Biological pathways involved in the aggressive behavior of the keratocystic odontogenic tumor and possible implications for molecular oriented treatment – An overview

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S U M M A R Y

In the classification of Head and Neck Tumors, published in 2005 by the World Health Organization Classification, the odontogenic keratocyst has been reclassified as a benign intraosseous neoplasm, calling it “keratocystic odontogenic tumor” (KCOT).

Significant differences on the molecular level between KCOT and other odontogenic cystic lesions suggest a different biological origin. Genetic and molecular research regarding odontogenic tumors, and KCOTs in particular, has led to an increasing amount of knowledge and understanding of their physiopathological pathways.

A review of the biological behavior of this recognized aggressive pathological entity of the jaws and a contemporary outline of the molecular (growth factors, p53, PCNA and Ki-67, bcl-2) and genetic (PTCH, SHH) alterations associated with this odontogenic neoplasm provides a better understanding of the mechanisms involved in its development and strengthen the current concept that the KCOT should, indeed, be regarded as a neoplasm.

Furthermore, markers known to be rapidly induced in response to growth factors, tumor promoters, cytokines, bacterial endotoxins, oncogenes, hormones and shear stress, such as COX-2, may also shed new light on the biological mechanisms involved in the development of these benign but sometimes aggressive neoplasms of the jaws.

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Introduction

The odontogenic keratocyst has been one of the most controversial pathological entities of the maxillofacial region since Philipsen first described it in 1956.1 Due to its clinicopathological features, the revised classification of Head and Neck Tumors, published in 2005 by the World Health Organization, reclassified the odontogenic keratocyst as a benign intraosseous neoplasm, recommending the term keratocystic odontogenic tumor (KCOT).2 Its typical histological features include a thin parakeratinized squamous epithelium, approximately 5–8 cells thick, covered by a thin corrugated layer of parakeratin.2–4 The basal layer exhibits a characteristic palisaded pattern with uniform nuclei.2,3,5 The epithelium can show budding of the basal layer into surrounding connective tissue with formation of detached microcyts, which have been termed daughter cysts.6 The fibrous cyst wall is relatively thin and usually lacks inflammatory cell infiltrate.5

Malignant transformation into squamous cell carcinoma, though rare, has been reported.7,8 Reported recurrences range from 0% to 100%.3,9–12 These marked discrepancies are thought to be related to the different lengths of postoperative follow-up periods, operative techniques employed or inclusion of cases with nevoid basal cell carcinoma syndrome (NBCCS).13,14 There is a wide variety of surgical approaches depending on the size and extent of the lesions, including decompression, curettage, marsupialization, enucleation or resection,7,12 with more meticulous surgical approaches correlating to a better prognosis.7,15

Significant differences on the molecular level between KCOT and other odontogenic cystic lesions suggest a different biological origin.4 KCOTs have a weak and discontinuous linear staining for laminin and collagen IV, suggesting unusual interactions between epithelium and connective tissue.16,17 Furthermore, greater suprabasal staining with proliferation markers, such as Ki-67 and proliferating cell nuclear antigen (PCNA)18 and more significant staining with p53 as compared to the other odontogenic cysts13,19 have
been reported. A series of genetic and molecular mechanisms appear to promote the development and progression of this tumor.

**Genetic mechanisms in the development and progression of KCOT**

Morphogenesis and cytodifferentiation of the teeth are under genetic control of regulators such as Sonic Hedgehog (SHH), bone morphogenetic protein (BMP), Wnt, HGF, and FGF and tumor-suppressor genes acting as regulators of cell growth. Inactivation of these genes by mutations and/or loss of heterozygosity (LOH) results in tumor development. Expression of Hedgehog signaling molecules – SHH, PTCH, smoothened (SMO), and GLI1 – has been detected in several odontogenic tumors, suggesting signaling molecules – SHH, PTCH, smoothened (SMO), and GLI1 – well known, although activation of this pathway may be related to Sonic Hedgehog (SHH) signal transduction pathway, controlling SMO to activate the Gli-family zinc-finger transcription factors which normally represses SMO, this inhibition is released, allowing receptor for SHH ligands. When SHH signal binds to PTCH, to form a transmembrane receptor complex which acts as the receptor of SHH ligands.

The PTCH encodes a transmembrane protein implicated in the Sonic Hedgehog (SHH) signal transduction pathway, controlling cell fates, patterning, and growth in numerous tissues, including teeth. PTCH is thought to combine with Smoothened (SMO) to form a transmembrane receptor complex which acts as the receptor for SHH ligands, resulting in upregulation of the transcription of cellular proliferation genes. Alterations, either inherited or sporadic, in the SHH signaling pathway genes might cause a number of developmental defects. aberrant activation of the SHH signaling pathway during adult life has been shown to be related to tumor formation.

The SHH signaling pathway in the development of KCOT is not well known, although activation of this pathway may be related to the clinical behavior and outcome of KCOT. The immunohistochemical analysis of the expression pattern of PTCH, SHH and SMO in sporadic KCOTs showed that the recurrence of KCOT is related to SMO expression. Yagyu et al. showed that the cases with strong SMO expression presented an higher Ki67 labeling than SMO-negative cases.

Recent studies demonstrated that the PTCH gene, a tumor-suppressor gene mapped onto chromosome 9q22.3-q31, is also involved in the etiology of KCOT. But sporadic KCOTs have also been shown in several studies to harbor germline mutations in the PTCH gene or loss of heterozygosity at 9q22.3-q3. Moreover, based on Knudson’s theory of homozygous tumor suppressor gene inactivation, suggested that when multiple cysts are present in NBCCS patients, a predisposing mutation has already occurred in the germ line, thus requiring only a single mutational event in the somatic cell to cause homozygous inactivation and neoplastic progression, whereas in sporadic cysts two independent mutational events are required in the somatic cell.

Barreto et al. supported the ‘two-hits’ hypothesis according to which the syndrome-related basal cell carcinomas (BCCs) and KCOTs probably arise from precursor cells that contain an hereditary ‘first hit’ and the allelic loss represents loss of the normal allele also known as “loss of heterozygosity” (LOH). Sporadic BCCs and KCOTs may, then, arise from susceptible cells in which two somatic ‘hits’ have occurred, one of which manifests as allelic loss. Thus, with the PTCH gene acting as a “gatekeeper gene”, KCOTs cells that lost the PTCH function become targets of other genetic alterations, such as dysregulation of the oncoproteins cyclin D1 and p53.

Although nonsense, frameshift, in-frame deletions, splice-site, and missense mutations have been associated with NBCC, haploinsufficiency of PTCH1, caused by interstitial deletion of 9q22.3, has also been associated with the syndrome. Recent studies show for the first time the physiological impact of constitutive heterozygous PTCH mutations in primary human keratinocytes and strongly argue for a yet elusive mechanism of haploinsufficiency as described by Santarosa and Ashworth.

Genotypic analysis performed by Agaram et al. using a panel of tumor-suppressor genes revealed a significant clonal loss of heterozygosity (LOH) of common tumor-suppressor genes such as p16, p53, PTCH and MCC in sporadic KCOTs.

**Proliferation mechanisms and biological markers**

**Growth factors**

Li et al. disclosed that the expression of epidermal growth factor receptor (EGFR) in odontogenic cyst was lower in epithelium adjacent to areas of inflammatory cell infiltration, with a most consistent staining of basal and suprabasal cells. The high levels of EGFR expression in KCOTs supported the view that they have an intrinsic growth potential not present in other odontogenic cysts. The lower EGFR expression reported both in the radicular cyst cells and the rests of Malassez from which they arise, contrasted with the maintenance of receptor expression in KCOTs which are derived from dental lamina remnants, which may reflect epithelial–mesenchymal interactions and growth factor/receptor modulation.

TGF-α has also been shown to be expressed mainly in the basal and suprabasal layers: 89% of the KCOTs expressed higher levels of TGF-α compared with 50% in both dentigerous and radicular cysts. Thus, expression levels of TGF-α, EGF and TGFβ suggest involvement of the growth factors in their pathogenesis.

Moreover, the immunohistochemical localization of HGF, TGF-β and their receptors in tooth germs and epithelial odontogenic tumors supports the hypothesis that these factors act on epithelial cells via paracrine and autocrine mechanisms.

**Angiogenesis**

Angiogenesis is an essential part of embryogenesis, wound healing, inflammation, and tumor progression, controlled by molecules such as VEGF, FGF, HGF, TGF-β, interleukin-8 (IL-8), and TNF-α. Immunohistochemical evaluation of microvessel density by

![Diagram of the Hedgehog pathway](image_url)
means of the vascular endothelial marker CD34 has shown higher vascularity in benign and malignant ameloblastomas than in tooth germs. Increased expression of VEGF has also been found in these odontogenic tumors. These features suggest that VEGF is an important mediator of tumor angiogenesis and upregulation of VEGF might be associated with tumorigenesis.

p53, PCNA and Ki-67

The proliferative activity of the lining epithelium of KCOTs has been the subject of various investigations aiming at the expression of p53, 68–71 proliferating cell nuclear antigen (PCNA), 72–75 and Ki67. 67,74 Such studies concluded that p53, PCNA and Ki67 are more strongly expressed in KCOTs than in other types of odontogenic cysts. 13

A number of immunohistochemical studies have examined KCOTs employing various markers of proliferation and of apoptosis. 13,57,68,70–76

Several studies have assessed the expression of p53 in relation to the proliferative activity of the lining epithelium of KCOTs. Although analysis of previously reported data must consider the different methods used, according to Ogden et al., the described clinical features (recurrence, association with NBCCS, frequent multiplicity, etc.) and the PCNA positivity in his sample's KCOTs are significant as to KCOTs p53 positivity. 68 Other studies have revealed remarkably high values of p53-negative ratios of cells in the lining epithelium, showing the highest p53-positive ratio in the intermediate layer, in agreement with other authors. 68,74 However, according to Sloatweg et al. the overexpression of p53 protein is related to the proliferative capacity of the KCOT rather than increased numbers of p53+ cells. 69

Li et al. concluded that immunocytochemical overexpression of p53 by KCOTs compared with the other odontogenic cysts was not the result of p53 gene mutation, but rather the result of overproduction and or stabilisation of normal p53 protein related to cell proliferation. 71

A relatively low p53-positive ratio and a high TUNEL-positive ratio have been reported exclusively in the surface layer, which may substantiate that the decrease in p53-reactivity correlates with apoptosis in the surface layer. It has been postulated that p53 transmits apoptotic signals via a complicated mechanism, and DNA strand breaks are sensed by kinases leading to the phosphorylation and activation of p53, in which case p53 functions not only as an apoptosis-related protein but also as a marker of cellular proliferation KCOTs. 13

Studies on KCOTs showing a maximum positivity for p53 in areas with an intense expression of the proliferation marker PCNA and Ki-67 57,68,69,79 support the concept that p53 overexpression in KCOTs probably results from an increase in wild-type p53 related to the increased cell proliferation observed in these lesions. 80,81

Li et al. 70 results indicated an higher proliferative activity, as shown by PCNA activity, in KCOTs linings, in accordance with their aggressive clinical behavior. Although they considered that the PCNA was associated with cell cycle related DNA synthesis, they were unable to determine whether the higher PCNA+ cell numbers in the epithelium represented a higher epithelial cell turnover rate or rather a prolonged cell cycle time. Furthermore, the number of PCNA+ cells per unit length of basement membrane was found similar to that of parakeratinised oral epithelium, which let them to conjecture whether KCOT experienced a greater lateral rather than vertical migration of cells that might explain the consistently narrow and regular KCOT epithelium concomitant with active cyst growth. 70

The predominant suprabasal distribution of PCNA+ cells was consistent with both their findings of EGFR expression in KCOT's suprabasal cells and the high levels of p53 protein activity in the suprabasal cells shown by Ogden et al. 68

Ki-67 expression has been shown to be higher in the epithelium of KCOTs 81 when compared to developmental and inflammatory cysts, with most of the Ki-67+ cells being detected in the suprabasal layers. 77,81 These results demonstrate that cells constituting the intermediate or suprabasal layers possess the highest proliferative activity in the KCOTs. The correlation between Ki-67 and PCNA reflects cell proliferation.

Apoptotic mechanisms

Previous reports comparing apoptosis-related factors in sporadic KCOTs and KCOTs associated with nevoid basal cell carcinoma have been published 13,57,71 and apoptotic cells have been found in the superficial cells of the lining epithelia of KCOTs through the TdT-mediated dUTP-biotin nick end labeling (TUNEL) method. 74,76 Among all proto-oncogenes, bcl-2, located at chromosome 18q21, is characteristically able to stop programmed cell death (apoptosis) without promoting cell proliferation. 77 Its gene product, the bcl-2 protein, acts as a cell death suppressor that facilitates cell survival by regulating apoptosis. 82,83

Investigations on the immunoreactivities of bcl-2 protein have been demonstrated in tooth germs, ameloblastomas, KCOTs and dentigerous cysts. 57,74,75,84–88 Recent studies report that bcl-2 positive cells are predominantly located basally, 77,77 thus supporting the concept that apoptosis does not occur in the basal cells of the lining epithelium. 57,74 TUNEL-positive cells have been detected exclusively in the surface layer of KCOTs, indicating marked levels of apoptosis. 77

Thus, bcl-2 inhibits apoptosis to facilitate cellular proliferation in the basal and suprabasal layers, whereas apoptosis maintains the homeostasis of the thickness of the lining epithelium and allows the synthesis of large amounts of keratin in the surface layer of KCOTs.

Considering that there is a regulated balance between cell proliferation, cell differentiation and cell death in this type of lesion, this may explain why KCOTs, though portraying a neoplastic behavior, with an increase potential to proliferate, do not tend to form tumor masses. Furthermore, Kolář et al. 5 has reported an higher expression of antiapoptotic as well as proapoptotic proteins bcl-2 and Bax, cell cycle-related protein p27Kip1, oncogene c-erbB-2 and proliferative potential measured by PCNA in KCOTs.

Inflammatory mechanisms

Loss of typical KCOT epithelial architecture adjacent to areas of inflammation and corresponding decrease of EGFR expression emphasised the importance of mesenchymal integrity in shaping the KCOT epithelium phenotype, a relationship well-demonstrated in earlier explant studies. 89

Nonetheless, the effect of inflammation in the epithelium of KCOT remains a subject of controversy, with contradictory results being portrayed. De Paula et al. 86 reported a statistically significant increase of PCNA+ and Ki-67+ cells and of AGNOR numbers in the linings of inflamed KCOTs compared to non-inflamed lesions, which was considered suggestive of a greater proliferative activity in the epithelial cells of inflamed KCOTs which could be associated with the disruption of the typical structure of odontogenic keratocyst linings.

Kaplan and Hirshberg 90 reported that the labeling indices for PCNA and Ki-67 yielded no significant differences between inflamed and non-inflamed KCOTs and no differences in labeling indices were observed between areas of classic and metaplastic epithelium with equal inflammation density.
Molecular oriented treatment of KCOT

The proliferating activity of the epithelial cells is strongly related to the aggressiveness of KCOTs.\textsuperscript{72} Immunohistochemical studies show that IL-1α and IL-6 are expressed in the epithelium of KCOTs,\textsuperscript{91} suggesting that these cytokines may play a crucial role in KCOTs growth. They stimulate bone resorption by inducing osteoclast-like cell formation and/or activation,\textsuperscript{92,93} and the production of prostaglandins,\textsuperscript{94,95} and collagenases.\textsuperscript{16,94,96–98}

IL-1 is known to stimulate the production of PGE\textsubscript{2} in KCOTs fibroblasts. Ogata et al.\textsuperscript{99} showed that IL-1α enhanced the expression of COX-2 mRNA and protein, and PGE\textsubscript{2} secretion in fibroblasts, thus protein kinase C (PKC)-dependent activation of extracellular signal-regulated protein kinase-1/2 (ERK1/2), p38 mitogen-activated protein kinase (MAPK), and c-Jun N-terminal kinase (JNK) signaling pathways. PKC inhibitor staurosporine inhibited IL-1α-induced phosphorylation of ERK1/2, p38, and JNK, and decreased IL-1α-induced COX-2 mRNA expression. He also demonstrated that IL-1α may stimulate COX-2 expression in KCOTs thru the NF-κB cascade.

Recent in vitro studies confirmed that IL-1 stimulates epithelial cell proliferation directly\textsuperscript{100} and/or indirectly by inducing the secretion of some factors such as keratinocyte growth factor (KGF) from the interacting fibroblasts.\textsuperscript{101}

These data might explain Ninomiya et al.\textsuperscript{102} results, which show that strong expression of IL-1α mRNA and protein, mainly detected in the epithelial cells of KCOTs, significantly decreases after marsupialization. In fact, Ki-67 labeling index of the epithelial cells in KCOTs, significantly decreases after marsupialization.\textsuperscript{100} Some studies have shown that IL-1α may increase the number of apoptotic cells in BCCs of pTT-treated tumor-free epidermis and by 76% in BCC tumor nests, while number of Ki-67-positive cells was decreased by 56% in pTT-treated KCOTs after the marsupialization, suggesting that marsupialization may reduce the size of KCOTs by inhibiting IL-1α expression and the epithelial cell proliferation.\textsuperscript{102}

Recent molecular-oriented studies related to PTCH pathway have provided some insights in the development of new drugs in the treatment of basal cell carcinoma.

Human tumors associated with mutations that activate SMO or that inactivate PTCH, causing excessive activity of the Hedgehog response pathway, react to plant-derived teratogen cyclopamine.\textsuperscript{103} Cyclopamine, and synthetic derivatives with improved potency, block activation of the Hedgehog response pathway as well as the abnormal cell growth associated with both types of oncogenic mutation, thus inhibiting the Hedgehog response. This study indicates that cyclopamine may act by influencing the balance between active and inactive forms of SMO.\textsuperscript{103}

Another study, by Arad et al.\textsuperscript{104} assessed the preventive effect of thymidine dinitucleotide (pTT) on basal cell carcinoma (BCC) in UV-irradiated Ptch-1(+/−) mice, a model of the Gorlin syndrome. After topical pTT treatment immunostaining revealed that the number of Ki-67-positive cells was decreased by 56% in pTT-treated tumor-free epidermis of KCOTs fibroblasts. Ogata et al.\textsuperscript{99} showed that IL-1α enhanced the expression of COX-2 mRNA and protein, and PGE\textsubscript{2} secretion in fibroblasts, thus protein kinase C (PKC)-dependent activation of extracellular signal-regulated protein kinase-1/2 (ERK1/2), p38 mitogen-activated protein kinase (MAPK), and c-Jun N-terminal kinase (JNK) signaling pathways. PKC inhibitor staurosporine inhibited IL-1α-induced phosphorylation of ERK1/2, p38, and JNK, and decreased IL-1α-induced COX-2 mRNA expression. He also demonstrated that IL-1α may stimulate COX-2 expression in KCOTs thru the NF-κB cascade.

Conflict of Interest Statement

None declared.

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