delocalization and survivin over-expression in KCOT have been reported in Fig. 4, showing for both a clear nuclear expression in the basal third of epithelial layer.

Discussion

Keratocystic Odontogenic Tumors (KCOTs), formerly Odontogenic Keratocysts, have been defined as benign intraosseous neoplasms of odontogenic origin (Barnes et al., 2005). Despite this, the neoplastic nature of KCOTs is not completely established and, as inhibition of apoptosis is among the most important mechanisms of tumorigenesis, studies regarding expression of apoptosis-related proteins in KCOTs may contribute to our understanding of these lesions (Andric et al., 2010). In this respect, the Wnt/ß-catenin pathway is one of the most crucial signaling pathways in cell proliferation and apoptosis. It is highly conserved throughout cell evolution and regulates the expression of Wnt target genes through the ß-catenin/T-cell factor (Tcf) (Moon et al., 2002; Nelson and Nusse, 2004). Abnormal Wnt/ß-catenin signaling and its accumulation in the nucleus plays a role in human carcinogenesis (Zhao et al., 2010). In brief, ß-catenin plays a dual role

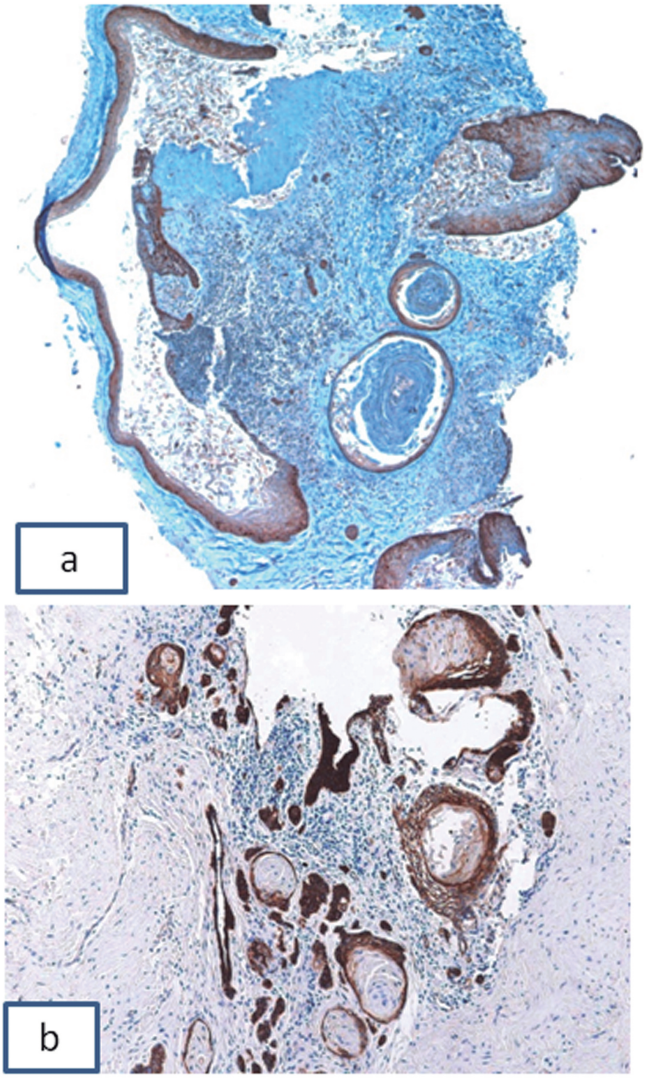


Fig. 3. Increasing clinical aggressivity is documented by progressive increase of daughter cysts and epithelial droplets in the connective wall in different types of KCOTs. **a.** Panoramic view of fig. 1A. **b.** Overview of fig.1D. Note that shedding of keratins into the connective cystic wall gives rise to phlogistic infiltration; the latter phenomenon is particularly evident in some SR-KCOTs and in NBCCS-KCOTs of our series. x 50

in cell pathway ; the first one is related to the cell-cell adhesion function of the *cadherin adhesion complex* (*CAC*), and the second one is due to its delocalization from the CAC and subsequent translocation into the nucleus, where it forms a complex with transcriptional factor (TCF), which in turn induces the expression of downstream target genes such as survivin, c-myc and cyclin D1 (Staal and Sen, 2008).

Survivin is a 16.5-kDa protein and is a unique member of the inhibitor of apoptosis gene family for which a function in the nucleus was identified (Altieri, 2003, 2010). Some reports have shown that high survivin expression is associated with adverse outcomes in patients with oral squamous cell carcinoma (Lin et al., 2005; Jane et al., 2006; Pannone et al., 2007). Functionally, survivin has been shown to inhibit apoptosis, and regulate cell division,. Because of its involvement in these processes, survivin is likely to be causally involved in tumor progression, and consequently, increased levels of survivin would be expected to predict an aggressive behavior of the disease.

The present study has evaluated the immunohistochemical expression of ß-catenin and survivin in sporadic primary, sporadic recurrent and NBCCS-associated KCOTs in order to understand the biological

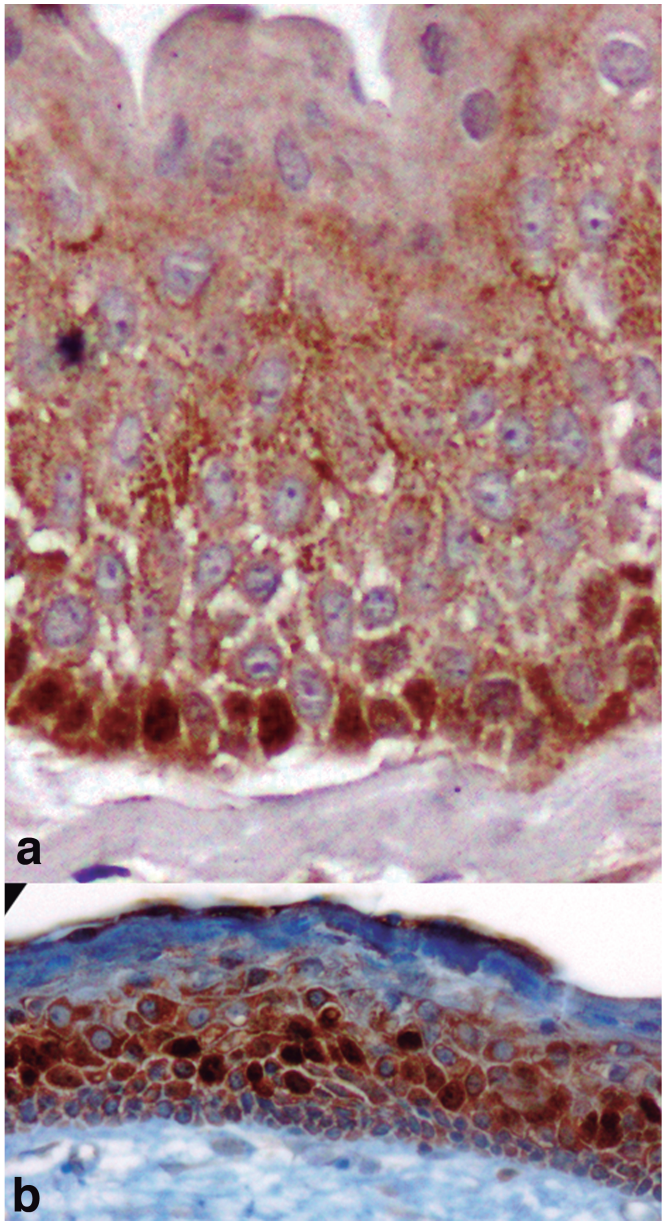


Fig. 4. Details of ß-catenin delocalization and survivin over-expression in KCOT. The pictures clearly show for both proteins a clear nuclear expression in the basal third of epithelial layer. a, x 600; b, x 400

behavior of the different types of KCOT. To our knowledge, there are only three published studies in this area, one investigating ß-catenin (Hakim et al., 2011) and two others survivin (Andric et al., 2010; de Oliveira et al. 2011) in KCOTs. A down-regulation in the expression of both ß-catenin and E-cadherin along with alteration of Wnt-1 and Wnt-10A expression in the epithelium of KCOT was reported by Hakim et al (2011). In particular, membrane expression of ß-catenin in all samples of sporadic and syndromic KCOT was significantly lower than that detected in dentigerous cyst linings. Our data agree with these findings in that NBCCS-KCOT and rKCOT linings appeared to show decreased membranous staining compared with those of pKCOT and DC. However, we detected increased cytoplasmic staining for ß-catenin in rKCOT and NBCCS-KCOTs compared to that seen in pKCOT and control dentigerous cysts, as well as nuclear expression exclusively in a proportion of recurrent and NBCCS-associated KCOT. These data suggest that the expression of delocalized ß-catenin may explain the different biological behavior of recurrent sporadic and NBCCS-associated KCOT which have a higher tendency to recur when compared to sporadic-primary KCOT.

Recently, nuclear accumulation of ß-catenin in the nucleus of squamous cell carcinoma of the tongue has been reported and it has been suggested that this is closely related to tumor progression and increased risk of recurrence (Li et al., 2009). Similarly, the apparent loss of ß-catenin membrane expression and increased cytoplasmic/nuclear staining detected in recurrent and NBCCS-associated KCOT could be important in the recurrence of these lesions. In this respect it has been demonstrated that Wnt-1 modulates cell-cell adhesion by stabilizing ß-catenin binding to the cell adhesion protein cadherin (Hinck et al., 1994). Since ß-catenin regulates, together with ß-catenin, the cadherin function, aberrant expression of ß-catenin and cadherin results in decreased cell-cell adhesion and disruption of tissue morphogenesis, which correlates with neoplastic cell invasion (Hakim et al., 2011).

As far as survivin is concerned, one previous study investigated the expression of survivin, in sporadic KCOT and apical cysts, in order to establish a possible relationship between survivin expression and the presence of human cytomegalovirus (CMV) within those cysts (Andric et al., 2010). Their results demonstrated that all KCOTs showed immunostaining for survivin, irrespective of the presence of CMV, while all the apical cysts were negative. The reported weak to patchy, strong survivin expression in the parakeratinized squamous epithelial lining was similar to that found in primary-sporadic KCOT in our study. Another investigation study evaluated the biological profile of odontogenic epithelium by immunolabeling of epidermal growth factor receptor (EGFR), Ki-67 and survivin in KCOTs, DCs, and pericoronal follicles (PF) (de Oliveira et al., 2011). Findings in KOTs demonstrated that survivin immunolabeling showed a greater percentage of positive cells in the suprabasal layer. The AA. (de Oliveira et al., 2011) thus argued that suprabasal proliferative compartment seems to be maintained as a result of the significant expression of survivin, thus inhibiting apoptosis. In line with these previous studies, our data extend current knowledge by demonstrating that recurrent sporadic and NBCCS-associated KCOT linings express higher levels of survivin than those of primary-sporadic cases or dentigerous control cysts. Furthermore, in NBCCS-KCOT linings, we observed some scattered cells, mainly in the suprabasal layers, showing nuclear staining for survivin and ß-catenin. Nuclear expression of survivin has been associated to cell cycle progression and with Ki-67 nuclear expression in malignant cancer of the oral cavity (Qi et al., 2010). Thus our data suggest that survivin over-expression and nuclear localization in NBCCS-KCOT linings could play a significant role in tumor recurrence coupling antiapoptotic properties with cell cycle deregulation.

Because epithelial invasion of the connective wall plays an important role in recurrence of NBCCS associated KCOT we documented this phenomenon in relation to ß-catenin-survivin expression in these very rare diseases. In fact, it is well known that delocalized ß-catenin could increase the expression level of survivin. In this study we show that a delocalized ß-catenin expression is paralleled by increased survivin expression in RS-KCOT and in NBCCS-KCOT. However, we specify that because of the rarity of the NBCCS disease, the evidence of our results should be considered in proportion to the consequent relative low number of cases presented.

Furthermore, we cannot exclude that ß-catenin and survivin expression may be due at least in part to focal phlogistic infiltration. As regards NBCCS associated KCOT we noticed that especially in areas with epithelial droplets and daughter cysts there was phlogistic infiltration, probably due to keratin shedding into the connective tissue wall. Further studies are needed to demonstrate a possible role of phlogistic background in the pathogenesis recurrence of KCOTs.

In summary, our data demonstrate ß-catenin and survivin cytoplasmic-nuclear overexpression in recurrent-sporadic and NBCCS-associated KCOT suggesting, indirectly, Wnt-pathway activation, together with apoptotic inhibition. These changes in cell-cycle regulation and apoptosis suggest that these pathways are involved, together with dysregulation of other cell-cycle factors (Vered et al., 2009), in their growth and recurrence.

References

Altieri D.C. (2003). Survivin in apoptosis control and cell cycle regulation in cancer. Prog. Cell Cycle Res. 5, 447-452.

Altieri D.C. (2010). Survivin and iap proteins in cell-death mechanisms. Biochem. J. 430, 199-205.

Andric M., Dozic B., Popovic B., Stefanovic D., Basta-Jovanovic G., Djogo N., Andjus P. and Milasin J. (2010). Survivin expression in

odontogenic keratocysts and correlation with cytomegalovirus infection. Oral Dis. 16, 156-159.

Barnes L., Eveson J., Reichart P. and Sidransky D. (2005). Pathology and genetics of head and neck tumours In: WHO classification of tumours series. L.I. Press

Clark P., Marker P., Bastian H.L. and Krogdahl A. (2006). Expression of p53, ki-67, and egfr in odontogenic keratocysts before and after decompression. J. Oral Pathol. Med. 35, 568-572.

de Oliveira M.G., Lauxen Ida S., Chaves A.C., Rados P.V. and Sant'Ana Filho M. (2008). Immunohistochemical analysis of the patterns of p53 and pcna expression in odontogenic cystic lesions. Medicina Oral Patologia Oral Cirugia Bucal 13, E275-280.

de Oliveira M.G., Lauxen Ida S., Chaves A.C., Rados P.V. and 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.

de Paula A.M., Carvalhais J.N., Domingues M.G., Barreto D.C. and Mesquita R.A. (2000). Cell proliferation markers in the odontogenic keratocyst: Effect of inflammation. J. Oral Pathol. Med. 29, 477-482.

Engels K., Knauer S.K., Metzler D., Simf C., Struschka O., Bier C., Mann W., Kovacs A.F. and Stauber R.H. (2007). Dynamic intracellular survivin in oral squamous cell carcinoma: Underlying molecular mechanism and potential as an early prognostic marker. J. Pathol. 211, 532-540.

Evans D.G. and Farndon P.A. (1993). Nevoid basal cell carcinoma syndrome. In: Genereviews. Pagon R.A., Bird T.D., Dolan C.R. and Stephens K. (eds). Seattle (WA).

Gadbail A.R., Patil R. and Chaudhary M. (2011). Co-expression of ki-67 and p53 protein in ameloblastoma and keratocystic odontogenic tumor. Acta Odontolog. Scand. 70, 529-535.

Gurgel C.A., Ramos E.A., Azevedo R.A., Sarmento V.A., da Silva Carvalho A.M. and dos Santos J.N. (2008). Expression of ki-67, p53 and p63 proteins in keratocyst odontogenic tumours: An immunohistochemical study. J. Mol. Histol. 39, 311-316.

Hakim S.G., Kosmehl H., Sieg P., Trenkle T., Jacobsen H.C., Attila Benedek G., Ribbat J. and Driemel O. (2011). Altered expression of cell-cell adhesion molecules beta-catenin/e-cadherin and related wnt-signaling pathway in sporadic and syndromal keratocystic odontogenic tumors. Clin. Oral Invest. 15, 321-328.

High A. and Zedan W. (2005). Basal cell nevus syndrome. Current Opin. Oncol. 17, 160-166.

Hinck L., Nelson W.J. and Papkoff J. (1994). Wnt-1 modulates cell-cell adhesion in mammalian cells by stabilizing beta-catenin binding to the cell adhesion protein cadherin. J. Cell Biol. 124, 729-741.

Jane C., Nerurkar A.V., Shirsat N.V., Deshpande R.B., Amrapurkar A.D. and Karjodkar F.R. (2006). Increased survivin expression in high-grade oral squamous cell carcinoma: A study in indian tobacco chewers. J. Oral Pathol. Med. 35, 595-601.

Kawahara A., Harada H., Abe H., Yamaguchi T., Taira T., Nakashima K., Mihashi H., Akiba J. and Kage M. (2011). Nuclear beta-catenin expression in basal cell adenomas of salivary gland. J. Oral Pathol. Med. 40, 460-466.

Kimonis V.E., Goldstein A.M., Pastakia B., Yang M.L., Kase R., DiGiovanna J.J., Bale A.E. and Bale S.J. (1997). Clinical manifestations in 105 persons with nevoid basal cell carcinoma syndrome. Am. J. Med. Genet .69, 299-308.

Kramer I.R. (1992). The world health organization: Histological typing of odontogenic tumours: An introduction to the second edition. J. Dent. Assoc. S. Afr. 47, 208-210.

Leonardi R., Matthews J.B., Caltabiano R., Greco M., Lombardo C., Loreto C., Santarelli A. and Lo Muzio L. (2010). Mmp-13 expression in keratocyst odontogenic tumour associated with nbccs and sporadic keratocysts. Oral Dis. 16, 795-800.

Li S., Jiao J., Lu Z. and Zhang M. (2009). An essential role for n-cadherin and beta-catenin for progression in tongue squamous cell carcinoma and their effect on invasion and metastasis of tca8113 tongue cancer cells. Oncol. Rep. 21, 1223-1233.

Li T.J., Browne R.M., Prime S.S., Paterson I.C. and Matthews J.B. (1996). P53 expression in odontogenic keratocyst epithelium. J. Oral Pathol. Med. 25, 249-255.

Lin C.Y., Hung H.C., Kuo R.C., Chiang C.P. and Kuo M.Y. (2005). Survivin expression predicts poorer prognosis in patients with areca quid chewing-related oral squamous cell carcinoma in taiwan. Oral Oncol. 41, 645-654.

Lippert B.M., Knauer S.K., Fetz V., Mann W. and Stauber R.H. (2007). Dynamic survivin in head and neck cancer: Molecular mechanism and therapeutic potential. Int. J. Cancer 121, 1169-1174.

Lo Muzio L., Staibano S., Pannone G., Bucci P., Nocini P.F., Bucci E. and De Rosa G. (1999). Expression of cell cycle and apoptosis-related proteins in sporadic odontogenic keratocysts and odontogenic keratocysts associated with the nevoid basal cell carcinoma syndrome. J. Dent. Res. 78, 1345-1353.

Lo Muzio L., Pannone G., Staibano S., Mignogna M.D., Grieco M., Ramires P., Romito A.M., De Rosa G. and Piattelli A. (2002). Wnt-1 expression in basal cell carcinoma of head and neck. An immunohistochemical and confocal study with regard to the intracellular distribution of beta-catenin. Anticancer Res. 22, 565-576.

Lo Muzio L., Pannone G., Leonardi R., Staibano S., Mignogna M.D., De Rosa G., Kudo Y., Takata T. and Altieri D.C. (2003). Survivin, a potential early predictor of tumor progression in the oral mucosa. J. Dent. Res. 82, 923-928.

Lo Muzio L., Staibano S., Pannone G., Mignogna M.D., Mariggio A., Salvatore G., Chieffi P., Tramontano D., De Rosa G. and Altieri D.C. (2001). Expression of the apoptosis inhibitor survivin in aggressive squamous cell carcinoma. Exp. Mol. Pathol. 70, 249-254.

Lo Muzio L., Santarelli A., Caltabiano R., Rubini C., Pieramici T., Fior A., Trevisiol L., Carinci F., Leonardi R., Bufo P., Lanzafame S. and Piattelli A. (2005). P63 expression in odontogenic cysts. Int. J. Oral Maxillof. Surg. 34, 668-673.

Lombardi T., Odell E.W. and Morgan P.R. (1995). P53 immunohistochemistry of odontogenic keratocysts in relation to recurrence, basal-cell budding and basal-cell naevus syndrome. Arch. Oral Biol. 40, 1081-1084.

Malcic A., Jukic S., Anic I., Pavelic B., Kapitanovic S., Kruslin B. and Pavelic K. (2008). Alterations of fhit and p53 genes in keratocystic odontogenic tumor, dentigerous and radicular cyst. J. Oral Pathol. Med. 37, 294-301.

Mendes R.A., Carvalho J.F. and van der Waal I. (2011). A comparative immunohistochemical analysis of cox-2, p53, and ki-67 expression in keratocystic odontogenic tumors. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endodont. 111, 333-339.

Moon R.T., Bowerman B., Boutros M. and Perrimon N. (2002). The promise and perils of wnt signaling through beta-catenin. Science 296, 1644-1646.

Moreira P.R., Guimaraes M.M., Guimaraes A.L., Diniz M.G., Gomes C.C., Brito J.A. and Gomez R.S. (2009). Methylation of p16, p21,

p27, rb1 and p53 genes in odontogenic keratocysts. J. Oral Pathol. Med. 38, 99-103.

Nelson W.J. and Nusse R. (2004). Convergence of wnt, beta-catenin, and cadherin pathways. Science 303, 1483-1487.

Nikitakis N.G., Scheper M.A., Papanikolaou V.S., Sklavounou A. and Sauk J.J. (2009). Immunohistochemical expression of the oncogenic molecules active stat3 and survivin in benign and malignant salivary gland tumors. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endodont. 107, 837-843.

Ogden G.R., Chisholm D.M., Kiddie R.A. and Lane D.P. (1992). P53 protein in odontogenic cysts: Increased expression in some odontogenic keratocysts. J. Clin. Pathol. 45, 1007-1010.

Pannone G., Bufo P., Serpico R., Rubini C., Zamparese R., Corsi F., Pedicillo M.C., Staibano S., De Rosa G. and Lo Muzio L. (2007). Survivin phosphorylation and m-phase promoting factor in oral carcinogenesis. Histol. Histopathol. 22, 1241-1249.

Pannone G., Bufo P., Santoro A., Franco R., Aquino G., Longo F., Botti G., Serpico R., Cafarelli B., Abbruzzese A., Caraglia M., Papagerakis S. and Lo Muzio L. (2010). Wnt pathway in oral cancer: Epigenetic inactivation of wnt-inhibitors. Oncol. Rep. 24, 1035-1041.

Pastorino L., Cusano R., Nasti S., Faravelli F., Forzano F., Baldo C., Barile M., Gliori S., Muggianu M., Ghigliotti G., Lacaita M.G., Lo Muzio L. and Bianchi-Scarra G. (2005). Molecular characterization of italian nevoid basal cell carcinoma syndrome patients. Human Mutat. 25, 322-323.

Qi G., Kudo Y., Ando T., Tsunematsu T., Shimizu N., Siriwardena S.B., Yoshida M., Keikhaee M.R., Ogawa I. and Takata T. (2010). Nuclear survivin expression is correlated with malignant behaviors of head and neck cancer together with aurora-B. Oral Oncol. 46, 263-270.

Sharifi-Sistani N., Zartab H., Babakoohi S., Saghravanian N., Jamshidi S., Esmaili H., Mohtasham N., Zamanzadeh M. and Abbaszadeh-Bidokhty H. (2011). Immunohistochemical comparison of the expression of p53 and mdm2 proteins in ameloblastomas and keratocystic odontogenic tumors. J. Craniofacial Surg. 22, 1652-1656.

Shear M. (2002). The aggressive nature of the odontogenic keratocyst: Is it a benign cystic neoplasm? Part 2. Proliferation and genetic studies. Oral Oncol. 38, 323-331.

Staal F.J. and Sen J.M. (2008). The canonical wnt signaling pathway plays an important role in lymphopoiesis and hematopoiesis. Eur. J. immunol. 38, 1788-1794.

Tanaka N., Odajima T., Ogi K., Ikeda T. and Satoh M. (2003). Expression of e-cadherin, alpha-catenin, and beta-catenin in the process of lymph node metastasis in oral squamous cell carcinoma. Br. J. Cancer 89, 557-563.

Uraguchi M., Morikawa M., Shirakawa M., Sanada K. and Imai K. (2004). Activation of wnt family expression and signaling in squamous cell carcinomas of the oral cavity. J. Dent. Res. 83, 327-332.

Vidya Priyadarsini R., Senthil Murugan R. and Nagini S. (2012). Aberrant activation of wnt/beta-catenin signaling pathway contributes to the sequential progression of dmba-induced hbp carcinomas. Oral Oncol. 48, 33-39.

Zhao L., Shan B., Du Y., Wang M., Liu L. and Ren F.Z. (2010). Periplocin from cortex periplocae inhibits cell growth and down-regulates survivin and c-myc expression in colon cancer in vitro and in vivo via beta-catenin/tcf signaling. Oncol. Rep. 24, 375-383.

Accepted March 18, 2013