Differential expression of TLR3 and TLR4 in keratocystic odontogenic tumor (KCOT): A comparative immunohistochemical study in primary, recurrent, and nevoid basal cell carcinoma syndrome (NBCCS)–associated lesions

Leonardi, R.; Perrotta, R.e.; Crimi, S.; Matthews, John; Barbato, E.; Dos Santos, J.n.; Rusu, M.; Bufo, P.; Bucci, P.; Pannone, G.

DOI:
10.1016/j.jcms.2015.03.016

Accepted Manuscript

Differential expression of TLR3 and TLR4 in keratocystic odontogenic tumor (KCOT): A comparative immunohistochemical study in primary, recurrent, and nevoid basal cell carcinoma syndrome (NBCCS)–associated lesions

Dr. R. Leonardi, R.E. Perrotta, S. Crimi, J.B. Matthews, E. Barbato, J.N. dos Santos, M. Rusu, P. Bufo, P. Bucci, G. Pannone

PII: S1010-5182(15)00070-0

DOI: 10.1016/j.jcms.2015.03.016

Reference: YJCMS 1999

To appear in: *Journal of Cranio-Maxillo-Facial Surgery*

Received Date: 10 December 2014

Revised Date: 3 February 2015

Accepted Date: 18 March 2015

Please cite this article as: Leonardi R, Perrotta RE, Crimi S, Matthews JB, Barbato E, Santos JNd, Rusu M, Bufo P, Bucci P, Pannone G, Differential expression of TLR3 and TLR4 in keratocystic odontogenic tumor (KCOT): A comparative immunohistochemical study in primary, recurrent, and nevoid basal cell carcinoma syndrome (NBCCS)–associated lesions, *Journal of Cranio-Maxillofacial Surgery* (2015), doi: 10.1016/j.jcms.2015.03.016.

Differential expression of TLR3 and TLR4 in keratocystic odontogenic tumor (KCOT): A comparative immunohistochemical study in primary, recurrent, and nevoid basal cell carcinoma syndrome (NBCCS)–associated lesions

R. Leonardi[1], R. E. Perrotta[2], S. Crimi[3], J.B. Matthews[4], E. Barbato[5], J.N. dos Santos[6], M. Rusu[7], P. Bufo[8], P. Bucci[9], G. Pannone[10]

[1]Department of Medical and Surgical Science, Section of Dentistry, University of Catania, Catania, Italy

[2]Department of Medical and Surgical Science, Section of Plastic Surgery, University of Catania, Catania, Italy

[3]Department of Maxillofacial Surgery, University of Messina, Messina, Italy

[4]Unit of Oral Pathology, School of Dentistry, University of Birmingham, Birmingham, United Kingdom

[5]Department of Oral and Maxillofacial Sciences, School of Dentistry, "Sapienza" University of Rome, Rome, Italy

[6]Universidade Federal Da Bahia Faculdade De Odontologia Laboratório De Patologia Cirúrgica, Brazil

[7]Division of Anatomy, Faculty of Dental Medicine, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

[8]Dipartimento di Neuroscienze, Scienze Riproduttive ed Odontostomatologiche, Institute of Oral Pathology, University of Napoli 'Federico II', Naples, Italy

[9]Direttore Struttura Complessa di Anatomia Patologica, Dipartimento di Medicina Clinica e Sperimentale, Università degli Studi di Foggia, Foggia, Italy

[10]Department of Clinical and Experimental Medicine, Institute of Pathology and Cytopathology, University of Foggia, Foggia, Ital.

Corresponding Author: Dr. Rosalia Leonardi, Department of Orthodontics, University of Catania, via S. Sofia n 78. Catania, Italy. Tel.: +39 95 3782459; e-mail: rleonard@unict.it

Key words: NBCCS, TLR3, TLR4, KCOTs.

Background: Toll-like receptors (TLRs) play an essential role in the activation of innate immunity and they can promote cancer cell survival and tumour progression. It has been claimed that TLRs can somehow predict the clinical behaviour in oral squamous cell carcinoma (OSCCs).
Aim: To elucidate the molecular basis underlying keratocystic odontogenic tumor (KOCTs) aggressive behaviour and recurrence we carried out this immunohistochemical study on TLR3 and TLR4 expression in sporadic primary KCOTs (sp-KCOTs), sporadic recurrent KCOTs (sp-KCOTs), and NBCCS-associated KCOTs (NBCCS-KCOTs).
Method: 40 cases of KOCTs removed from 23 men and 17 women were the sample. Paraffin-embedded blocks were processed for immunohistochemistry. Sections were incubated with TLR3 and TLR4 antibodies and immunoreactivity evaluated on a semi-quantitative score.
Results: Both TLR3 and TLR4 were expressed in KCOTs epithelium, although with a different extent. TLR3 was not expressed in sp-KCOTs and sr-KCOTs, but it showed a faint staining in NBCCS-KCOTs. On the other hand, both cytoplasmic and nuclear staining for TLR4 was detected in all the 3 types of lesions; however being significantly more expressed in sr-KCOT and NBCCS-KCOTs ($p<0.0001$).
Our results, demonstrated an association between TLR4, but not TLR3 expression to recurrence behaviour of KCOTs. In fact, TLR4 was up-regulated in sr-KCOTs and NBCCS-KCOTs but not in sp-KCOTs.
Conclusions: According these findings it seems conceivable to assume that the up-regulation of TLR4 in some KCOTs can be correlated somehow to their tendency recurrence.

INTRODUCTION

Keratocystic odontogenic tumor (KCOT), previously called odontogenic keratocyst (OCK), is a developmental cyst characterized by an aggressive nature and high rate of recurrence, especially compared with other odontogenic cysts (Ahmed Haji Omar et al., 2014; Shear, 2002a; b; c).

KCOTs are normally sporadic primary lesions (sp-KCOTs), but they can be associated with nevoid basal cell carcinoma syndrome (NBCCS-KCOTs), in which case multiple lesions can be present. Interestingly, these two varieties of KOCTs are known to differ in their biological behaviour, i.e., in regard to growth speed and recurrence rate, as outlined in previous studies (Shear, 2002c). In fact, even though recurrence rates are capricious and have been reported to vary from 2.5% to 62.5%, they are generally higher in NBCCS-KCOTs (Woolgar et al., 1987a; b).

To date, many efforts have been carried out to determine the reasons for these high recurrence rates and aggressive behaviour. Consequently, many studies have been performed to identify predictive factors that could elucidate the behaviour of KCOTs. These studies have dealt with clinical and histological factors as well as molecular pathways (Leonardi et al., 2010; Leonardi et al., 2013; Lo Muzio et al., 2005; Shear, 1985; 2002b; Woolgar et al., 1987b). According to several investigations, differences in cellular proliferation rates and/or in the expression of oncoproteins and tumour suppressor genes have been described (de Paula et al., 2000; Lin et al., 2005), being much more highly expressed in NBCCS-KCOTs than in sp-KCOTs. However, whether these factors and, especially, epigenetic alterations are really related to KCOTs behaviour is a question that is still not answered (Gomes et al., 2009).

Several studies have addressed the relevance of innate immune response in cancer progression, as it has been argued that tumour progression could be

partly due to a failure of the innate immune response (Duray et al., 2010). Toll-like receptors (TLRs) have recently been reported to play an essential role in the activation of innate immunity. These receptors are expressed in a large number of immune cells as well as nonimmune cells such as epithelial cells (Zhang et al., 2009), and they are also present in many types of cancer.

To date, 11 human TLRs have been identified. There are two kinds of ligands for TLRs: exogenous pathogen–associated molecular patterns (PAMPs), which are components of microbes, and endogenous damage–associated molecular patterns (DAMPs), released from injured or inflamed tissues (Ioannou and Voulgarelis, 2010). Hence, the TLR-mediated immune response can be activated in the absence of foreign microbes (Tsan and Gao, 2004). DAMPs can be released from cells that have been affected by various stimuli and have entered a potentially neoplastic phase, as well as from cells that have undergone malignant transformation. On these premises, a large number of studies have investigated the role of TLRs in the pathogenesis of a range of malignant neoplasms (Bodelon et al., 2014; Rich et al., 2014).

As far as head and oral cavity tissues are concerned, some interesting studies have pointed out the role in TLRs to predicting the clinical behaviour of these types of cancer. According to these researches, an up-regulation of TLR5 activity may lead to more aggressive, invasive behaviour of oral carcinoma compared to skin cancer (Omar et al., 2014). Furthermore, another study demonstrated that the pattern of expression of TLR4 and TLR9 increased significantly from mild to severe oral epithelial dysplasia (OED), reflecting the progression of OED in oral squamous cell carcinoma (Kotrashetti et al., 2013). Also, a high/strong expression of TLR2, TLR4, and TLR9 seems to predict an invasive tumour growth of oral tongue squamous cell carcinoma (OTSCC) (Makinen et al., 2014).

Considering the roles of TLRs, in an attempt to elucidate the molecular basis underlying KOCTs aggressive behaviour and recurrence, we carried out this immunohistochemical study on TLR3 and TLR4 expression in sporadic primary KCOTs, sporadic recurrent KCOTs, and NBCCS-associated KCOTs.

MATERIAL AND METHODS

Specimens

After receiving approval by each institutional ethics committee, 40 cases of KOCTs were retrieved from the files of the Oral Pathology Unit, School of Dentistry, University of Birmingham, UK; the Faculdade De Odontologia Laboratório De Patologia Cirúrgica Universidade Federal Da Bahia, Brazil; and Pathological Anatomy, University of Foggia, Italy. The KOCTs were surgically removed from 23 men and 17 women with a mean age (± standard deviation) of 32 ± 8.7 years; range, 16–49). A total of 36 KOCTs were in the mandible and four in the maxilla. All of 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; as 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 as 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 (Evans and Farndon, 1993; High and Zedan, 2005). 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). All pathological diagnoses were made comparing the clinical, radiological, and histological data; all conformed to the parameters recommended by Kimonis (Kimonis et al., 1997) and Kramer and the World Health Organization classification of odontogenic cysts and tumors (Barnes L et al., 2005; Kramer, 1992). Noninflamed follicular dentigerous cysts (DC, n = 7) served as controls.

Immunohistochemistry

Serial sections (4 μm) 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.

Antibodies for TLR3 (TLR3.7; sc-32232), and TLR4 (H-80; sc-10741) were obtained from Santa Cruz Biotechnology. (Santa Cruz, California, USA). The dilution for each antibody was established based on negative and positive controls (1/10 for TLR3 and1/40 for TLR4,) Immunohistochemistry was performed on these sections using a Ventana Benchmark XT autostainer (Ventana Medical Systems, Tucson, Arizona, USA). Deparaffinization was done in xylene and rehydration with graded alcohol series. A standard linked streptavidin–biotin horseradish peroxidase technique (LSAB-HRP) technique with an iView DAB Detection Kit (Ventana Medical Systems, Tucson, Arizona, USA) was used as the staining detection system. Sections were counterstained with type-II Gill's hematoxylin, dehydrated with ethanol, and permanently coverslipped. Positive controls for immunohistochemistry consisted of tissue sections of oral squamous cell carcinomas (OSCCs). A negative control was performed in all cases by substituting the primary antibody with normal mouse serum.

Evaluation of immunohistochemistry

The slides were examined under a light microscope (Olympus Cx31; Tokyo, Japan) at ×400 magnification. Immunoreactivity was evaluated 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 four following categories 0 (0 to <5% immunostained cells), 1 (6% to <40% immunostained cells, 2 (41% to <70% immunostained cells), and 3 (>71% immunostained cells). The percentage of positive cells was determined from the analysis of 200 cells in 10 random areas at ×40 magnification. For each patient, the highest immunoscore was selected for further statistical analysis.

Statistical analysis

Means and standard deviations were obtained for the extent score (ES) for staining in each sample and for each antibody. The data were analyzed using the Kruskal–Wallis test, which allowed comparison of TLR3 and TLR4 expression scores among epithelial linings of the 3 cyst types and in the DC. Statistical computation was conducted using SPSS release 16.0 (SPSS Inc., Chicago, Illinois, 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, epithelial buds, and satellite cysts. In addition, discrete focal mononuclear inflammatory infiltrates were seen in 14 lesions.

Both TLR3 and TLR4 were expressed in KCOTs epithelium, although to a different extent; furthermore, the pattern of immunoreactivity showed extensive variation among each type of lesions (Figure 1, Table 1). TLR3 was not expressed in sp-KCOTs and sr-KCOTs. In NBCCS-KCOTs, TLR3 showed a faint staining, mainly concentrated in scattered cells of the basal and para-basal layers, with no staining of the luminal layers.

On the other hand, both cytoplasmic and nuclear staining for TLR4 was detected in all lesions qualitatively. Cytoplasmic staining was most pronounced in sp-KCOTs, whereas cytoplasmic and nuclear staining was most prominent in sr-KCOTs and NBCCS-KCOTs. In sp-KCOTs, immostaining was apparent in some scattered cells of the basal layer. In sr-KCOTs and NBCCS-KCOTs, almost every cell of the basal and para-basal layers was immunolabeled by anti-TLR4 antibody. In some instances also, the basal third of epithelial layer was immunopositive for TLR4 antibody.

There were no statistically significant differences in the expression of TLR3 among NBCCS-KOCTs, sr-KOCTs, sp-KOCTs, and DC ($p = 0.0517$). On the other hand, TLR4 ES showed a statistically significant difference ($p < 0.0001$) in the three cyst types, being more expressed in sr-KCOT and NBCCS-KCOTs, versus sp-KOCTs and DC (Table 1). Details of TLR3 and TLR4 expression in KCOTs and DC are given in Figures 1 and 2.

DISCUSSION

KCOT is an aggressive cystic lesion. Furthermore, although some OKC tumors do not recur after marsupialization or other conservative approaches, some lesions show aggressive behaviour. Several attempts have been made to elucidate this biologic behaviour, and investigations have tried to identify the molecular basis underlying the tendency of these lesions toward recurrence. Studies have demonstrated a high proliferative activity of these lesions and the expression of proteins associated with the inhibition of apoptosis. However, research has dealt with TLRs, which have been demonstrated to play a crucial role in cancer cells: in fact, they can promote cancer cell survival and tumor progression. In accordance, we carried out this immunohistochemical study with the intent to correlate any possible associations between TLR3 and TLR4 in sp-KCOTs, sr-KCOTs, and NBCCS-KCOTs, to improve the knowledge of the underlying mechanisms behind the aggressiveness of the disease as well as to provide new predictive markers that could be of assistance in choosing treatment modalities (Gomes et al., 2009; Luo et al., 2012).

Among the family of TLRs, the present study has included TLR3 and TLR4, because they have been significantly associated with a higher probability of biochemical recurrence in prostate carcinomas (Gonzalez-Reyes et al., 2011), and they are up-regulated in oral squamous cell carcinoma (OSCC) and oral tongue squamous cell carcinoma (OTSCC) (Ahmed Haji Omar et al., 2014; Kotrashetti et al., 2013; Luo et al., 2012; Rich et al., 2014). However, for the sake of clarity, it should be underlined that both TLR3 and TLR4 can act as

pro-tumorigenic, with their signals mediating tumor invasion and metastasis by enhancing cell migration; meanwhile, they can have also an anticancer effect in some situations (Rich et al., 2014).

Our results demonstrated an association between TLR4, but not TLR3, and recurrence behaviour of KCOTs. In fact, TLR4 was up-regulated in sr-KCOTs and NBCCS-KCOTs but not in sp-KCOTs and DC; on the other hand, a faint expression of TLR3 was observed in the 3 KCOT types and DC. According to our findings, it can be postulated that TLR3, being down-regulated in KCOTs, cannot induce apoptosis as described for some cancer cells (Luo et al., 2012; Salaun et al., 2006); on the other hand, it has been found that activation of TLR4 signalling results in increased growth of those cancer cells (Kelly et al., 2006).

In fact, TLR3, like Fas, can engage the extrinsic apoptotic machinery through the recruitment of caspase-8 to itself (Estornes et al., 2012). Furthermore, it has been demonstrated that ligation of TLR3 with its agonist directly reduces the viability of OSCC cells also (Luo et al., 2012)

On the other hand, TLR4 expressed on cancer cells can suppress the antitumor functions of infiltrating immune cells and can thus alter the inflammatory response in a manner that promotes cancer progression (Nair et al., 2013). Furthermore, in some instances, the expression levels of TLR4 in situ seems to be correlated with tumor differentiation (Sun et al., 2012), in that that poorly differentiated tumors expressed little TLR4. Accordingly, TLR4 is highly expressed in well-differentiated and moderately well–differentiated tumors. In this respect, it has been reported that the expression of TLR4 increased with increasing degrees of OED (Kotrashetti et al., 2013), and its up-regulation reflected the progression of OED to OSCC. High expression of TLR4 has been demonstrated in well-differentiated and moderately differentiated carcinomas (WDSCCs and MDSCCs), whereas on poorly differentiated carcinomas (PDSCCs) there was only a faint expression. Similar results were obtained in separate immunohistochemical studies conducted by Szczepanski et al. (Szczepanski et al., 2009) on head and neck cancers and on oral cancer, in which a strong positive reaction for TLR4 was characteristic for well-

differentiated or moderately differentiated tumours relative to moderate or weak staining intensity in poorly differentiated tumours (Nair et al., 2013). TLR4 has been associated also with the invasive potential of early-stage OTSCC (Makinen et al., 2014).

The net effect of TLR4 signalling activation by its respective ligands results in activation of transcriptional factors such as nuclear factor–κB (NF-κB) and activator protein 1 (AP-1) through myeloid differentiation factor 88 (MyD88) –dependent and MyD88-independent pathways (Rich et al., 2014). NF-κB regulates, in turn, the expression of various genes, the products of which are involved in tumorigenesis (Basith et al., 2012; de Andrade Santos et al., 2011).

Interestingly, it has been reported in a previous study that the more aggressive biologic behavior of OKCs, compared with radicular cysts (RC) and dentigerous cysts (DC) is related to the higher expression of NF-κB in these lesions (de Andrade Santos et al., 2011).

For the sake of clarity, it should be mentioned that there is a drawback in our study. Tissue from primary KCOTs, before becoming a recurrent lesion, was not available; thus we carried out our investigation in sp-KCOTs and sr-KCOTs, which were not paired. However, findings from this research may prompt further studies; in fact, in future investigations it will be worthwhile to evaluate both and compare the paired findings.

CONCLUSION

Based on our findings, it can be hypothesed that the up-regulation of TLR4 in some KCOTs can be correlated with their tendency toward recurrence. This finding can improve the current knowledge of molecular pathways involved in KCOTs and in some way help oral surgeons to predict which lesions will or will not recur.

Conflict of interest statement

None of the authors has a financial interest in any of the products, devices, or drugs mentioned in this article.

REFERENCES

Ahmed Haji Omar A, Korvala J, Haglund C, Virolainen S, Hayry V, Atula T, Kontio R, Rihtniemi J, Pihakari A, Sorsa T, Hagström J, Salo T: Toll-like receptors -4 and -5 in oral and cutaneous squamous cell carcinomas. J Oral Pathol Med, 2014. [Epub ahead of print].

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

Basith S, Manavalan B, Yoo TH, Kim SG, Choi S: Roles of toll-like receptors in cancer: a double-edged sword for defense and offense. Arch Pharm Res 35: 1297-1316, 2012

Bodelon C, Madeleine MM, Johnson LG, Du Q, Galloway DA, Malkki M, Petersdorf EW, Schwartz SM: Genetic variation in the TLR and NF-kappaB pathways and cervical and vulvar cancer risk: a population-based case-control study. Int J Cancer 134: 437-444, 2014

de Andrade Santos PP, de Aquino AR, Oliveira Barreto A, de Almeida Freitas R, Galvao HC, de Souza LB: Immunohistochemical expression of nuclear factor kappaB, matrix metalloproteinase 9, and endoglin (CD105) in odontogenic keratocysts, dentigerous cysts, and radicular cysts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 112: 476-483, 2011

de Paula AM, Carvalhais JN, Domingues MG, Barreto DC, Mesquita RA: Cell proliferation markers in the odontogenic keratocyst: effect of inflammation. J Oral Pathol Med 29: 477-482, 2000

Duray A, Demoulin S, Hubert P, Delvenne P, Saussez S. Immune suppression in head and neck cancers: a review. Clin Dev Immunol: 701657, 2010

Estornes Y, Toscano F, Virard F, Jacquemin G, Pierrot A, Vanbervliet B, Bonnin M, Lalaoui N, Mercier-Gouy P, Pachéco Y, Salaun B, Renno T, Micheau O, Lebecque S: dsRNA induces apoptosis through an atypical death complex associating TLR3 to caspase-8. Cell Death Different 19: 1482-1494, 2012

Evans DG, Farndon PA. Nevoid basal cell carcinoma syndrome. In: Pagon RA, Bird TD, Dolan CR, Stephens K, editors. GeneReviews. Washington: Seattle; 1993

Gomes CC, Diniz MG, Gomez RS: Review of the molecular pathogenesis of the odontogenic keratocyst. Oral Oncol 45: 1011-1014, 2009

Gonzalez-Reyes S, Fernandez JM, Gonzalez LO, Aguirre A, Suarez A, Gonzalez JM, Escaff S, Vizoso FJ: Study of TLR3, TLR4, and TLR9 in prostate carcinomas and their association with biochemical recurrence. Cancer Immunol Immunother 60: 217-226, 2011

High A, Zedan W. Basal cell nevus syndrome. Curr Opin Oncol 17: 160-166, 2005

Ioannou S, Voulgarelis M: Toll-like receptors, tissue injury, and tumourigenesis. Mediators Inflamm 581837. doi: 10.1155/2010/581837, 2010

Kelly MG, Alvero AB, Chen R, Silasi DA, Abrahams VM, Chan S, Visintin I, Rutherford T, Mor G: TLR-4 signaling promotes tumor growth and paclitaxel chemoresistance in ovarian cancer. Cancer Res 66: 3859-3868, 2006

Kimonis VE, Goldstein AM, Pastakia B, Yang ML, Kase R, DiGiovanna JJ, Bale AE, Bale SJ: Clinical manifestations in 105 persons with nevoid basal cell carcinoma syndrome. Am J Med Genet 69: 299-308, 1997

Kotrashetti VS, Nayak R, Bhat K, Hosmani J, Somannavar P: Immunohistochemical expression of TLR4 and TLR9 in various grades of oral epithelial dysplasia and squamous cell carcinoma, and their roles in tumor progression: a pilot study. Biotech Histochem 88: 311-322, 2013

Kramer IR: The World Health Organization: histological typing of odontogenic tumours: an introduction to the second edition. J Dent Assoc S Afr 47: 208-210, 1992

Leonardi R, Matthews JB, Caltabiano R, Greco M, Lombardo C, Loreto C *et al.* MMP-13 expression in keratocyst odontogenic tumour associated with NBCCS and sporadic keratocysts. Oral Dis 16: 795-800, 2010

Leonardi R, Matthews JB, Loreto C, Musumeci G, Campisi G, Lo Muzio L, dos Santos JN, Pastorino L, Bufo P, Pannone G: 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. Histol Histopathol 28: 1175-1184, 2013

Lin CY, Hung HC, Kuo RC, Chiang CP, Kuo MY. Survivin expression predicts poorer prognosis in patients with areca quid chewing-related oral squamous cell carcinoma in Taiwan. Oral Oncol 41: 645-654, 2005

Lo Muzio L, Santarelli A, Caltabiano R, Rubini C, Pieramici T, Fior A, Trevisiol L, Carinci F, Leonardi R, Bufo P, Lanzafame S, Piattelli A: p63 Expression in odontogenic cysts. Int J Oral Maxillofac Surg 34: 668-673, 2005

Luo Q, Hu S, Yan M, Sun Z, Chen W, Chen F. Activation of Toll-like receptor 3 induces apoptosis of oral squamous carcinoma cells in vitro and in vivo. Int J Biochem Cell Biol 44: 1266-1275, 2012

Makinen LK, Atula T, Hayry V, Jouhi L, Datta N, Lehtonen S, Ahmed A, Mäkitie AA, Haglund C, Hagström J: Predictive role of toll-like receptors 2, 4, and 9 in oral tongue squamous cell carcinoma. Oral Oncol 51: 96-102, 2015

Nair S, Kotrashetti VS, Nayak R, Bhat K, Somannavar P, Hosmani J. HSP70 induces TLR4 signaling in oral squamous cell carcinoma: an immunohistochemical study. J Cancer Res Ther 9: 624-629, 2013

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

Rich AM, Hussaini HM, Parachuru VP, Seymour GJ. Toll-like receptors and cancer, particularly oral squamous cell carcinoma. Front Immunol 5: 464, doi: 10.3389/fimmu.2014.00464. eCollection 2014

Salaun B, Coste I, Rissoan MC, Lebecque SJ, Renno T. TLR3 can directly trigger apoptosis in human cancer cells. J Immunol 176: 4894-4901, 2006

Shear M. The odontogenic keratocyst: recent advances. Dtsch Zahnarztl Z 40: 510-513, 1985

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

Shear M. The aggressive nature of the odontogenic keratocyst: is it a benign cystic neoplasm? Part 3. Immunocytochemistry of cytokeratin and other epithelial cell markers. Oral Oncol 38: 407-415, 2002b

Shear M. The aggressive nature of the odontogenic keratocyst: is it a benign cystic neoplasm? Part 1. Clinical and early experimental evidence of aggressive behaviour. Oral Oncol 38: 219-226, 2002c

Sun Z, Luo Q, Ye D, Chen W, Chen F. Role of toll-like receptor 4 on the immune escape of human oral squamous cell carcinoma and resistance of cisplatin-induced apoptosis. Mol Cancer 11: 33, doi: 10.1186/1476-4598-11-33, 2012

Szczepanski MJ, Czystowska M, Szajnik M, Harasymczuk M, Boyiadzis M, Kruk-Zagajewska A, Szyfter W, Zeromski J, Whiteside TL: Triggering of Toll-like receptor 4 expressed on human head and neck squamous cell carcinoma promotes tumor development and protects the tumor from immune attack. Cancer Res 69: 3105-3113, 2009

Tsan MF, Gao B. Endogenous ligands of Toll-like receptors. J Leukoc Biol 76: 514-519, 2004

Woolgar JA, Rippin JW, Browne RM. A comparative histological study of odontogenic keratocysts in basal cell naevus syndrome and control patients. J Oral Pathol 16: 75-80, 1987a

Woolgar JA, Rippin JW, Browne RM. A comparative study of the clinical and histological features of recurrent and non-recurrent odontogenic keratocysts. J Oral Pathol 16: 124-128, 1987b

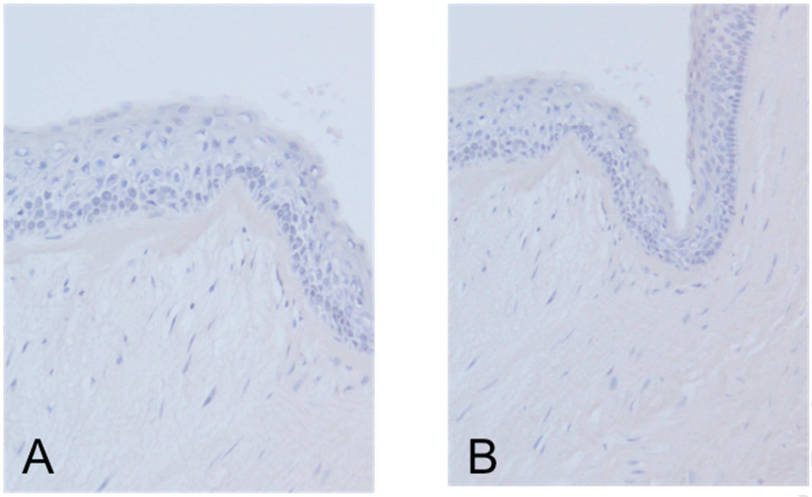
Zhang YB, He FL, Fang M, Hua TF, Hu BD, Zhang ZH, Cao Q, Liu RY: Increased expression of Toll-like receptors 4 and 9 in human lung cancer. Mol Biol Rep 36: 1475-1481, 2009

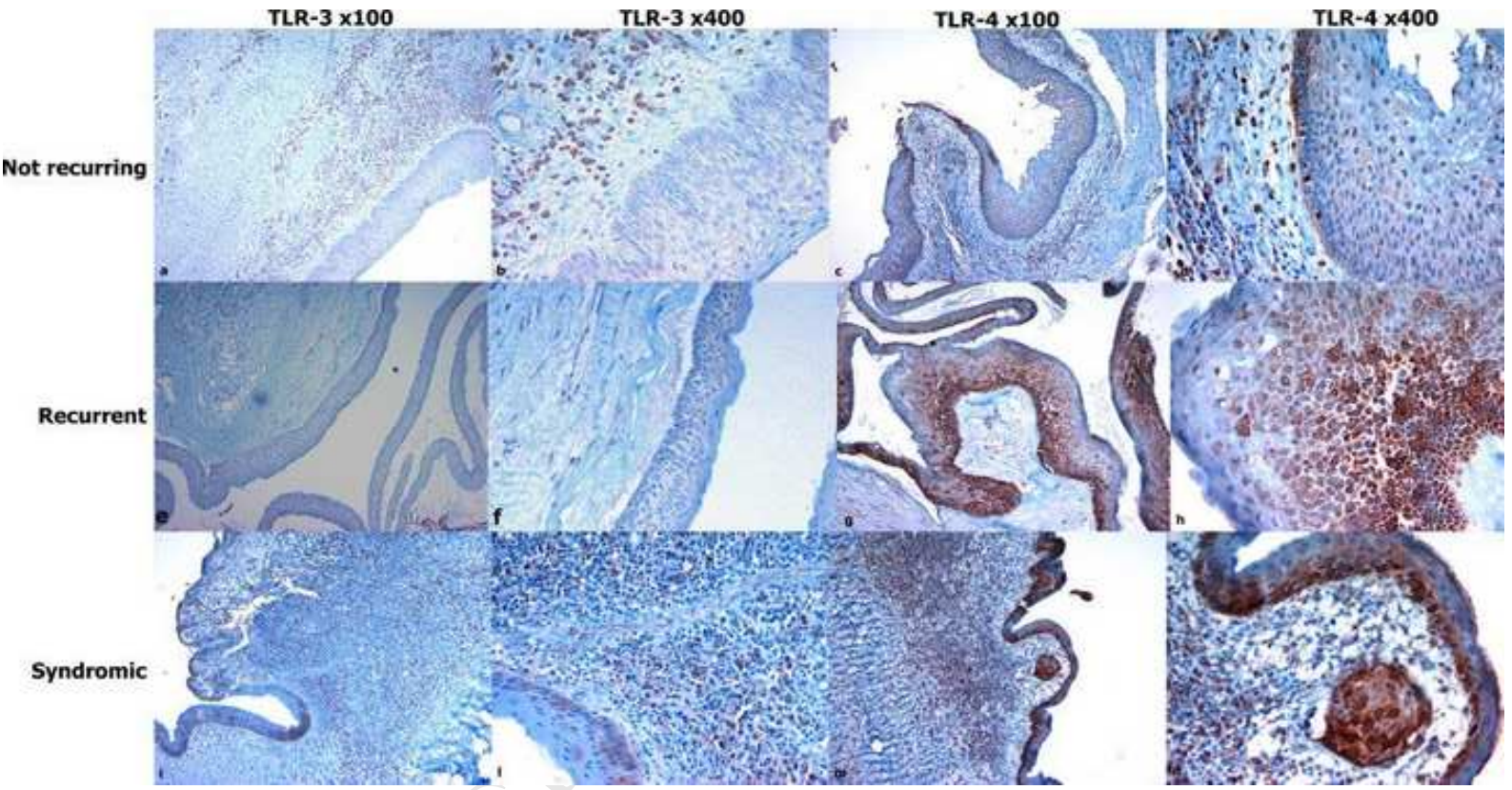
Figure 1. Immunohistochemistry for TLR3 and TLR4 in sporadic primary KCOTs (sp-KCOTs), sporadic recurrent KCOTs (sr-KCOTs), and NBCCS-associated KCOTs (NBCCS-KCOTs) at magnifications of ×100 and ×400.

Figure 2. Immunohistochemistry for TLR3 (A) and TLR4 (B) in noninflamed follicular dentigerous cyst (DC), at a magnification of ×400. An immunoreaction product is apparent.

	TLR3	TLR4
DC	0.179 ± 0.46	0.187 ± 0.53
sp-KCOTs	0.117 ± 0.33	0.470 ± 0.51
sr-KCOTs	0.444 ± 0.52	2.333 ± 0.50
NBCCS-KCOTs	0.666 ± 0.22	2.417 ± 0.51

Table 1. Means and standard deviations obtained respectively for each antibody in dentigerous cysts (DC), sporadic primary keratocystic odontogenic tumors (sp-KCOTs), sporadic recurrent keratocystic odontogenic tumors (sr-KCOTs), and nevoid basal cell carcinoma syndrome–associated keratocystic odontogenic tumors (NBCCS-KCOTs).




TLR-3 x100
TLR-3 x400
TLR-4 x100
TLR-4 x400
Not recurring
Recurrent
Syndromic
a
b
c
e
f
g
h
i
j