原文題目(出處)：	Biological pathways involved in the aggressive behavior of the keratocystic odontogenic tumor and possible implications for molecular oriented treatment – an overview Oral Oncol 2010;46:19-24.
原文作者姓名：	Mendes RA, Carvalho JFC, van der Waal Isaac
通訊作者學校：	Department of Oral Surgery, Faculty of Dental Medicine, University of Porto, Porto, Portugal
報告者姓名(組別)：	陳靜怡 CR
報告日期：	99/4/13

內文：

Introduction

1. 演變 (1) First described by Philipsen in 1956
   (2) Reclassified as a benign intraosseous neoplasm by WHO in 2005
   → **Keratocystic odontogenic tumor** (KCOT)
2. 病理特徵
   (1) Thin parakeratinized squamous epithelium, approximately 5-8 cells thick, corrugation, wavy appearance
   (2) Nuclei of basal cells arranging in palisaded pattern
   (3) Budding into the underlying fibrous connective tissue → daughter cyst
   (4) Relatively thin fibrous wall and lacking of inflammatory cell infiltrate
3. Malignant transformation to SCC→ 有報告，數量很少
4. Recurrence → 0~100% in different studies, 差異原因
   (1) Follow up 時間長短不同
   (2) 採用手術不同
   (3) 將nevoid basal cell carcinoma syndrome (NBCCS)的cases也列入
5. 手術方法
   (1) Decompression
   (2) Curettage
   (3) Marsupialization
   (4) Enucleation
   (5) Resection
   More meticulous surgical approaches correlating to a better prognosis
6. KCOT與其它odontogenic cystic lesion的差別
   (1) KCOT have a weak and discontinuous linear stating for laminin and collagen IV, suggesting unusual interactions between epithelium and connective tissue
   (2) Greater suprabasal staining with proliferation markers, such as Ki-67 and proliferating cell nuclear antigen (PCNA) and more significant staining with p53

Genetic mechanisms in the development and progression of KCOT

1. Morphogenesis and cytodifferentiation of the teeth under genetic control of
   (1) SHH – Sonic Hedgehog pathway, vertebrate organogenesis
   (2) BMP – Bone Morphogenetic Protein
   (3) Wnt – Wg (wingless)+Int, embryogenesis
   Regulating cell growth
   (1) HGF – Hepatocyte growth factor, paracrine cellular growth
   (2) FGF – Fibroblast growth factor, angiogenesis
   (3) Tumor suppressor gene

2. The growth of odontogenic tumor – SHH signaling pathway
   Mutation and/or Loss of heterozygosity (LOH) → tumor growth
   Expression of SHH, PTCH (patched homolog, tumor suppressor gene), SMO (smoothened), GLI1 (transcription factor) detected in several odontogenic tumors → playing a role in epithelial-mesencymal interactions and cell proliferation during the growth of odontogenic tumors
   PTCH (transmembrane protein)參與SHH signal transduction pathway, 控制 cell fates, patterning, and growth
   Mechanism:
   PTCH與SMO結合形成transmembrane receptor complex as receptor for SHH ligands, PTCH會抑制SMO的作用, 當SHH與PTCH結合(抑制PTCH), PTCH就解除對SMO的抑制, SMO會啟動Gli1, 造成upregulation of transcription of cellular proliferation genes
3. SHH signaling pathway 與development of KCOT的關係
   → not well known
   IHC analysis:
   (1) In sporadic KCOTs, recurrence is related to SMO expression
   (2) Yagyuu et al – SMO(+), higher Ki-67 labeling
4. PTCH與development of KCOT的關係
   (1) PTCH gene – chromosome 9q22.3-q31, involved in etiology of KCOT shown in recent studies
   (2) Knudson's theory of homozytous tumor suppressor gene inactivation (Two hit hypothesis)
   此對偶基因其中一個先天就有mutation (first hit), 後天只需一次變異(second hit)即可造成loss of heterozygosity, gene inactivation and neoplastic progression
   → syndrome(NBCCS) related multiple cysts (Lench et al)
   此對偶基因兩個皆正常, 後天需有兩次獨立事件造成兩個都mutation才能使gene inactivation → Sporadic KCOT
   PTCH mutation – nonsense, frameshift, in-frame deletions, splice-site, misssense, haploinsufficiency
5. Genotypic analysis
   Agaram et al – LOH of p16, p53, PTCH, and MCC in sporadic KCOTs

Proliferation mechanisms and biological markers

1. Growth factor
   (1) Epidermal growth factor receptor(EGFR)
   Li et al,
   - EGFR的染色表現 : consistent staining of basal and suprabasal cells
   - 在inflammatory cell infiltration旁的epithelium, EGFR的表現比較低
   - KCOTs : higher EGFR expression
     表示KCOTs有其它odontogenic cyst沒有的intrinsic growth potential
     KCOT derived from dental lamina remnants,
   - Radicular cyst and the rests of Malassez : lower EGFR expression
   - 這可能反映出epithelial-mesenchymal interactions(radicular cyst與KCOT的epithelium來源不同)兩者epithelium and growth factor/ receptor modulation

   (2) TGF-α
   - Expression mainly in the basal and suprabasal layers

- 89% of KCOTs v.s.50% of dentigerous and radicular cysts

Expression of TGF-α, EGF and EGFR表示growth factor有參予這些lesions的pathogenesis

(3) HGF, TGF-β and their receptors

- 在tooth germs與epithelial odontogenic tumor 中都有出現
- Act on epithelial cells via paracrine and autocrine mechanism (hypothesis)

(4) VEGF

- Control angiogenesis, 其它控制angiogenesis的factor包括FGF, HGF, TGF-β, interleukin-8 (IL-8), and TGF-α
- CD34 expression: higher in benign and malignant ameloblatoma than in tooth germs
- Increased expression of VEGF also found in these odontogenic tumors → an important mediator of tumor angiogenesis and upregulation of VEGF might be associated with tumorigenesis

2. PCNA, p53 and Ki-67

Proliferative activity of the lining epithelium of KCOTs → studies aiming at p53, PCNA and Ki-67, conclusion: p53, PCNA and Ki-67 strongly expressed in KCOTs than in other types of odontogenic cysts

(1) p53

- Kichi et al – remarkably high values of p53-positivity ratios of cells in the lining epithelium, intermediate layer: the highest ratio
- Slootweg et al – overexpression of p53 protein is related to the proliferative capacity of the KCOT rather than increased numbers of p53(+) cells
- Li et al – 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 stabilization of normal p53 product related to cell proliferation

  Wild type p53 – 抑制cell proliferation

  1. Apoptosis
  2. Arrest cell cycle, 停在 G1/S, repair DNA damage

  Mutant type p53 – 增加 cell proliferation
- Low p53(+) ratio and high TUNEL(+) ratio in surface layer → high apoptosis in the surface layer
- Not only as an apoptosis-related protein but also a marker of cellular proliferation KCTOs
- p53高, PCNA與Ki-67也高 → increase in wild type p53 related to increased cell proliferation

(2) PCNA

- Higher PCNA(+) cells in KCOTs → represented a higher epithelial cell turnover rate or a prolonged cell cycle time ?
- PCNA(+) cell per unit length of basement membrane與在parakeratinised oral epithelium相近 → 推論: KCOT 的lateral migration比vertical migration強, 這可以解釋KCOT的epithelium呈現cystic growth而非tumor mass

(3) Ki-67

- Higher expression in epithelium of KCOTs
- Most of Ki-67(+) cells in the suprabasal layers

3. Apoptotic mechanism

(1) 以TdT-mediated dUTP-biotin nick end labeling (TUNEL)方法來檢測，可在KCOT epithelium的superficial cells發現apoptotic cells

(2) bcl-2

- bcl-2為可停止apoptosis的proto-oncogene，研究發現bcl-2 protein可在tooth germs, ameloblastomas, KCOTs與dentigerous cysts表現
- 最近的研究顯示bcl-2(+) cells主要分布在basal layer

bcl-2(+) cells分布在basal layer (stop apoptosis), TUNEL(+) cells分布在表面(apoptosis) →維持epithelium thickness，形成大量keratin在表面

KCOTs在cell proliferation, cell differentiation與cell death存在一種平衡，所以KCOTs有neoplastic behavior以及increased potential to proliferate，但不會形成tumor mass.

4. Inflammatory mechanisms

Inflammation對KCOTs的影響目前仍維持矛盾

(1) De Paula et al

Ki-67(+)與PCNA(+) cells在inflamed KCOTs比non-inflamed KCOTs多 → inflamed KCOTs 有比較高的proliferation能力

(2) Kaplan and Hirshberg

Inflamed 與non-inflamed KCOTs在PCNA與Ki-67的表現上無顯著差異

Molecular oriented treatment of KCOT

1. IL-1 α and IL-6
   (1) Expressed in epithelium of KCOTs
   (2) Play a crucial role in KCOTs growth
   (3) Stimulating bone resorption by inducing osteoclast-like cell formation and/or activation, and the production of prostaglandin and collagenases
   (4) Ogata et al – IL-1 α 會增進fibroblast表現COX-2 mRNA與其protein，及分泌 PGE_2
   (5) Recent studies – IL-1會刺激epithelial cell proliferation by inducing the secretion of keratinocyte growth factor (KGF) from the interacting fibroblasts
   (6) Ninomiya et al – strong expression of IL-1 α mRNA and protein in the epithelial cells of KCOT, after marsupialization →significantly decreased, Ki-67 labeling index也隨之下降

   Marsupialization 可藉由抑制IL-1 α 的表現與epithelial cell proliferation來減少KCOT的size.
2. Mutations that activating SMO or inactivating PTCH 會導致Hedgehog pathway的過度活動，而Cyclopamine and synthetic derivatives可阻擋 activation of the hedgehog response pathway 與abormal mitoses，研究指出cyclopamine會影響SMO active與inactive form之間的平衡
3. Arad et al – thymidine dinucleotide (pTT)

   pTT對Gorlin syndrome的basal cell carcinoma的預防效果(UV irradiated Ptch(+/-) mice), topical application

   Results: Ki-67(+) (proliferation) 表現在tumor(-) epithelium 下降56%
   在BCC tumor nests 下降76%
   TUNEL(+) (apoptosis) 表現在BCC tumor nests上升213%
   COX-2(+) (proliferation)表現在BCC tumor nests下降80%
4. William et al – CUR61414

   A novel inhibitor of the Hedgehog pathway → suppress proliferation and induce

apoptosis of basaloid nests in the BCC models, 對normal skin cell無作用, 可做為BCC與KCOT可能的治療方法

5. Zhang et al
   SHH的antagonists 對KCOTs可能是有效的治療方法, 作法包括
   (1) 置入wild type的PTCH
   (2) 置入合成的SMO antagonist
   (3) 抑制SHH pathway下游的transcription factor(Gli family)
   他們認為intracystic injection of SMO antagonist是最有潛力的治療選擇
6. Stolina et al
   抑制COX-2會導致大量的tumor lymphocytic infiltration與減少tumor growth, 他們假設藉由減少免疫抑制的cytokine(IL-10)的分泌, COX-2 inhibitor可達到antitumor response

Conclusions

Both 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. 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, indeed, shed new light on the biological mechanisms involved in the development of these benign but yet aggressive neoplasms of the jaws.