Alterations in the LINE-1 methylation pattern in patients with lichen simplex chronicus

Surasak Yooyongsatit,[1] Kriangsak Ruchusatsawat,[2] Pakpoom Supiyaphun,[3] Nopadon Noppakun,[4] Apiwat Mutirangura[5] and Jongkonnee Wongpiyabovorn[6]

Summary

Background: **Long interspersed element-1 (LINE-1) and short interspersed element (Alu) retrotransposons have been identified to influence the human genome by modifications in gene expression. Variations in LINE-1 and Alu methylation have been shown to be associated with many diseases, predominantly malignancies and autoimmune diseases. Moreover, the degree and pattern of LINE-1 methylation are related to risk, prognosis and aggressiveness of several cancers. However, a similar study has not been performed in lichen simplex chronicus (LSC).**

Objective: **To evaluate DNA methylation status of repetitive sequences in LSC.**

Methods: **We determined the level and pattern of LINE-1 and Alu methylation in keratinocytes from patients with LSC (n=10) compared to normal controls (n=13), by the improved combined bisulfite restriction analysis of LINE-1 and Alu (COBRA-LINE-1 and Alu). COBRA-LINE-1 classifies LINE-1 loci according to the methylation patterns of two CpG dinucleotides in the 5'UTR into four categories: hypermethylated ($^{m}C^{m}C$), hypomethylated ($^{u}C^{u}C$), and two forms of partially methylated loci ($^{u}C^{m}C$ and $^{m}C^{u}C$).**

Results: **The %$^{m}C^{m}C$ of LINE-1 was significantly decreased in keratinocytes from patients with LSC (p=0.012). Moreover, the %$^{m}C^{u}C$ was significantly lower in LSC than controls (p=0.029). Conversely, %$^{u}C^{m}C$ was significantly higher LSC than controls (p=0.004). A receiver-operating characteristic (ROC) curve analysis demonstrated that % $^{m}C^{m}C$, % $^{m}C^{u}C$ and % $^{u}C^{m}C$ were highly sensitive and specific for LSC with an optimal cut-off value. There were no significant differences in Alu methylation in keratinocytes from LSC patients.**

Conclusion: **Changes in the LINE-1 pattern were revealed in the epidermis from patients with LSC. A particular LINE-1 methylation pattern is indicative of LSC and might be used as a diagnostic tool.** ***(Asian Pac J Allergy Immunol 2012;31:51-7)***

Key words: *Methylation, Methylation pattern, LINE-1, Alu, Lichen simplex chronicus*

From 1. Medical Microbiology, Interdisciplinary Program, Graduate School Chulalongkorn University, Bangkok 10330, Thailand
2. National Institute of Health, Department of Medical Sciences, Nontaburi 11000, Thailand
3. Department of Otolaryngology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand
4. Division of Dermatology, Department of Internal Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand
5. Center of Excellence in Molecular Genetics of Cancer and Human Diseases, Department of Anatomy, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand
6. Division of Immunology, Department of Microbiology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand
Corresponding author: Jongkonnee Wongpiyabovorn
E-mail: jongkonnee.w@chula.ac.th
Submitted date: 9/7/2012
Accepted date: 18/8/2012

Introduction

Lichen simplex chronicus is an inflammatory skin disorder classified as an endogenous eczema. The disease is characterized by lichenification of the skin as a result of repeated scratching.[1] Clinically, the disease should be differentially diagnosed from other skin diseases and underlying skin diseases such as fungal skin infection and psoriasis should be ruled out. Sometimes, a skin biopsy is required to diagnose the disease. The histological features of LSC consist of epidermal hyperplasia with hyperkeratosis and hyperkeratosis. The exact etiology of the disease is unclear. Environmental,

psychological and immunological factors influence the development and course of the disease. Recently, an epigenetic phenomenon has been implicated in the pathogenesis of eczema. Down-regulation of DNA methyltransferase-1 (DNMT-1) was illustrated in peripheral blood mononuclear cells from patients with atopic dermatitis with high serum IgE levels.[2] A recent study on monocytes from patients with atopic eczema revealed global DNA hypomethylation and hypomethylation of the *FCER1G* promoter with an inverse correlation to mRNA expression.[3] Subsequently, LSC was associated with atopic disorders.[4] We hypothesize that epigenetic changes are involved in the pathogenesis of LSC.

The human genome comprises about 34% non-long terminal repeat (non-LTR) retrotransposons.[5] The major components of non-LTR retrotransposons are LINE-1 (long interspersed element-1; 17%) and Alu (11%). Both LINE-1 and Alu are widely inserted in the genome and have numerous probable functional outcomes.[6,7] DNA methylation has been shown to be an important mechanism in silencing LINE-1 for maintaining genomic stability. Hypomethylation of LINE-1 and Alu have been suggested to be the cause of global hypomethylation and genomic instability in many malignancies and autoimmune diseases.[7-24] Moreover, the intensity of LINE-1 hypomethylation has been directly related to high cancer risk, prognosis and the aggressiveness of many cancers.[8,15,17,25,26] Current data show that specific patterns of LINE-1 methylation are more efficient than the overall LINE-1 methylation level in distinguishing many cancers from normal tissue. For example, %^{u}C^{u}C has better power than overall %LINE-1 methylation to differentiate cancers of the colon, liver, lung and nasopharynx from normal controls.[27]

To date, there has been no information available on DNA methylation in LSC. Thus, we determined the DNA methylation level and pattern of LINE-1 and Alu in keratinocytes from LSC patients and compared to normal controls using improved COBRA methods. The LINE-1 methylation patterns were classified as hypermethylated, hypomethylated and two partially methylated loci (^{m}C^{m}C, ^{u}C^{u}C, ^{m}C^{u}C and ^{u}C^{m}C, respectively). Then, the level and pattern of LINE-1 methylation was assessed with respect to the disease.

Methods

Patients and healthy controls

Ten patients with lichen simplex chronicus diagnosed by an experienced dermatologist and skin biopsy (seven males, three females) at King Chulalongkorn Memorial Hospital and 13 normal subjects (one male, twelve females) were registered in the study. All patients were free from systemic skin therapies for at least four weeks or topical skin therapies for at least two weeks prior to sample collection. Patients with family or personal history of autoimmune disease or cancer were excluded from the study. Normal skin samples were collected from elective plastic surgery procedures. The study was approved by the ethical committee of King Chulalongkorn University. All participants provided informed consent. The demographic data of the LSC subjects and normal controls are shown in Table 1.

Cell isolation

The epidermal skin from paraffin-embedded tissues of 10 patients with LSC and 13 normal controls were isolated using the PALM MicroLaser Microdissection System (P.A.L.M. MicroLaser Technologies AG, Burnried, Germany). The dissected epidermis was removed from the slide by the cutter pulse and collected in a microtube.

DNA preparation and bisulfite modification

DNA was extracted from all samples using the QIAamp DNA mini kit™ (QIAGEN). Then, 500 ng of DNA was bisulfite-treated using the EZ DNA methylation Kit™ (Zymo Research, Orange, CA, USA) according to the manufacturer's specifications. The bisulfite-treated DNA samples were stored at -20˚C until analysis.

Table 1. Patient and control subject demographics

Sample group	Sex (Male/Female)	Age (years) (Mean ± SD)
Normal controls (n= 13)	1/12	49.62 ± 9.63
Patients with LSC (n= 10)	7/3	49.11 ± 19.47

Combined bisulfite restriction analysis (COBRA) of LINE-1 and Alu

DNA methylation of LINE-1 and Alu was assessed using the combined bisulfite restriction analysis (COBRA) method of interspersed repetitive elements as previously described.[8, 27] For LINE-1 and ALU COBRA, 2 μL of modified DNA was amplified by Hot-start PCR at 95˚C for 15 min. For LINE-1, DNA was amplified for 35 cycles of 95˚C for 1 min, 50˚C for 1 min and 72˚C for 1 min with a final extension at 72˚C for 7 min. For Alu, DNA was amplified for 35 cycles of 95˚C for 45 sec, 60˚C for 45 sec and 72˚C for 45 sec with a final extension at 72˚C for 7 min. After amplification, 8 μL of the PCR products were digested with the restriction enzymes *Taq*I and *Tas*I (MBI Fermentas). Each reaction was incubated overnight at 65˚C and then DNA fragments were separated on 8% polyacrylamide gels and stained with the SYBR green nucleic acid stain. The intensity of DNA fragment fluorescence was assessed using a PhosphoImager I apparatus and Image Quant software (Molecular Dynamics). DNA templates from HeLa, Jurkat and Daudi cell lines were used for interassay variation normalization as positive controls in all experiments.

Methylation analysis

By the COBRA-LINE-1 method, LINE-1 loci were categorized into four groups based on the methylation status of two CpG dinucleotides at the 5' and 3' ends of the sequence. These four groups consisted of: (1) two unmethylated CpGs ($^{u}C^{u}C$) at LINE-1 loci; (2) two methylated CpGs ($^{m}C^{m}C$) at LINE-1 loci; (3) 5' methylated and 3' unmethylated CpGs ($^{m}C^{u}C$) at LINE-1 loci; and (4) 5' unmethylated and 3' methylated CpGs ($^{u}C^{m}C$) at LINE-1 loci. The level of overall LINE-1 methylation and the loci from each group were analyzed by the percentage of the intensity of COBRA-digested LINE-1 products. Fragmented DNA sequences after enzymatic digestion for COBRA LINE-1 were separated into five fragments including 160, 98, 80, 62 and 18 bp. The 160 bp and the 98 bp fragments represented $^{m}C^{u}C$ and $^{u}C^{u}C$, respectively. The 80 bp fragments represented a mixture of $^{m}C^{m}C$ and $^{u}C^{m}C$. Eventually, it was determined that the 62 bp fragments represented a mixture of $^{u}C^{u}C$ and $^{u}C^{m}C$ (Figure 1). The number of CpG dinucleotides was calculated by dividing the intensity of each band by the corresponding size of the double-stranded DNA fragment as follows: A = 160 bp fragment intensity/160, B = 98 bp fragment intensity/94, C = 80 bp fragment intensity/79 and D = 62 bp fragment intensity/62. Then, the LINE-1 methylation levels were determined according to the following formulas: LINE-1 methylation level percentage = 100×(C+A)/(C+A+A+B+D); $\%^{m}C^{u}C) = 100\times(A)/(((C-D+B)/2)+A+D)$; $\%^{u}C^{m}C = 100\times(D-B)/((C-D+B)/2)+A+D$; $\%^{u}C^{u}C = 100\times B/(((C-D+B)/2)+A+D)$; $\%^{m}C^{m}C = 100\times((C-D+B)/2)/(((C-D+B)/2)+D+A)$.[27, 28]

For Alu analysis, methylated and unmethylated Alu bands were found at 57 bp and 78 bp. The %intensity of Alu methylated bands represented the %methylation of Alu.

Statistical analysis

The methylation status was compared between groups by an independent sample t-test (two-tailed) using the SPSS software package for Windows version 15.0 (SPSS Inc., Chicago, IL, USA).

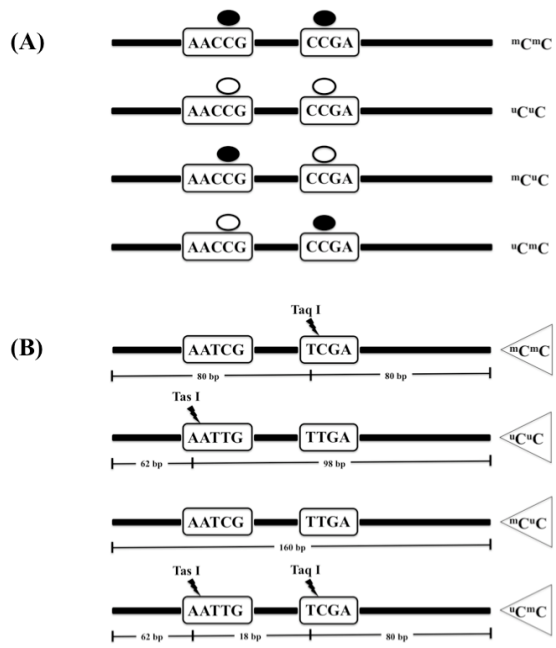

Figure 1. LINE-1 methylation patterns detected by COBRA-LINE-1 PCR. (A) The methylation patterns of two CpG dinucleotides at the 5'UTR of LINE-1 were separated by COBRA-LINE-1 into four products: $^{m}C^{m}C$, $^{u}C^{u}C$, $^{u}C^{m}C$ and $^{m}C^{u}C$. Black dots represent methylated cytosines and white dots represent unmethylated cytosines in the sequence of LINE-1. (B) Following bisulfite treatment and the PCR reaction, the amplicons were digested with the *TaqI* and *TasI* restriction enzymes. Two 80 bp nucleotide fragments were produced by *TaqI*-positive amplicons, while 62 and 98 bp nucleotide fragments were produced by *TasI*-positive amplicons.

A *P* value of <0.05 was considered to be significant. A ROC curve analysis was performed to verify the possibility of using COBRA-LINE-1 methylation status to discriminate LSC patients from healthy controls.

Results

DNA methylation pattern of LINE-1 in lichen simplex chronicus

By the COBRA-LINE-1 analysis, there was no significant difference in the overall LINE-1 methylation level in keratinocytes from patients with LSC compared to normal controls. There was no significant difference in the overall LINE-1 methylation level and patterns between male and female patients with LSC (data not shown). However, the number of hypermethylated loci ($^{m}C^{m}C$) and partially methylated loci ($^{m}C^{u}C$) of LINE-1 in LSC patients (23.26%) was significantly lower than in normal controls (% methylation LSC vs. normal = 23.26 vs. 27.71 and 18.13 vs. 19.58, respectively; p =0.012 and p =0.029, respectively). Alternatively, the partially methylated loci ($^{u}C^{m}C$) of LINE-1 in LSC (21.13%) was significantly higher than normal controls (% methylation LSC vs. normal = 21.13 vs. 15.31) (p =0.004) (Table 2).

In the Alu analysis, there was no significant difference in Alu methylation in the epidermis from LSC patients (n =10) and normal controls (n =10) (Table 3).

Table 2. Methylation level and patterns of LINE-1 in keratinocytes from patients with LSC

LINE-1 methylation levels and patterns	Normal controls (mean ± SD)	LSC (mean ± SD)	*P*-value (Normal keratinocytes compared to patients with LSC)
	Keratinocyte (n= 13)	Keratinocyte (n= 10)	
^{m}C (%)	45.15 ± 3.67	42.90 ± 1.15	0.055
$^{m}C^{m}C$ (%)	27.71 ± 4.61	23.26 ± 2.38	0.012
$^{u}C^{m}C$ (%)	15.31 ± 3.79	21.13 ± 4.79	0.004
$^{m}C^{u}C$ (%)	19.58 ± 1.38	18.13 ± 1.58	0.029
$^{u}C^{u}C$ (%)	37.40 ± 3.41	37.47 ± 2.51	0.960
Partial (%)	34.89 ± 3.46	39.27 ± 4.31	0.013

Table 3. Methylation level of Alu in keratinocytes from patients with LSC

Alu methylation levels	Normal controls (mean ± SD)	LSC (mean ± SD)
	Keratinocyte (n= 10)	Keratinocyte (n= 10)
% Alu methylation	30.99 ± 3.17	30.01 ± 1.76

LINE-1 methylation pattern as a biomarker for lichen simplex chronicus

The ROC curve analysis was performed to determine the probable use of the LINE-1 methylation pattern to discriminate LSC patients from normal controls. The $^{m}C^{m}C$ pattern was appropriate for LSC detection with a cut-off value of ≤26.26% (sensitivity = 100.00 and specificity = 69.23). In addition, the cut-off value ≤18.02% for the $^{m}C^{u}C$ pattern with sensitivity = 60.00% and specificity = 92.31% and the cut-off value >18.39% for the $^{u}C^{m}C$ pattern with sensitivity = 60.00% and specificity = 92.31% were suitable to discriminate LSC patients from normal controls (Figure 2).

Discussion

Recent publications have elucidated the difference in global and specific promoter DNA methylation in a number of skin diseases, including atopic eczema.[3, 29] Furthermore, alterations in LINE-1 and Alu methylation have been reported in various malignancies, including squamous cell carcinoma.[10, 21, 22, 30-33] So far, there has been no information on DNA methylation in LSC. The present study demonstrated alterations in the LINE-1 methylation pattern in keratinocytes from LSC patients. Our data demonstrate that the overall LINE-1 methylation level in LSC showed a trend to be decreased in LSC. Moreover, certain patterns of LINE-1 methylation ($^{m}C^{m}C$, $^{m}C^{u}C$, $^{u}C^{m}C$) were more significantly associated with LSC than overall LINE-1 methylation. ROC analysis confirmed that %$^{m}C^{m}C$, %$^{m}C^{u}C$ and %$^{u}C^{m}C$ are highly specific for LSC detection with cut-off values of ≤26.26%, ≤18.02% and >18.39%, respectively. Previous studies in various cancers showed that % $^{u}C^{u}C$ has better power than % overall LINE-1 methylation and can be used as a biomarker for disease detection.[27, 28]

Another study in bladder cancer reported that %$^{m}C^{u}C$ was better than other forms of LINE-1 methylation for cancer detection.[34] Thus, %$^{m}C^{m}C$, %$^{m}C^{u}C$ and %$^{u}C^{m}C$ have potential use in discriminating LSC from normal skin. However, further study is needed to identify the significance and biological relevance of the different forms of LINE-1 methylation.

Several studies in cancer have reported hypomethylation of both LINE-1 and Alu, which represent the genomic instability of these diseases. We did not demonstrate a significant change in Alu methylation in keratinocytes from patients with LSC. This finding represented both the common and variable pathogenesis of LSC and cancer. Unlike cancer, acanthosis and hyperkeratosis of the epidermis in LSC occur as a result of chronic rubbing of the skin. Moreover, it should be noted that genomic instability does not occur in LSC.

LINE-1 has been identified and shown to affect the human genome by modifying gene expression. Several studies have revealed LINE-1 hypomethylation is associated with genomic instability in cancers. The LINE-1 methylation pattern at different loci has been reported to be distinctive between loci.[35] Moreover, intragenic LINE-1 could promote gene repression in cancer, depending on the degree and site of LINE-1 hypomethylation. A postranscriptional mechanism by AGO2 and siRNA has been identified to be the mechanism by which hypomethylation of intragenic LINE-1 suppresses genes in cancer.[32] In atopic

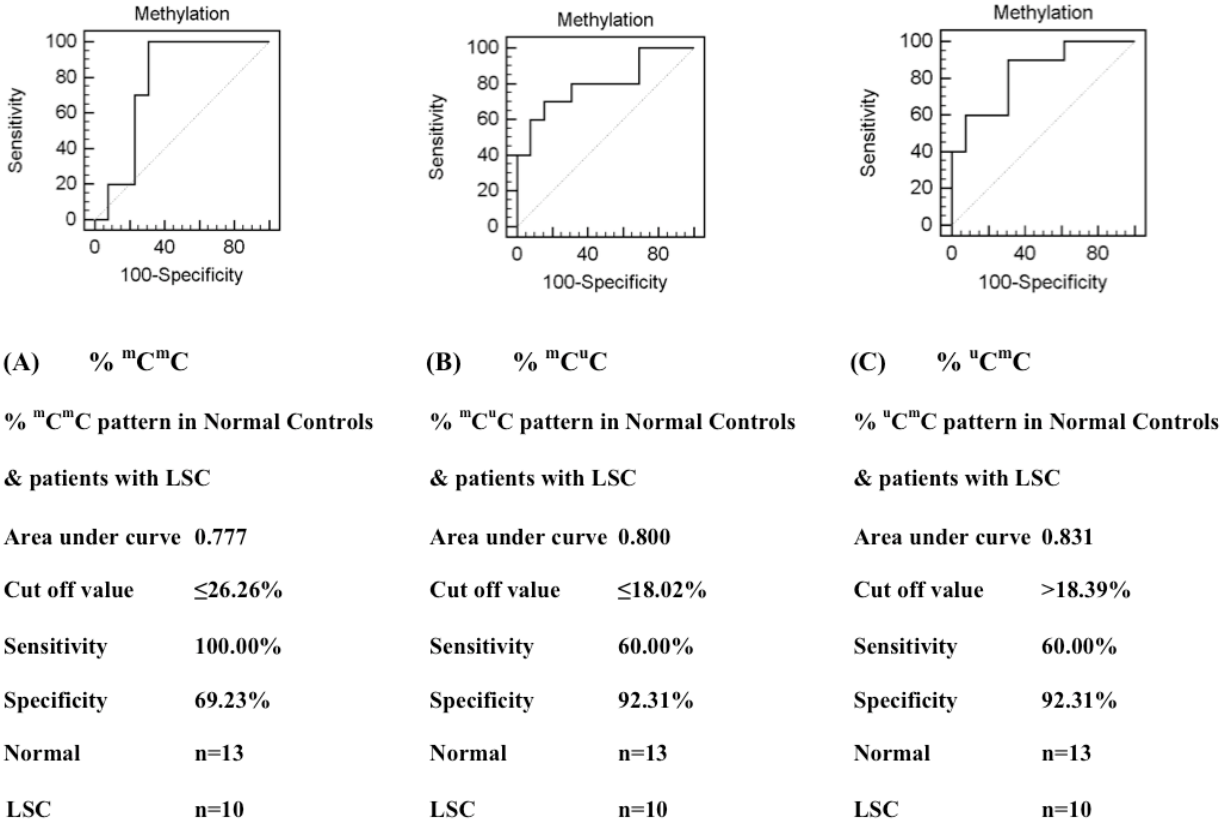

Figure 2. ROC curve analysis of LINE-1 methylation in LSC. (A) The %$^{m}C^{m}C$ of LINE-1 methylation compared between LSC and normal keratinocytes with a criterion value of ≤26.26% (sensitivity = 100.00% and specificity = 69.23%). (B) The %$^{m}C^{u}C$ of LINE-1 methylation compared between LSC and normal keratinocytes with a criterion value of ≤18.02% (sensitivity = 60.00% and specificity = 92.31%). (C) The %$^{u}C^{m}C$ of LINE-1 methylation compared between LSC and normal keratinocytes with a criterion value of >18.39% (sensitivity = 60.00% and specificity = 92.31%).

eczema, both global and specific promoter hypomethylation with transcriptional silencing of the gene have also been described in monocytes. Although we do not know the significance of alterations in the LINE-1 methylation pattern in LSC, it is likely that changes in LINE-1 methylation may be involved in the pathogenesis of the disease. Further study is needed to clarify the mechanism, which would involve changing the LINE-1 methylation pattern and investigating the possible consequences in LSC.

In conclusion, alterations in LINE-1 methylation patterns were detected in keratinocytes from patients with LSC. A distinctive pattern of LINE-1 methylation was revealed in LSC, and $^{m}C^{m}C$, $^{m}C^{u}C$ and $^{u}C^{m}C$ were very highly sensitive and specific for detecting LSC. Therefore, they are appropriate for use as biomarkers for this disease.

Acknowledgement

This work was supported by a research grant from the National Research University Project of CHE and the Ratchadaphiseksomphot Endowment Fund (HR1163A), the Development and Promotion of Science and Technology Talents Project (DPST), the Thailand Research Fund TRG 5480010, the National Research Council of Thailand and the 90th Year Anniversary of Chulalongkorn University (Ratchadaphiseksomphot Endowment Fund). AM is supported by a Research Chair Grant (2011) from the National Science and Technology Development Agency (NSTDA), Thailand.

References

1. Brown SJ, McLean WH. Eczema genetics: current state of knowledge and future goals. J Invest Dermatol. 2009;129:543-52.
2. Nakamura T, Sekigawa I, Ogasawara H, Mitsuishi K, Hira K, Ikeda S, et al. Expression of DNMT-1 in patients with atopic dermatitis. Arch Dermatol Res. 2006;298:253-6.
3. Liang Y, Wang P, Zhao M, Liang G, Yin H, Zhang G, et al. Demethylation of the FCER1G promoter leads to FcepsilonRI overexpression on monocytes of patients with atopic dermatitis. Allergy. 2012;67:424-30.
4. Lynch PJ. Lichen simplex chronicus (atopic/neurodermatitis) of the anogenital region. Dermatol Ther. 2004;17:8-19.
5. Cordaux R, Batzer MA. The impact of retrotransposons on human genome evolution. Nat Rev Genet. 2009;10:691-703.
6. Han JS, Szak ST, Boeke JD. Transcriptional disruption by the L1 retrotransposon and implications for mammalian transcriptomes. Nature. 2004;429:268-74.
7. Chisholm KM, Aubert SD, Freese KP, Zakian VA, King MC, Welcsh PL. A genomewide screen for suppressors of Alu-mediated rearrangements reveals a role for PIF1. PLoS One. 2012;7:e30748.
8. Chalitchagorn K, Shuangshoti S, Hourpai N, Kongruttanachok N, Tangkijvanich P, Thong-ngam D, et al. Distinctive pattern of LINE-1 methylation level in normal tissues and the association with carcinogenesis. Oncogene. 2004;23:8841-6.
9. Schulz WA. L1 retrotransposons in human cancers. J Biomed Biotechnol. 2006;2006:83672.
10. Estecio MR, Gharibyan V, Shen L, Ibrahim AE, Doshi K, He R, et al. LINE-1 hypomethylation in cancer is highly variable and inversely correlated with microsatellite instability. PLoS One. 2007;2:e399.
11. Hsiung DT, Marsit CJ, Houseman EA, Eddy K, Furniss CS, McClean MD, et al. Global DNA methylation level in whole blood as a biomarker in head and neck squamous cell carcinoma. Cancer Epidemiol Biomarkers Prev. 2007;16:108-14.
12. Cho NY, Kim BH, Choi M, Yoo EJ, Moon KC, Cho YM, et al. Hypermethylation of CpG island loci and hypomethylation of LINE-1 and Alu repeats in prostate adenocarcinoma and their relationship to clinicopathological features. J Pathol. 2007;211:269-77.
13. Matsuzaki K, Deng G, Tanaka H, Kakar S, Miura S, Kim YS. The relationship between global methylation level, loss of heterozygosity, and microsatellite instability in sporadic colorectal cancer. Clin Cancer Res. 2005;11:8564-9.
14. Perrin D, Ballestar E, Fraga MF, Frappart L, Esteller M, Guerin JF, et al. Specific hypermethylation of LINE-1 elements during abnormal overgrowth and differentiation of human placenta. Oncogene. 2007;26:2518-24.
15. Pattamadilok J, Huapai N, Rattanatanyong P, Vasurattana A, Triratanachat S, Tresukosol D, et al. LINE-1 hypomethylation level as a potential prognostic factor for epithelial ovarian cancer. Int J Gynecol Cancer. 2008;18:711-7.
16. Moore LE, Pfeiffer RM, Poscablo C, Real FX, Kogevinas M, Silverman D, et al. Genomic DNA hypomethylation as a biomarker for bladder cancer susceptibility in the Spanish Bladder Cancer Study: a case-control study. Lancet Oncol. 2008;9:359-66.
17. Smith IM, Mydlarz WK, Mithani SK, Califano JA. DNA global hypomethylation in squamous cell head and neck cancer associated with smoking, alcohol consumption and stage. Int J Cancer. 2007;121:1724-8.
18. Subbalekha K, Pimkhaokham A, Pavasant P, Chindavijak S, Phokaew C, Shuangshoti S, et al. Detection of LINE-1s hypomethylation in oral rinses of oral squamous cell carcinoma patients. Oral Oncol. 2009;45:184-91.
19. Karouzakis E, Gay RE, Michel BA, Gay S, Neidhart M. DNA hypomethylation in rheumatoid arthritis synovial fibroblasts. Arthritis Rheum. 2009;60:3613-22.
20. Choi IS, Estecio MR, Nagano Y, Kim do H, White JA, Yao JC, et al. Hypomethylation of LINE-1 and Alu in well-differentiated neuroendocrine tumors (pancreatic endocrine tumors and carcinoid tumors). Mod Pathol. 2007;20:802-10.
21. Roman-Gomez J, Jimenez-Velasco A, Agirre X, Castillejo JA, Navarro G, San Jose-Eneriz E, et al. Repetitive DNA

hypomethylation in the advanced phase of chronic myeloid leukemia. Leuk Res. 2008;32:487-90.

22. Lee HS, Kim BH, Cho NY, Yoo EJ, Choi M, Shin SH, et al. Prognostic implications of and relationship between CpG island hypermethylation and repetitive DNA hypomethylation in hepatocellular carcinoma. Clin Cancer Res. 2009;15:812-20.
23. Kim SJ, Kelly WK, Fu A, Haines K, Hoffman A, Zheng T, et al. Genome-wide methylation analysis identifies involvement of TNF-alpha mediated cancer pathways in prostate cancer. Cancer Lett. 2011;302:47-53.
24. Daskalos A, Nikolaidis G, Xinarianos G, Savvari P, Cassidy A, Zakopoulou R, et al. Hypomethylation of retrotransposable elements correlates with genomic instability in non-small cell lung cancer. Int J Cancer. 2009;124:81-7.
25. Ogino S, Nosho K, Kirkner GJ, Kawasaki T, Chan AT, Schernhammer ES, et al. A cohort study of tumoral LINE-1 hypomethylation and prognosis in colon cancer. J Natl Cancer Inst. 2008;100:1734-8.
26. Saito K, Kawakami K, Matsumoto I, Oda M, Watanabe G, Minamoto T. Long interspersed nuclear element 1 hypomethylation is a marker of poor prognosis in stage IA non-small cell lung cancer. Clin Cancer Res. 2010;16:2418-26.
27. Kitkumthorn N, Tuangsintanakul T, Rattanatanyong P, Tiwawech D, Mutirangura A. LINE-1 methylation in the peripheral blood mononuclear cells of cancer patients. Clin Chim Acta. 2012;413:869-74.
28. Pobsook T, Subbalekha K, Sannikorn P, Mutirangura A. Improved measurement of LINE-1 sequence methylation for cancer detection. Clin Chim Acta. 2011;412:314-21.
29. Ruchusatsawat K, Wongpiyabovorn J, Protjaroen P, Chaipipat M, Shuangshoti S, Thorner PS, et al. Parakeratosis in skin is associated with loss of inhibitor of differentiation 4 via promoter methylation. Hum Pathol. 2011;42:1878-87.
30. Cadieux B, Ching TT, VandenBerg SR, Costello JF. Genome-wide hypomethylation in human glioblastomas associated with specific copy number alteration, methylenetetrahydrofolate reductase allele status, and increased proliferation. Cancer Res. 2006;66:8469-76.
31. Sunami E, de Maat M, Vu A, Turner RR, Hoon DS. LINE-1 hypomethylation during primary colon cancer progression. PLoS One. 2011;6:e18884.
32. Aporntewan C, Phokaew C, Piriyapongsa J, Ngamphiw C, Ittiwut C, Tongsima S, et al. Hypomethylation of intragenic LINE-1 represses transcription in cancer cells through AGO2. PLoS One. 2011;6:e17934.
33. Lin CH, Hsieh SY, Sheen IS, Lee WC, Chen TC, Shyu WC, et al. Genome-wide hypomethylation in hepatocellular carcinogenesis. Cancer Res. 2001;61:4238-43.
34. Patchsung M, Boonla C, Amnattrakul P, Dissayabutra T, Mutirangura A, Tosukhowong P. Long interspersed nuclear element-1 hypomethylation and oxidative stress: correlation and bladder cancer diagnostic potential. PLoS One. 2012;7:e37009.
35. Phokaew C, Kowudtitham S, Subbalekha K, Shuangshoti S, Mutirangura A. LINE-1 methylation patterns of different loci in normal and cancerous cells. Nucleic Acids Res. 2008;36:5704-12.