ORAL DISEASES

Oral Diseases (2010) 16, 286–291. doi:10.1111/j.1601-0825.2009.01640.x

http://www.blackwellmunksgaard.com

ORIGINAL ARTICLE

LINE-1 methylation difference between ameloblastoma and keratocystic odontogenic tumor

N Kitkumthorn[1], A Mutirangura[2]

[1]*Department of Oral Pathology, Faculty of Dentistry, Mahidol University, Bangkok;* [2]*Center of Excellence in Molecular Genetics of Cancer and Human Diseases, Department of Anatomy, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand*

OBJECTIVE: Global hypomethylation is a common epigenetic event in cancer. Keratocystic odontogenic tumor (KCOT) and ameloblastoma are different tumors but posses the same tissue in origin. Here, we investigated long interspersed nuclear element-1 (LINE-1 or L1) methylation status between ameloblastoma and KCOT.
MATERIALS AND METHODS: We studied the methylation levels of the long interspersed nucleotide element-1 (LINE-1) in ameloblastoma and KCOT. After collecting ameloblastoma cells and epithelium lining cells of KCOT by laser capture microdissection from paraffin embedded tissue, combined bisulfite restriction analysis of LINE-1 (COBRALINE-1) was performed to measure LINE-1 methylation levels.
RESULTS: The LINE-1 methylation level in KCOT (53.16 ± 12.03%) was higher than that in ameloblastoma (36.90 ± 16.52%), with a statistical significance of $P = 0.001$. The ranges of LINE-1 methylation of both lesions were not associated with either age or sex.
CONCLUSION: We found LINE-1 hypomethylation levels between ameloblastoma and KCOT are different. Therefore, global methylations between these tumors are processed differently.
Oral Diseases (2010) **16**, 286–291

Keywords: ameloblastoma; keratocystic odontogenic tumor; LINE-1; methylation level; COBRALINE-1

Introduction

Ameloblastoma is one of the most common tumors originating in the odontogenic epithelium, accounting for 10% of odontogenic tumors and 1% of all oral and maxillofacial tumors (Regezi *et al*, 1978). This type of lesion has received attention because it is characterized by a benign with locally aggressive behavior and a high tendency to recur. Other clinical characteristics include slow growth, no metastasis, and an 80% occurrence in the mandible, with a marked predilection for the posterior region (Regezi *et al*, 1978; Reichart *et al*, 1995; Melrose, 1999; Mendenhall *et al*, 2007).

Theoretically, ameloblastoma may arise from cell rests of the enamel organ, the developing enamel, the epithelial lining of an odontogenic cyst, or basal cells of the oral mucosa. However, the actual cause is not known. Molecular study of genetic changes in ameloblastoma has been limited. Heikinheimo *et al*, (2002) demonstrated different gene expression in ameloblastoma compared with tooth germs using cDNA microarray. The genes for sonic hedgehog (*SHH*), TNF receptor-associated factor 3 (*TRAF3*), Rho GTPase-activating protein 4 (*ARHGAP4*), deleted in colorectal carcinoma (*DCC*), cadherins 12 and 13 (*CDH12* and *13*), teratocarcinoma-derived growth factor-1 (*TDGF1*), and transforming growth factor-β1 (*TGFB1*) were underexpressed in all ameloblastoma samples. On the other hand, the fos oncogene and tumor necrosis factor receptor 1 (*TNFRSF1A*) were overexpressed genes.

Keratocystic odontogenic tumor (KCOT) is the name now recommended by the WHO working group; the tumor was formerly known as an 'odontogenic keratocyst' (Reichart *et al*, 2006). The recent name change is derived from the locally aggressive behavior of this tumor. KCOT has specific histopathologic features, particularly a thin friable wall which leads to high recurrence rates. Multiple KCOTs are frequently associated with nevoid basal cell carcinoma syndrome, and the malignant transformation of KCOTs has also been reported (Shear, 2002; Gonzalez-Alva *et al*, 2008).

Keratocystic odontogenic tumor arises from the dental lamina or its remnants. It may be an extension of the basal cells of the oral epithelial lining, as in the case of ameloblastoma. A number of genetic studies point to the role of the *PTCH* gene in the etiology of KCOT. *PTCH* gene mutation has been shown to be an important step in its pathogenesis, and is thought to be involved in the development of KCOT (Barreto *et al*, 2000; Shear, 2002; Diniz *et al*, 2009). Recently, Moreira *et al* reported 30% promoter methylation of *P21* in KCOT (Moreira *et al*, 2009).

Correspondence: Nakarin Kitkumthorn, Department of Oral Pathology, Faculty of Dentistry, Mahidol University, Bangkok, Thailand. Tel/Fax: +662 203 6470, E-mail: nakarinkit@hotmail.com
Received 19 August 2009; revised 21 September 2009; accepted 30 September 2009

Ameloblastoma and KCOT invade locally and are resorbed in the adjacent roots of teeth. They infiltrate medullary spaces, erode cortical bone, and might extend into adjacent tissue. Ameloblastoma is considered to be more aggressive. The widely accepted treatment of ameloblastoma is surgical resection, at least 1 cm past the radiological margins. In the case of KCOT, the treatment varies from marsupialization or enucleation to en-bloc resection; but the ideal treatment should be enucleation or curettage followed by applying Carnoy's solution (Schmidt and Pogrel, 2001).

Global hypomethylation is the most common epigenetic change in cancer, occurring in the coding region of genes and repetitive sequences (Piyathilake *et al*, 2001; Chalitchagorn *et al*, 2004; Hoffmann and Schulz, 2005). As about 80% of CpG dinucleotides are located in repetitive sequences, global hypomethylation is considered to be responsible for the majority of the hypomethylation of repetitive sequences (Chalitchagorn *et al*, 2004; Yang *et al*, 2004, 2006; Weisenberger *et al*, 2005; Phokaew *et al*, 2008). Long interspersed nuclear element-1 (LINE-1 or L1) sequences are highly repeated, interspersed retrotransposons, and constitute at least 17% of the human genome (Kazazian and Moran, 1998; Lander *et al*, 2001). LINE-1 elements are normally heavily methylated and contain much of the CpG methylation found in tumor tissue. Therefore, hypomethylated DNA of these elements might determine as a genome wide hypomethylation characterized of human cancer (Chalitchagorn *et al*, 2004; Hoffmann and Schulz, 2005).

Recently, we developed combined bisulfite restriction analysis polymerase chain reaction (PCR) of LINE-1s (COBRALINE-1), which represents the whole genome methylation status (Chalitchagorn *et al*, 2004). We also studied common cancer types, and found global hypomethylation in cancers of the head and neck, esophagus, stomach, liver, colon, cervix, bladder and ovary. By contrast, thyroid cancer, renal cell carcinoma and lymphoma did not show global hypomethylation. Importantly, LINE-1 hypomethylation level is associated with the degree of cancer advancement, and can be used as a prognostic marker for several cancers: non-small cell lung cancer (Daskalos *et al*, 2009), chronic myeloid leukemia (Roman-Gomez *et al*, 2005), epithelial ovarian cancer (Pattamadilok *et al*, 2008), cervical cancer (Shuangshoti *et al*, 2007), hepatocellular carcinoma (Tangkijvanich *et al*, 2007), prostate adenocarcinoma (Cho *et al*, 2007) and oral squamous cell carcinoma (Subbalekha *et al*, 2009). We are the first group to study LINE-1 in ameloblastoma, comparing with KCOT. the epigenetic research of ameloblastoma and KCOT are limited. (Moreira *et al*, 2009); therefore, this work provides additional molecular information and also global methylation status.

Materials and methods

All tissue samples, prepared from January 1, 2007 to December 31, 2008, were retrieved at the Department of Oral Pathology at Mahidol University. Histopathologic review was performed by an oral pathologist to confirm the diagnosis. After fixation of 4-μm-thick sections and hematoxylin and eosin (H&E) staining, laser capture microdissection was used to collect approximately 1500 tumor cells of ameloblastoma and epithelium lining cells of KCOT (Figure 1). DNA was extracted from the microdissected tissue fragments using proteinase K digestion and phenol–chloroform extraction protocols.

Combined Bisulfite Restriction Analysis of LINE-1 was performed as previously described (Chalitchagorn *et al*, 2004), with some modifications. Briefly, denaturing of genomic DNA was performed in 0.22 M NaOH at 37°C for 10 min; then 30 μl of 10 mM hydroquinone and 520 μl of 3 M sodium bisulfite were added for 16–20 h at 50°C. After that, the DNA was purified and incubated in 0.33 M NaOH at 25°C for 3 min, ethanol precipitated, and then washed with 70% ethanol and resuspended in 20 μl of H_2O. Bisulfited DNA (2 μl) was subjected to 40 cycles of PCR with two primers (5′-CCGTAAGGGGTTAGGGAGTTTTT-3′ and 5′-RTAAAACCCTCCRAACCAAATATAAA-3′) at an annealing temperature of 50°C. The amplicons were digested with *TaqI* or *TasI* in TaqI buffer (MBI Fermentas, Burlington, ON, Canada) at 65°C overnight, and then electrophoresed in 8% non-denaturing polyacrylamide gel. Later, the intensities of the bands were measured by a PhosphorImager, using ImageQuant software (Molecular Dynamics, Amersham Pharmacia Biotech, and Piscataway, NJ, USA) (Figure 2). The LINE-1 amplicon size was 160 bp. The methylated amplicons, *TaqI* positive, yielded two 80 bp DNA fragments; whereas the unmethylated amplicons, *TasI* positive, yielded 63 and 97 bp fragments. The percentage of LINE-1 methylation level was calculated by the formula; the intensity of methylated LINE-1 cut by *TaqI*, divided by the amount of the unmethylated LINE-1 cut by *TasI*- and the *TaqI*-positive amplicons DNA from the same stocks of HeLa, Daudi, and Jurkat cell lines were included in all experiments. They were used as control to validate the inter-assay variation. There were limited variations of methylation levels of these cell lines. All experiments were adjusted so all controls demonstrated the same methylation levels.

Statistical significance was determined using one-way ANOVA, *t*-test, and Pearson's correlation using SPSS software, version 11.5 (SPSS Inc., Chicago, IL, USA). A correlation was designated as statistically significant at $P < 0.05$.

Results

Laser capture microdissection was performed in 20 cases of ameloblastoma and KCOT, as shown in Figure 1. The ameloblastoma group was divided by histological appearance: as a plexiform pattern (four cases) and a follicular pattern (16 cases). The histological pattern was not analyzed as a result of the low number of plexiform pattern ameloblastoma.

As shown in Table 1, the average age range (year) of the ameloblastoma group was 41 (19–81) years, while the KCOT group was 25 (12–62) years. The gender ratio

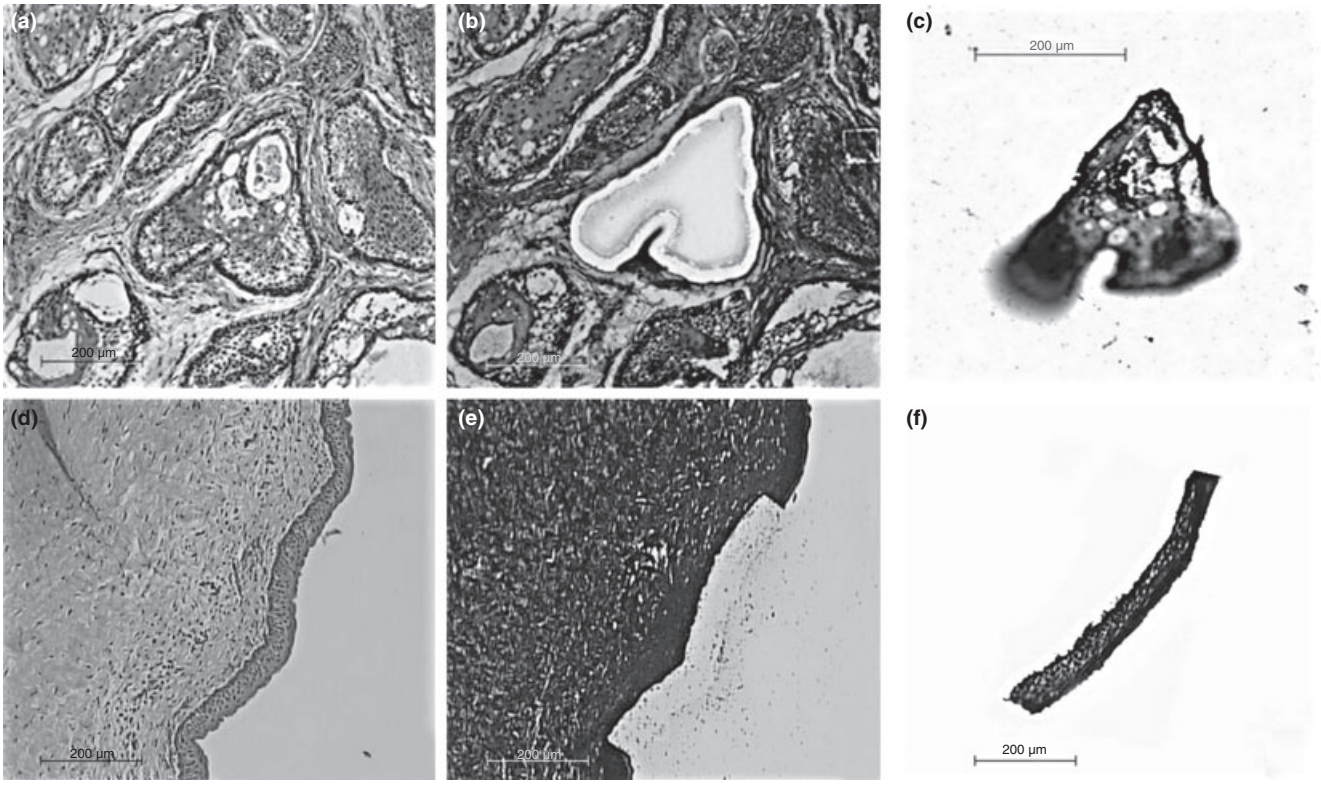

Figure 1 Laser capture microdissection. Formalin-fixed, paraffin-wax-embedded sections of ameloblastoma and keratocystic odontogenic tumor (KCOT), stained with hematoxylin and eosin, before laser capture microdissection (**a, d**), after microdissection (**b, e**), and the captured tumor cells (**c, f**)

(male:female) in the ameloblastoma and KCOT groups were 13:7 and 8:12, respectively. The average age ranges (year) of the male ameloblastoma group, female ameloblastoma group, male KCOT group and female KCOT group were 38 (19–81), 50 (41–70), 31 (12–62) and 25 (14–45), respectively.

The percentage of LINE-1 methylation in ameloblastoma and KCOT was 36.90 ± 16.52% and 53.16 ± 12.02%, respectively. The LINE-1 methylation level in KCOT was higher than that in ameloblastoma, with a statistical significance at $P = 0.001$ (95% CI; 6.67–25.84) (Figure 3a). For gender distribution, a lower LINE-1 methylation level was found in the female ameloblastoma group (26.12 ± 12.17%) compared with the male ameloblastoma group (42.71 ± 15.93%), with statistical significance at $P = 0.028$ (95% CI; 2.03–31.14). By contrast, the male KCOT group was found to have a lower methylation level (49.01 ± 11.08%) compared with the female group (55.92 ± 12.29%) without statistical significance ($P = 0.209$) (95% CI; −18.09 to 4.28) (Figure 3b). The LINE-1 methylation level was not correlated with age distribution in either the ameloblastoma or the KCOT group ($R = 0.08674$, $P = 0.2075$

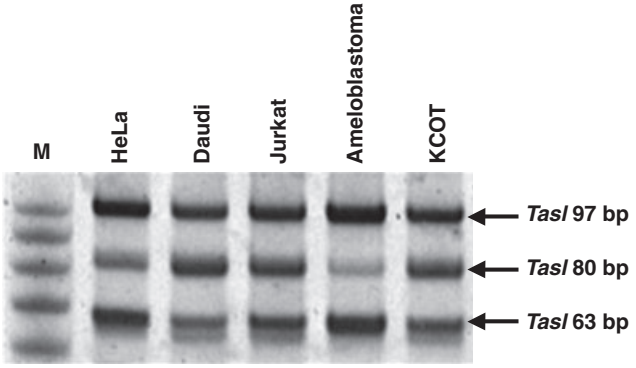

Figure 2 An example of combined bisulfite restriction analysis of long interspersed nucleotide element-1 (LINE-1) (COBRALINE-1). M, 10-bp DNA size marker; HeLa, Daudi and Jurkat are positive controls for adjusting inter-assay variation of ameloblastoma and keratocystic odontogenic tumor (KCOT) as follows: *TasI* 63 and 97, unmethylated amplicons; *TaqI* 80, methylated amplicons

Table 1 Percentage of long interspersed nucleotide element-1 (LINE-1) methylation in ameloblastoma and keratocystic odontogenic tumor (KCOT)

	n	*Mean age, years (range)*	*% Methylation (±s.d.)*
Ameloblastoma	20	41 (19–81)	36.90 (±16.52)
Male	13	38 (19–81)	42.71 (±15.93)
Female	7	50 (41–70)	26.12 (±12.17)
Keratocystic odontogenic tumor (KCOT)	20	25 (12–62)	53.16 (±12.03)
Male	8	31 (12–62)	49.01 (±11.08)
Female	12	25 (14–45)	55.92 (±12.29)

[95% CI; −0.6521 to 0.1703] and $R = 0.07014$, $P = 0.2591$ [95% CI; −0.6332 to 0.2014], respectively) (Figure 3c, d).

Discussion

Ameloblastoma and KCOT are common tumors of odontogenic epithelial origin which have different degrees of aggressive behavior as well as recurrence rates. At present, the etiologic factors of both lesions have not yet been precisely recognized. Our work represents additional molecular data of the global methylation status of ameloblastoma and KCOT. Besides the pathological features of these lesions, this work also analyzes LINE-1 methylation differences.

One characteristic of most tumor cells is focal promoter hypermethylation, as well as generalized genomic hypomethylation (Feinberg, 2004). There may be several consequences from global hypomethylation. Genomic instability is a fundamental aspect of cancer, and is proposed to be caused by this epigenetic process (Eden *et al*, 2003; Mutirangura, 2008; Pornthanakasem *et al*, 2008). LINE-1s are human retrotransposable interspersed repetitive sequences scattered along the human genome. The global hypomethylation process that occurs in cancerous cells can generally deplete LINE-1 methylation levels. Nevertheless, LINE-1 methylation can be influenced differentially, depending on where the particular sequences are located in the genome (Phokaew *et al*, 2008). Many studies have reported LINE-1 biological properties in tumorigenesis, such as: inducing genomic instability; creating DNA double-stranded breaks; increasing mutation events; altering gene expression; and inducing cell proliferation and differentiation (Eden *et al*, 2003; Gaudet *et al*, 2003; Kaneda *et al*, 2004; Wilson *et al*, 2007).

In this study, we used bisulfate-treated DNA, and analyzed global methylation using a modified COBRA-LINE-1 technique (Chalitchagorn *et al*, 2004). This technique generated short PCR products and covered LINE-1 distribution along the genome sequence. Hence, this method is efficient and suitable for a small amount of DNA derived from laser-captured, microdissected, paraffin-embedded tissue.

A wide range of LINE-1 methylation levels are displayed in individuals with ameloblastoma and KCOT. These data are similar to our previous report on variable LINE-1 methylation levels between individuals in normal thyroid and esophagus tissues (Chalitchagorn *et al*, 2004). When KCOT and ameloblastoma were compared, it was found that the percentage of LINE-1 methylation level determined by band intensities was statistically significantly lower in ameloblastoma than in KCOT. The global hypomethylation of ameloblastoma demonstrated in this work was the same as in our previous report on cancers of the urinary bladder, head and neck, liver, lung, prostate gland, stomach, colon, breast and esophagus (Chalitchagorn *et al*, 2004).

In several cancers, a higher level of LINE-1 hypomethylation is presented in the late stages of malignant progression than in the early stages; and a prominent global methylation level is also observed to be lower in tumor tissues than in normal tissues (Roman-Gomez *et al*, 2005; Cho *et al*, 2007; Shuangshoti *et al*, 2007; Tangkijvanich *et al*, 2007; Pattamadilok

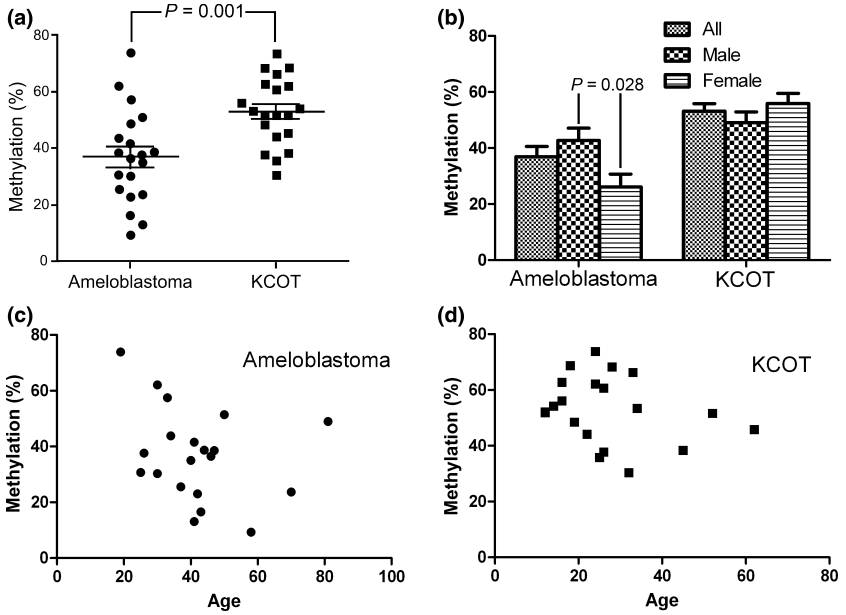

Figure 3 Methylation status of long interspersed nucleotide element-1 (LINE-1). (**a**) LINE-1 methylation levels of ameloblastoma and keratocystic odontogenic tumor (KCOT). A significantly high percentage of LINE-1 methylation is detected in KCOT ($P = 0.001$). (**b**) LINE-1 methylation level differentiated by sex distribution. (**c, d**) LINE-1 methylation level differentiated by age distribution. Data in (**a**) and (**b**) represent means ± s.e.m.

et al, 2008; Daskalos *et al*, 2009; Subbalekha *et al*, 2009). That means that LINE-1 hypomethylation should be a progressive process during tumor development, and presumably can further predict the malignant behavior of tumor types. For odontogenic tumors, KCOT can transform into ameloblastoma, the latter being highly malignant and with a worse prognosis. It is interesting to study if LINE-1 methylation altered according to the transformation. It is also important to study in a larger cohort to evaluate whether LINE-1 hypomethylation levels are prognostic marker of ameloblastoma.

Our study demonstrated a significant reduction of the methylation level of LINE-1 elements in ameloblastoma compared with KCOT. Ameloblastoma and KCOT are different tumor types but both are originated from odontogenic epithelium. It is also interesting to further determine how the alteration of LINE-1 methylation level is related to the tumor development of this epithelium. In conclusion, our findings provide a basis for further studies which could improve our understanding of the epigenetic changes in the ameloblastoma and KCOT development process.

Acknowledgements

We would like to thank Mr. Dusit Bumalee for his help in preparing the tissue samples; Dr. Pornrutsami Jintaridth for statistical assistance; and the Research Center, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, for providing the laser capture microdissection system. This work was supported by Center of Excellence in Molecular Genetics of Cancer and Human Diseases, Department of Anatomy, Faculty of Medicine, Chulalongkorn University, TRF-MRG young scientific researcher grant No.MRG5180242 and TRF senior research scholar grant.

Author contributions

N Kitkumthorn contributed in study concepts, study design, definition of intellectual content, experimental studies, data acquisition and manuscript preparation. A Mutirangura contributed in data analysis, manuscript editing and review.

References

Barreto DC, Gomez RS, Bale AE, Boson WL, De Marco L (2000). PTCH gene mutations in odontogenic keratocysts. *J Dent Res* **79:** 1418–1422.

Chalitchagorn K, Shuangshoti S, Hourpai N *et al* (2004). Distinctive pattern of LINE-1 methylation level in normal tissues and the association with carcinogenesis. *Oncogene* **23:** 8841–8846.

Cho NY, Kim BH, Choi M *et al* (2007). Hypermethylation of CpG island loci and hypomethylation of LINE-1 and Alu repeats in prostate adenocarcinoma and their relationship to clinicopathological features. *J Pathol* **211:** 269–277.

Daskalos A, Nikolaidis G, Xinarianos G *et al* (2009). Hypomethylation of retrotransposable elements correlates with genomic instability in non-small cell lung cancer. *Int J Cancer* **124:** 81–87.

Diniz MG, Borges ER, Guimaraes AL *et al* (2009). PTCH1 isoforms in odontogenic keratocysts. *Oral Oncol* **45:** 291–295.

Eden A, Gaudet F, Waghmare A, Jaenisch R (2003). Chromosomal instability and tumors promoted by DNA hypomethylation. *Science (New York, NY)* **300:** 455.

Feinberg AP (2004). The epigenetics of cancer etiology. *Semin Cancer Biol* **14:** 427–432.

Gaudet F, Hodgson JG, Eden A *et al* (2003). Induction of tumors in mice by genomic hypomethylation. *Science (New York, NY)* **300:** 489–492.

Gonzalez-Alva P, Tanaka A, Oku Y *et al* (2008). Keratocystic odontogenic tumor: a retrospective study of 183 cases. *J Oral Sci* **50:** 205–212.

Heikinheimo K, Jee KJ, Niini T *et al* (2002). Gene expression profiling of ameloblastoma and human tooth germ by means of a cDNA microarray. *J Dent Res* **81:** 525–530.

Hoffmann MJ, Schulz WA (2005). Causes and consequences of DNA hypomethylation in human cancer. *Biochem Cell Biol (Biochimie et biologie cellulaire)* **83:** 296–321.

Kaneda A, Tsukamoto T, Takamura-Enya T *et al* (2004). Frequent hypomethylation in multiple promoter CpG islands is associated with global hypomethylation, but not with frequent promoter hypermethylation. *Cancer Sci* **95:** 58–64.

Kazazian HH Jr, Moran JV (1998). The impact of L1 retrotransposons on the human genome. *Nat Genet* **19:** 19–24.

Lander ES, Linton LM, Birren B *et al* (2001). Initial sequencing and analysis of the human genome. *Nature* **409:** 860–921.

Melrose RJ (1999). Benign epithelial odontogenic tumors. *Semin Diagn Pathol* **16:** 271–287.

Mendenhall WM, Werning JW, Fernandes R, Malyapa RS, Mendenhall NP (2007). Ameloblastoma. *Am J Clin Oncol* **30:** 645–648.

Moreira PR, Guimaraes MM, Guimaraes AL *et al* (2009). Methylation of P16, P21, P27, RB1 and P53 genes in odontogenic keratocysts. *J Oral Pathol Med* **38:** 99–103.

Mutirangura A (2008). Methylation status of endogenous DNA double strand breaks: possible connection between genomic hypomethylation and instability. *Asian Biomed* **2:** 445–449.

Pattamadilok J, Huapai N, Rattanatanyong P *et al* (2008). LINE-1 hypomethylation level as a potential prognostic factor for epithelial ovarian cancer. *Int J Gynecol Cancer* **18:** 711–717.

Phokaew C, Kowudtitham S, Subbalekha K, Shuangshoti S, Mutirangura A (2008). LINE-1 methylation patterns of different loci in normal and cancerous cells. *Nucleic Acids Res* **36:** 5704–5712.

Piyathilake CJ, Frost AR, Bell WC *et al* (2001). Altered global methylation of DNA: an epigenetic difference in susceptibility for lung cancer is associated with its progression. *Hum Pathol* **32:** 856–862.

Pornthanakasem W, Kongruttanachok N, Phuangphairoj C *et al* (2008). LINE-1 methylation status of endogenous DNA double-strand breaks. *Nucleic Acids Res* **36:** 3667–3675.

Regezi JA, Kerr DA, Courtney RM (1978). Odontogenic tumors: analysis of 706 cases. *J Oral Surg* **36:** 771–778.

Reichart PA, Philipsen HP, Sonner S (1995). Ameloblastoma: biological profile of 3677 cases. *Eur J Cancer* **31B:** 86–99.

Reichart PA, Philipsen HP, Sciubba JJ (2006). The new classification of head and neck tumours (WHO) – any changes? *Oral Oncol* **42:** 757–758.

Roman-Gomez J, Jimenez-Velasco A, Agirre X *et al* (2005). Promoter hypomethylation of the LINE-1 retrotransposable elements activates sense/antisense transcription and marks the progression of chronic myeloid leukemia. *Oncogene* **24:** 7213–7223.

Schmidt BL, Pogrel MA (2001). The use of enucleation and liquid nitrogen cryotherapy in the management of odontogenic keratocysts. *J Oral Maxillofac Surg* **59:** 720–725; discussion 726–7.

Shear M (2002). 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.

Shuangshoti S, Hourpai N, Pumsuk U, Mutirangura A (2007). Line-1 hypomethylation in multistage carcinogenesis of the uterine cervix. *Asian Pac J Cancer Prev* **8:** 307–309.

Subbalekha K, Pimkhaokham A, Pavasant P *et al* (2009). Detection of LINE-1s hypomethylation in oral rinses of oral squamous cell carcinoma patients. *Oral Oncol* **45:** 184–191.

Tangkijvanich P, Hourpai N, Rattanatanyong P, Wisedopas N, Mahachai V, Mutirangura A (2007). Serum LINE-1 hypomethylation as a potential prognostic marker for hepatocellular carcinoma. *Clin Chim Acta; Int J Clin Chem* **379:** 127–133.

Weisenberger DJ, Campan M, Long TI *et al* (2005). Analysis of repetitive element DNA methylation by MethyLight. *Nucleic Acids Res* **33:** 6823–6836.

Wilson AS, Power BE, Molloy PL (2007). DNA hypomethylation and human diseases. *Biochim Biophys Acta* **1775:** 138–162.

Yang AS, Estecio MR, Doshi K, Kondo Y, Tajara EH, Issa JP (2004). A simple method for estimating global DNA methylation using bisulfite PCR of repetitive DNA elements. *Nucleic Acids Res* **32:** e38.

Yang AS, Doshi KD, Choi SW *et al* (2006). DNA methylation changes after 5-aza-2′-deoxycytidine therapy in patients with leukemia. *Cancer Res* **66:** 5495–5503.