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

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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 (mCmC), hypomethylated (uCuC), and two forms of partially methylated loci (uCmC and mCuC).

Results: The %mCmC of LINE-1 was significantly decreased in keratinocytes from patients with LSC (p=0.012). Moreover, the %mCUc was significantly lower in LSC than controls (p=0.029). Conversely, %uCmC was significantly higher LSC than controls (p=0.004). A receiver-operating characteristic (ROC) curve analysis demonstrated that %mCmC, %mCUc and %UCuC 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

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, \(^3\%\)\(^\text{C}\text{C}\) has better power than overall \(^\%\)\(^\text{LINE-1}\) 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 (\(^\text{C}\text{mC}, \text{C}\text{C}, \text{mC}\text{C}\) and \(^\text{C}\text{mC}\), 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\(^\text{™}\) (QIAGEN). Then, 500 ng of DNA was bisulfite-treated using the EZ DNA Methylation Kit\(^\text{™}\) (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**

<table>
<thead>
<tr>
<th>Sample group</th>
<th>Sex (Male/Female)</th>
<th>Age (years) (Mean ± SD)</th>
</tr>
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<tbody>
<tr>
<td>Normal controls (n= 13)</td>
<td>1/12</td>
<td>49.62 ± 9.63</td>
</tr>
<tr>
<td>Patients with LSC (n= 10)</td>
<td>7/3</td>
<td>49.11 ± 19.47</td>
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**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. 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 TaqI and TasI (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.

**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).
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 (\(\text{mCmC}\)) and partially methylated loci (\(\text{mCuC}\)) 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 (\(\text{CuCM}\)) 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

<table>
<thead>
<tr>
<th>LINE-1 methylation levels and patterns</th>
<th>Normal controls (mean ± SD)</th>
<th>LSC (mean ± SD)</th>
<th>P-value (Normal keratinocytes compared to patients with LSC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{mC}) (%)</td>
<td>45.15 ± 3.67</td>
<td>42.90 ± 1.15</td>
<td>0.055</td>
</tr>
<tr>
<td>(\text{mCmC}) (%)</td>
<td>27.71 ± 4.61</td>
<td>23.26 ± 2.38</td>
<td>0.012</td>
</tr>
<tr>
<td>(\text{mCuC}) (%)</td>
<td>15.31 ± 3.79</td>
<td>21.13 ± 4.79</td>
<td>0.004</td>
</tr>
<tr>
<td>(\text{CuCM}) (%)</td>
<td>19.58 ± 1.38</td>
<td>18.13 ± 1.58</td>
<td>0.029</td>
</tr>
<tr>
<td>(\text{CmC}) (%)</td>
<td>37.40 ± 3.41</td>
<td>37.47 ± 2.51</td>
<td>0.960</td>
</tr>
<tr>
<td>Partial (%)</td>
<td>34.89 ± 3.46</td>
<td>39.27 ± 4.31</td>
<td>0.013</td>
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Table 3. Methylation level of Alu in keratinocytes from patients with LSC

<table>
<thead>
<tr>
<th>Alu methylation levels</th>
<th>Normal controls (mean ± SD)</th>
<th>LSC (mean ± SD)</th>
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<tbody>
<tr>
<td>% Alu methylation</td>
<td>Keratinocyte (n= 10)</td>
<td>Keratinocyte (n= 10)</td>
</tr>
<tr>
<td></td>
<td>30.99 ± 3.17</td>
<td>30.01 ± 1.76</td>
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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 \(\text{mCmC}\) pattern was appropriate for LSC detection with a cut-off value of \(\leq26.26\%\) (sensitivity = 100.00 and specificity = 69.23). In addition, the cut-off value \(\leq18.02\%\) for the \(\text{mCuC}\) pattern with sensitivity = 60.00% and specificity = 92.31% and the cut-off value \(>18.39\%\) for the \(\text{CuCM}\) 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 (\(\text{mCmC}\), \(\text{mCuC}\), \(\text{CuCM}\)) were more significantly associated with LSC than overall LINE-1 methylation. ROC analysis confirmed that %\(\text{mCmC}\), %\(\text{mCuC}\) and %\(\text{CuCM}\) are highly specific for LSC detection with cut-off values of \(\leq26.26\%\), \(\leq18.02\%\) and \(>18.39\%\), respectively. Previous studies in various cancers showed that % \(\text{mC}\) 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\(^{m}\)C was better than other forms of LINE-1 methylation for cancer detection.\(^{34}\) Thus, %\(^{m}\)C\(^{m}\)C, %\(^{u}\)C\(^{m}\)C and %\(^{u}\)C\(^{u}\)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 posttranscriptional mechanism by AGO2 and siRNA has been identified to be the mechanism by which hypomethylation of intragenic LINE-1 suppresses genes in cancer.\(^{32}\)

![Figure 2](image)

**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 \(\leq 26.26\%\) (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 \(\leq 18.02\%\) (sensitivity = 60.00% and specificity = 92.31%). (C) The %\(^{u}\)C\(^{u}\)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 \( ^{mCmC} \), \( ^{mCmC} \) and \( ^{CmC} \) were very highly sensitive and specific for detecting LSC. Therefore, they are appropriate for use as biomarkers for this disease.

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References
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