การศึกษาดีเอนเอเมทิลเลชั่นทั้งยีโนมและบทบาทของสภาวะเมทิลเลชั่นที่ลำดับเบสของ LINE-1 และ Alu ในโรคสะเก็ดเงิน

นายสุรศักดิ์ อยู่ยงสะถิต

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
สาขาวิชาจุลชีววิทยาทางการแพทย์ (สหสาขาวิชา)
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ปีการศึกษา 2554
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GENOME-WIDE ANALYSIS OF DNA METHYLATION AND ROLE OF METHYLATION STATUS AT LINE-1 AND ALU SEQUENCES IN CHRONIC PLAQUE-TYPE PSORIASIS

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A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Medical Microbiology
(Interdisciplinary Program)
Graduate School
Chulalongkorn University
Academic Year 2011

Thesis Title	GENOME-WIDE ANALYSIS OF DNA METHYLATION AND ROLE OF METHYLATION STATUS AT LINE-1 AND ALU SEQUENCES IN CHRONIC PLAQUE-TYPE PSORIASIS
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สุรศักดิ์ อยู่ยงสะถิต : การศึกษาดีเอนเอเมทิลเลชั่นทั้งยีโนมและบทบาทของสภาวะเมทิลเลชั่นที่ลำดับเบส ของ LINE-1 และ Alu ในโรคสะเก็ดเงิน. (GENOME-WIDE ANALYSIS OF DNA METHYLATION AND ROLE OF METHYLATION STATUS AT LINE-1 and ALU SEQUENCES IN CHRONIC PLAQUE-TYPE PSORIASIS) อ. ที่ปรึกษาวิทยานิพนธ์หลัก : รศ.พญ.ดร.จงกลนี วงศ์ปิยะบวร, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม : ศ.นพ.ดร.อภิวัฒน์ มุทิรางกูร, 131 หน้า.

ลำดับเบสซ้ำชนิด LINE-1 และ Alu ถูกจัดจำแนกว่ามีบทบาทต่อการเปลี่ยนแปลงในยีโนมของมนุษย์ผ่านหลายกลไกด้วยกัน เช่นกลไกการเปลี่ยนแปลงการแสดงออกของยีน การเปลี่ยนแปลงระดับเมทิลเลชั่นของลำดับเบสซ้ำ LINE-1 และ Alu สัมพันธ์กับการเกิดโรคหลายชนิด โดยเฉพาะโรคมะเร็งและโรคภูมิต้านทานเนื้อเยื่อตนเอง ยิ่งไปกว่านั้นระดับและรูปแบบเมทิลเลชั่นระดับต่ำของ LINE-1 ยังสัมพันธ์กับความเสี่ยงสูงในการเป็นโรคมะเร็ง, การพยากรณ์โรค และการรุกรานของมะเร็งหลายชนิด แต่อย่างไรก็ตามยังไม่มีการศึกษาเรื่องนี้ในโรคสะเก็ดเงิน เราจึงทำการศึกษาระดับเมทิลเลชั่นของลำดับเบสซ้ำในโรคสะเก็ดเงิน เราศึกษาระดับ และรูปแบบดีเอนเอเมทิลเลชั่น ของ LINE-1และ Alu ในเซลล์ผิวหนังและเซลล์เม็ดเลือดของผู้ป่วยโรคสะเก็ดเงินจำนวน 29 ราย เปรียบเทียบกับคนปกติจำนวน 33 รายและผู้ป่วยโรคมะเร็งผิวหนังจำนวน 8 ราย จากวิธี COBRA-LINE-1 and Alu ที่ถูกพัฒนาให้ดีขึ้น COBRA-LINE-1 แบ่ง LINE-1 loci ตามรูปแบบของเมทิลเลชั่นของนิวคลีโอไทด์ 2 ตำแหน่งบริเวณ CpG ที่ 5'UTR เป็นสี่กลุ่มดังนี้ hypermethylated ($^{m}C^{m}C$), hypomethylated ($^{u}C^{u}C$), และ 2 รูปแบบของ partially methylated loci ($^{u}C^{m}C$ and $^{m}C^{u}C$) ผลสืบเนื่องจากการเปลี่ยนแปลงทาง epigenetic ในผิวหนังผู้ป่วยโรคสะเก็ดเงินกับ LINE-1 ในยีน และการแสดงออกของยีนถูกทดสอบใน 2 ไมโครอะเร (GSE13355 และ GSE14905) ผลการศึกษาพบสภาวะเมทิลเลชั่นระดับต่ำใน LINE-1 แต่ไม่พบใน Alu ของเซลล์ผิวหนังผู้ป่วยโรคสะเก็ดเงินอย่างมีนัยสำคัญทางสถิติ (p-value=0.044) (ระดับเมทิลเลชั่นในโรคสะเก็ดเงินเทียบกับกลุ่มควบคุม = 41.64 และ 45.15) นอกจากนั้นยังพบเปอร์เซ็นต์เมทิลเลชั่นในรูปแบบ %$^{u}C^{u}C$ ของยีน LINE-1 มีระดับที่สูงขึ้นในโรคสะเก็ดเงินกว่ากลุ่มควบคุมอย่างมีนัยสำคัญทางสถิติ (p-value=0.045) (ระดับเมทิลเลชั่นในโรคสะเก็ดเงินเทียบกับกลุ่มควบคุม = 41.11 และ 37.40) จากการวิเคราะห์ด้วยโปรแกรมทางสถิติ (ROC curve analysis) แสดงค่าความไว = 69.23% และค่าความจำเพาะ = 69.23% ในโรคสะเก็ดเงินเมื่อใช้ค่า cut-off ของเปอร์เซ็นต์เมทิลเลชั่นในรูปแบบ %$^{u}C^{u}C$ ของยีน LINE-1 ที่ > 39.67% ยิ่งไปกว่านั้น เปอร์เซ็นต์เมทิลเลชั่นในรูปแบบ %$^{u}C^{m}C$ ของยีน LINE-1 ลดต่ำลงอย่างมีนัยสำคัญทางสถิติในผู้ป่วยโรคสะเก็ดเงินที่มีความรุนแรงของโรคสูงกว่าผู้ป่วยโรคสะเก็ดเงินที่มีความรุนแรงของโรคต่ำ (p-value=0.022) (ระดับเมทิลเลชั่นในผู้ป่วยที่มีความรุนแรงของโรคสูงเทียบกับผู้ป่วยที่มีความรุนแรงของโรคต่ำ = 9.10 และ 18.80) การวิเคราะห์ทางสถิติ (ROC curve analysis) แสดงให้เห็นความน่าจะเป็นที่เปอร์เซ็นต์เมทิลเลชั่นในรูปแบบ %$^{u}C^{m}C$ ของยีน LINE-1 สามารถใช้เป็นเครื่องหมายทางชีวภาพชี้วัดความรุนแรงของโรคสะเก็ดเงินที่ค่าความไว = 83.33% และค่าความจำเพาะ = 100.00% โดยใช้ cut-off ที่ $\leq$ 10.87% สำหรับการวิเคราะห์ข้อมูลการแสดงออกของยีนในไมโครอะเรพบความสัมพันธ์ระดับสูงระหว่างยีนที่มี LINE-1 กับยีนที่ลดระดับการแสดงออกอย่างมีนัยสำคัญทางสถิติ (p-value=3.84 x 10^{-27} และ p-value=2.14 x 10^{-21} ตามลำดับ) การเปลี่ยนแปลงรูปแบบของ LINE-1 และการลดระดับการแสดงออกพบในผิวหนังโรคสะเก็ดเงิน การเปลี่ยนแปลงนี้อาจเป็นหนึ่งในกลไกซึ่งเปลี่ยนแปลงการแสดงออกของยีน และนำไปสู่การเปลี่ยนแปลง phenotype ในผิวหนังโรคสะเก็ดเงิน ดังนั้นเปอร์เซ็นต์เมทิลเลชั่นในบางรูปแบบได้แก่ %$^{u}C^{u}C$ และ %$^{u}C^{m}C$ ของยีน LINE-1 อาจนำมาใช้เป็นเครื่องหมายทางชีวภาพในโรคสะเก็ดเงินต่อไป

สาขาวิชา จุลชีววิทยาทางการแพทย์ ลายมือชื่อนิสิต .....

ปีการศึกษา 2554 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก .....

ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม .....

5087868220 : MAJOR MEDICAL MICROBIOLOGY

KEYWORDS : DNA METHYLATION / LINE-1 / ALU / REPETTIVE ELEMENTS / PSORIASIS

SURASAK YOOYONGSATIT: GENOME-WIDE ANALYSIS OF DNA METHYLATION AND ROLE OF METHYLATION STATUS AT LINE-1 and ALU SEQUENCES IN CHRONIC PLAQUE-TYPE PSORIASIS. ADVISOR : ASSOC.PROF. JONGKONNEE WONGPIYABOVORN, M.D.,Ph.D., CO-ADVISOR : PROF. APIWAT MUTIRANGURA, M.D.,Ph.D., 131 pp.

Long interspersed element-1 (LINE-1 or L1) and short interspersed elements (Alu) retrotransposons are identified to influence human genome in many fashions such as modifications in gene expression. Alterations in methylation of LINE-1 and Alu have been demonstrated to associate with many diseases, particularly in malignancies and autoimmune diseases. Moreover, level and pattern of LINE-1 hypomethylation were related to high cancer risk, prognosis and aggressiveness of many several cancers. But there is no study in Psoriasis. To evaluate DNA methylated status of repetitive sequence in psoriasis. We determined DNA methylation level and pattern of LINE-1 and Alu in keratinocyte and various hematopoietic cells from psoriasis (n=29) compared to normal controls (n=33) and squamous cell carcinoma (SCC) (n=8), by the improved combined bisulfite restriction analysis of LINE-1 and Alu (COBRA-LINE-1 and Alu). COBRA-LINE-1 classified LINE-1 loci due to methylation patterns of 2 CpG dinucleotides at 5'UTR into four categories: hypermethylated ($^{m}C^{m}C$), hypomethylated ($^{u}C^{u}C$), and 2 forms of partially methylated loci ($^{u}C^{m}C$ and $^{m}C^{u}C$). The consequences of epigenetic changes of psoriatic skin in intragenic LINE-1 in gene expression were tested on 2 expression microarrays (GSE13355 and GSE14905). We found hypomethylation of LINE-1 but not Alu in keratinocyte from psoriasis (*p*-value=0.044) (% methylation in psoriasis vs. normal = 41.64 VS. 45.15). The percentage of $^{u}C^{u}C$ ($\%^{u}C^{u}C$) of LINE-1 was significantly higher in keratinocyte from psoriasis than healthy subjects (*p*-value=0.045) (% methylation in psoriasis vs. normal = 41.11 VS. 37.40). A receiver-operating characteristic (ROC) curve analysis demonstrated the cut-off value >39.67% of $\%^{u}C^{u}C$ with sensitivity = 69.23% and specificity = 69.23% for psoriasis. Moreover, $\%^{u}C^{m}C$ of LINE-1 was significantly lower in severe psoriasis than mild psoriasis (*p*-value=0.022) (% methylation in severe vs. mild psoriasis = 9.10 VS. 18.80). ROC curve analyses displayed the possible of $\%^{u}C^{m}C$ as biomarker for psoriasis severity with a cut-off value of $\leq$ 10.87% (sensitivity = 83.33% and specificity = 100.00%). Genome-wide expression array analysis revealed a higher prevalence of down regulation when genes containing LINE-1s than without (*p*-value=3.84 x 10^{-27} and *p*-value=2.14 x 10^{-21}, respectively). Changes in LINE-1 pattern and reduction in level can be observed in psoriatic skin. This change is one of the mechanisms that alter gene expression leading to phenotypic change of psoriatic skin. Consequently, certain classes of LINE-1 methylation patterns, $\%^{u}C^{u}C$ and $\%^{u}C^{m}C$ may be used as novel biomarker for psoriasis.

Department : Microbiology Student's Signature .....

Field of Study : Medical microbiology Advisor's Signature .....

Academic Year : 2011 Co-advisor's Signature .....

ACKNOWLEDGEMENTS

I would like to thank everyone who encouraged and supported me to complete this dissertation. This dissertation could not have been written without the support of Development and Promotion of Science and Technology Talents Project (DPST), the 90th Year Anniversary of Chulalongkorn University (Ratchadaphiseksomphot Endowment Fund), National Research University project from the Commission of Higher Education and the Ratchadaphiseksomphot Endowment Fund (HR1163A), the Thailand Research Fund (TRF) senior research scholar grant, Center of Excellence in Molecular Genetics of Cancer and Human Diseases, Department of Anatomy, Faculty of Medicine, Chulalongkorn University and the Research Chair Grant 2011 from the National Science and Technology Development Agency (NSTDA), Thailand

This thesis would not be accomplished without support of many people whose name could not be listed here due to the limited space. However, my gratitude is not less than those listed below.

I wish to express my deepest gratitude to my advisor and co-advisors, Assoc. Prof. Jongkonnee Wongpiyabovorn, and Prof. Apiwat Mutirangura for their patience and talent in teaching, encouraging and guiding during these years. I have enjoyed the inspiring atmosphere they have created in our research group.

I wish to express my deepest gratitude to Dr. Kriangsak Ruchusatsawat and Miss Phantipa Protjaroen for their technical help and support the methylation array data.

I express my sincere thanks to all of my colleagues in research units for their fellowship, technical help and support during this work. I really appreciate Mr. Jeerawat Nakkuntod, Miss Supranee buranapraditkun for her kindly suggestion of all techniques.

Finally, with all my heart, I would like to thank my family for their unconditional love and support. I am forever humbled in gratitude that they lead my life so elegantly and perfectly.

CONTENTS

PAGE

PAGE

PAGE

LIST OF TABLES

TABLE PAGE

LIST OF FIGURES

FIGURE PAGE

LIST OF ABBREVIATIONS

COBRA	Combined Bisulfite Restriction Analysis
CU-DREAM	Connection Up- or Down-Regulation Expression Analysis of Microarray Program
CU-DREAMX	Connection Up- or Down-Regulation Expression Analysis of Microarray Extension Program
DNMTs	DNA Methyltransferases
GEO	Gene Expression Omnibus
IRSs	Interspersed Repetitive Sequences
TEs	Transposable Elements
LINE-1	Long Interspersed Nuclear Element type 1
SINE-1	Short Interspersed Nuclear Element type 1
LTRs	Long Terminal Repeats
ORE	Normal Oral Epithelium
PBMCs	Peripheral Blood Mononuclear Cells
SCC	Squamous Cell Carcinoma
RA	Rheumatoid arthritis
Bp	Base pair
IFN	Interferon
IL	Interleukin
KC	Keratinocyte
MHC	Major Histocompatibility Complex
PCR	Polymerase Chain Reaction

PASI	Psoriasis Area And Severity Index
ROC	A Receiver Operating Characteristic
AUC	Area Under Curve

CHAPTER I

INTRODUCTION

Human genome composed of transposable elements (TEs) approximately 45%. Human mobile elements or transposable elements (TEs) are known as jumping genes that able to move within and sometimes between the genome from site to site. TEs can be divided into DNA transposons and retrotransposons. And retrotransposons can be subdivided into LTR and Non-LTR retrotransposons. Non-LTR retrotransposons constitute about 34% of human genome [1]. Two highest densities of non-LTR retrotransposons are long interspersed nuclear elements-1 (LINE-1) (16.9%) and short interspersed nuclear elements (SINE or Alu) (10.6%). LINE-1 and Alu widely inserts in human genome and has a lot of probable functional outcomes [2-6]. Non-LTR retrotransposons is the only TEs that definitely influence human genome in many fashions such as generating insertion mutation, genomic instability and alteration in gene expression [1, 2, 7, 8]. Several studies shown DNA methylation is an important mechanism in silencing retrotransposable elements such as LINE-1 for maintaining genomic stability [9-11]. Different methylation level and pattern of LINE-1 at each loci were observed in different normal tissue type (4). Hypomethylation of LINE-1 and Alu were suggested to be cause of global hypomethylation and genomic instability in many malignancies and autoimmune diseases [6, 12-32]. In malignancies, a positive correlation of LINE-1 methylation levels of most loci with genome-wide levels but hypermethylation of gene promoters were found [33-35]. Moreover, the degree of LINE-1 hypomethylation was directly related to high cancer risk, prognosis and aggressiveness of many several cancers [12, 15, 20, 21, 24, 36, 37]. Recently, LINE-1 methylation pattern has been reported to be a biomarker in various cancers. The percentage of $^{U}C^{U}C$ (%$^{U}C^{U}C$) has

better power than overall %LINE-1 methylation to differentiate cancer of colon, liver, lung and nasopharynx from normal controls [38]. In autoimmune diseases, LINE-1 hypomethylation was found in lymphocyte of patients with systemic lupus erythematosus and synovial fibroblast of rheumatoid arthritis [26, 39].

Psoriasis is a common chronic skin disease that occurs worldwide. The disease characterized by abnormal proliferation and differentiation of keratinocyte and inflammation in both epidermis and dermis. Multifactorial genetic disorder and environmental factors are important in phenotypic expression of the disease. Recently, epigenetic change has been implicated in the pathogenesis of psoriasis. A number of studies reported alteration of methylation in promoter region with reverse mRNA expression of several genes in psoriatic skin and PBMC [40-44]. Increase of 5-methylcytocine in both epidermis and PBMC from psoriasis also has been observed [45]. Moreover, global histone H4 hypoacethylation in psoriatic PBMC has been reported [46]. Recently, alteration of genome-wide CpG methylation in psoriatic skin with anti-tumor necrotic factor-alpha (TNF-α) has been reported [47]. Furthermore, genome-wide DNA methylation has been identified as epigenetic dysregulation in monozygotic twins discordant for psoriasis [48]. So far, there is no information about methylation status of LINE-1 and Alu in psoriasis.

Therefore, we determined DNA methylation level and different methylation pattern of long interspersed element 1 s (LINE-1) and short interspersed element (SINE or Alu) in keratinocyte and various hematopoietic cells from psoriasis compared to normal controls, SCC and eczema by COBRA methods. The LINE-1 methylation patterns were classified to hypermethylated ($^{m}C^{m}C$), hypomethylated ($^{u}C^{u}C$) and 2 partially methylated loci ($^{m}C^{u}C$ and $^{u}C^{m}C$). Then, the association of LINE-1 methylation level and pattern were investigated with severity of psoriasis. The methylation status of different loci of LINE-1 was also studied. A receiver-operating characteristic (ROC) analysis was used to evaluate the detection of

sensitivity and specificity in each LINE-1 patterns. Moreover, we analyzed the correlation between LINE-1 and Alu methylation pattern according to demographic data, disease and clinical parameter of patients with psoriasis. The associations of LINE-1 methylation level and pattern with response to methotrexate treatment in psoriasis were also evaluated in all cell-types. Besides, we detected LINE-1 mRNA expression in psoriatic epidermis compared to normal epidermis.

By bioinformatics, we assessed the correlation between gene-containing LINE-1 or promoter methylated genes and differential expression in psoriasis expression array by the CU-DREAMX program. We also determined the correlation of differential expression in 2 expression microarrays such as GSE14905 and GSE13355 by CU-DREAM program.

Research question

Dose DNA hypomethylation at LINE-1 and Alu insertion sequences occur in chronic plaque-type psoriasis?

Hypothesis

DNA hypomethylation at LINE-1 and Alu insertion sequences occurs in chronic plaque-type psoriasis.

The objective of this thesis study was:

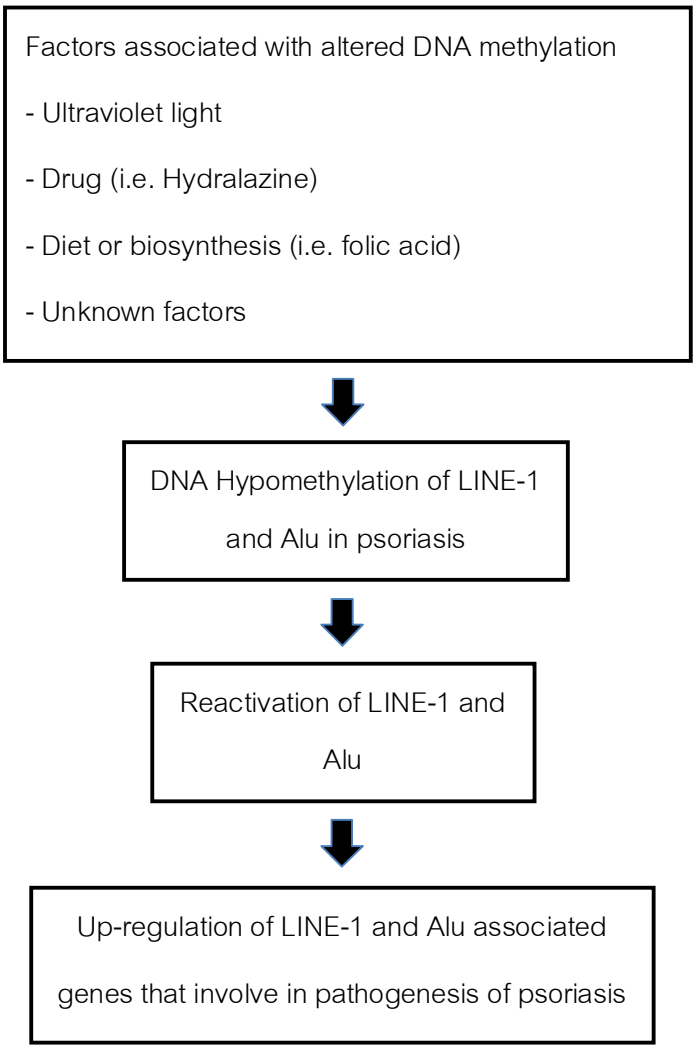
I. To investigate DNA methylation level and pattern at LINE-1 and Alu sequences in chronic plaque-type psoriasis.

II. To investigate the altered LINE-1 DNA methylation pattern affect clinical outcome of chronic plaque-type psoriasis.

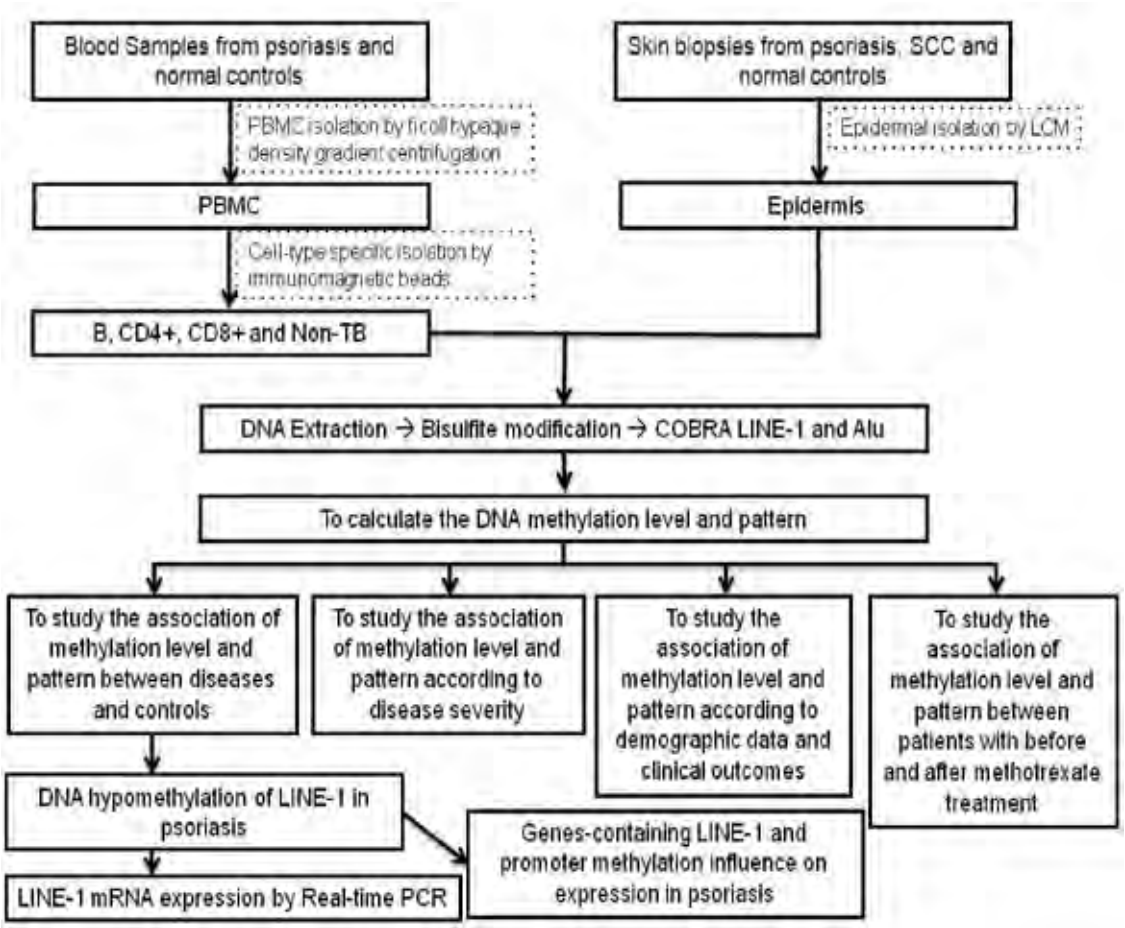
III. To investigate the gene-containing LINE-1 affects expression in chronic plaque-type psoriasis.

IV. To investigate the association of promoter methylation of LINE-1 containing gene and expression in chronic plaque-type psoriasis.

Conceptual framework



Research framework



CHAPTER II

LITERATURE REVIEW

Psoriasis

Psoriasis is a common chronic inflammatory autoimmune skin disorder that varies in age and onset of disease, severity, course, duration and clinical morphology from one individual to another. For many patients, the improvement of psoriasis symptoms is increased in the summer and worsens in the winter. In recent years, many reports have been made in elucidating the molecular mechanisms of psoriasis pathogenesis. However, the mechanisms of psoriasis remain unresolved, including nature of the disease such as epithelial and immune defect, inflammatory processes cause of the autoimmune disorder, systemic factors and the role of genetic and environmental factors influence on the disease pathogenesis and progression.

Clinically, it is characterized by raised, sharply demarcated, oval erythematous plaques covered with adherent silvery scales in different sizes and shapes. The disease affects skin, nails, mucous membranes and joints (Figure 1).

Histologically, it was considered to be a disease of keratinocyte hyperproliferation and parakeratotic differentiation. Incompletion occurs in squamous corneocytes. Hyper-proliferative epidermis with immaturity of keratinocytes is found in scales of psoriasis patients and abnormal keratinocyte cornification with retention of nuclei in the stratum corneum of skin termed as parakeratosis. The basal keratinocytes of psoriatic patients increase mitotic rate as compared with in normal epidermis. Mitotic activity of basal keratinocytes is increased by as much as a factor of 50 in skin of psoriasis. Consequently, the epidermal skin is thickened termed as acanthosis with elongation of rete ridges. Hence,

the characteristic scale or flakes of psoriasis lesion causes poorly adherent of stratum corneum. Infrequently, a few of small pustules can be identified in especially inflamed psoriasis lesions. The infiltration of inflammatory cells consists variously of dendritic cells, macrophages and T-lymphocytes in the dermal skin and neutrophils, with some T-lymphocytes in the epidermal skin. The CD3+ and CD8+ T lymphocytes are detected around blood capillaries of the epidermis and dermis. Furthermore, the CD11c+ dendritic cells are detected mostly within the upper layer of the dermal skin. The redness of psoriatic lesions is due to increase the numbers of tortuous blood capillaries (Figure 2 and 3) [49-54].

Figure 1: Clinical features of psoriasis [53, 55-57]. Sharply demarcated erythematous plaques covered with silvery scale in different sizes and shapes are hallmarks of psoriasis pathogenesis. There are tendency sites including the elbows, knees and sacral region. The lesions may cover the entirety of the skin. Moreover, the lesion may also affect nails and joints.

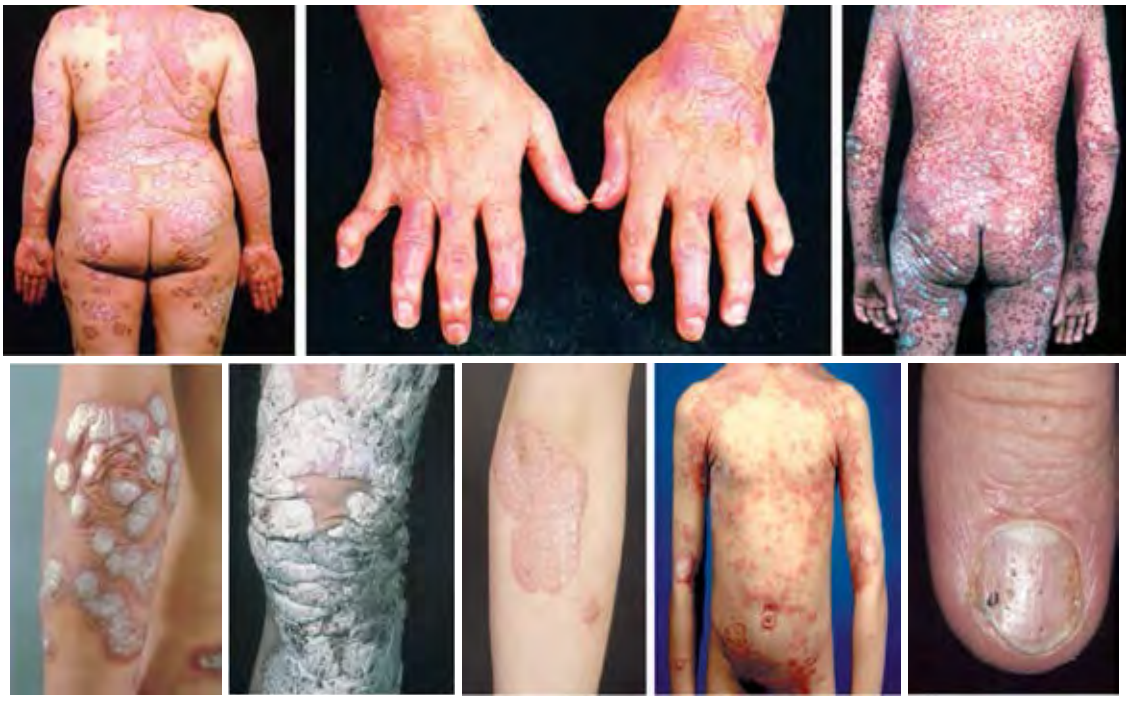


Figure 2: Histopathological features of psoriasis. (A) Normal skin, (B) psoriatic skin slough off (arrow) and Munro microabscesses are transported to the upper layers of the stratum corneum , (C) Histological hallmark of psoriasis, neutrophils transmigrate through the epidermis (Neutrophilic parakeratosis), (D) Thickening of the epidermis, parakeratosis, rete ridges elongation and a mixed immune infiltrate, (E) CD3+ T lymphocytes (3,3' –diaminobenzidine and hematoxylin), (F) CD8+ T lymphocytes (3,3' –diaminobenzidine and hematoxylin), (G) CD11c+ dendritic cells (3,3' –diaminobenzidine and hematoxylin) [53, 57].

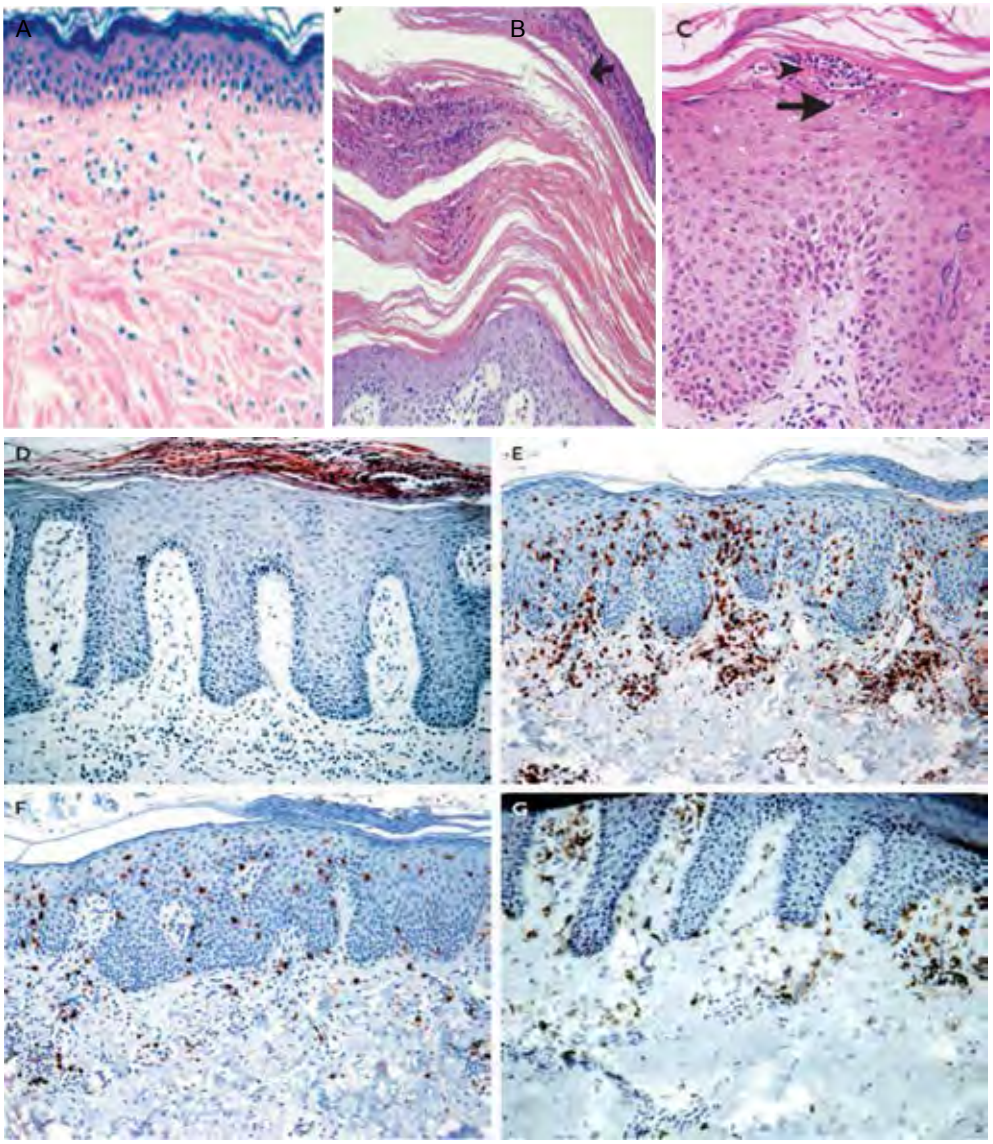
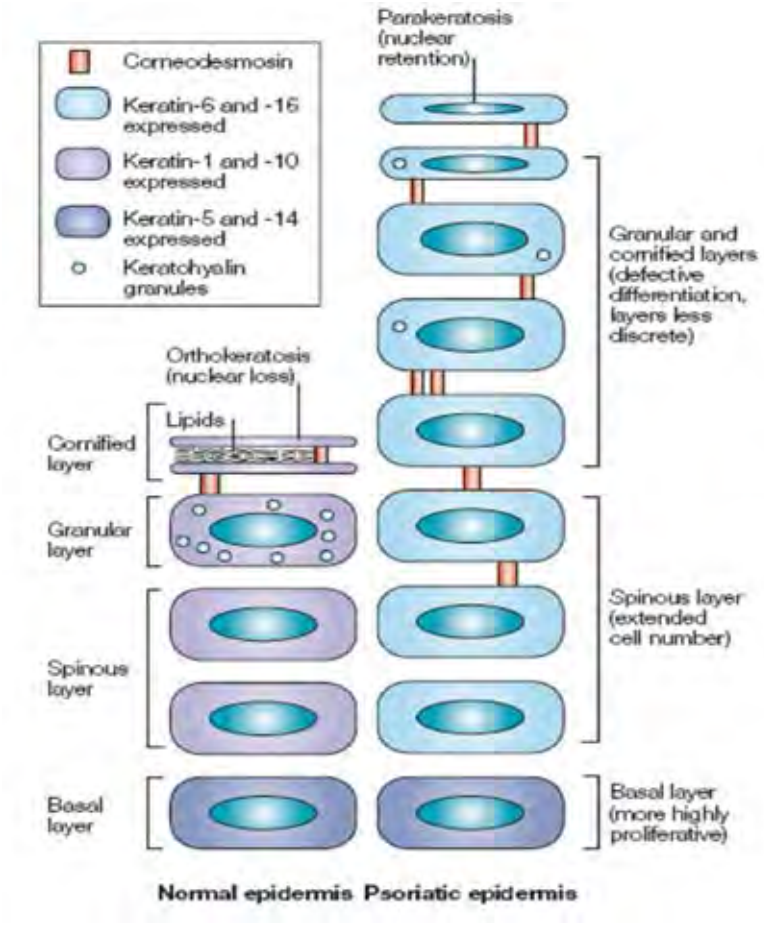


Figure 3: Differentiation of keratinocytes in psoriatic plaques compared with normal skin (or uninvolved skin of psoriasis) [51]



Epidemiology

Psoriasis is a worldwide disease. The prevalence rates of psoriasis are estimated around 2-3% of world's populations [55, 58]. Ethnic backgrounds and geographical areas also influence the prevalence of psoriasis [57, 59]. The annual incidence of psoriasis in USA was showed an age and sex adjusted incidence of 60.4/100 000 for Rochester, Minnesota. The prevalence was adjusted 3.2% in men and 2.5% in women. There is more prevalent in Caucasian populations (estimated prevalence about 1.5-3.0%), especially in

Northern European. Generally, psoriasis is more common in colder northern weathers than in tropical regions. Ethnic factors also influence the prevalence rate of psoriasis. The high prevalence rates of psoriasis has been reported 11.8% from Kazach'ye that locate in Arctic region of the former Soviet Union but the lowest prevalence rates has been accounted 0% from Samoa. The prevalence of psoriasis in America was estimated to be around 1.4-4.6% while in Canada it was to be 4.7%. The prevalence rate is 3.0-4.8% in Norway, 0.7% in American black and 0.7% in East Africa. In Asia, The population has the largest quantities with abundant races and sub-races. The prevalence rates are 0.5%-1.5% in India, 4-5.5% in Malaysia, 0.29-1.18% in Japan 3.1% in Kuwait, 0.4% in Henan district of China and 2% in Thai, respectively [54, 55, 59].

Classification of psoriasis

In textbooks, It is also classified by a major types of lesion into 2 groups including Non-pustular and Pustular psoriasis, as well as is categorized by a lesional morphology (table 1) [55]. The common clinical form of psoriasis is chronic plaque-type psoriasis or psoriasis vulgaris, which affects approximately 85% to 90% of all patients with psoriasis [52, 53].

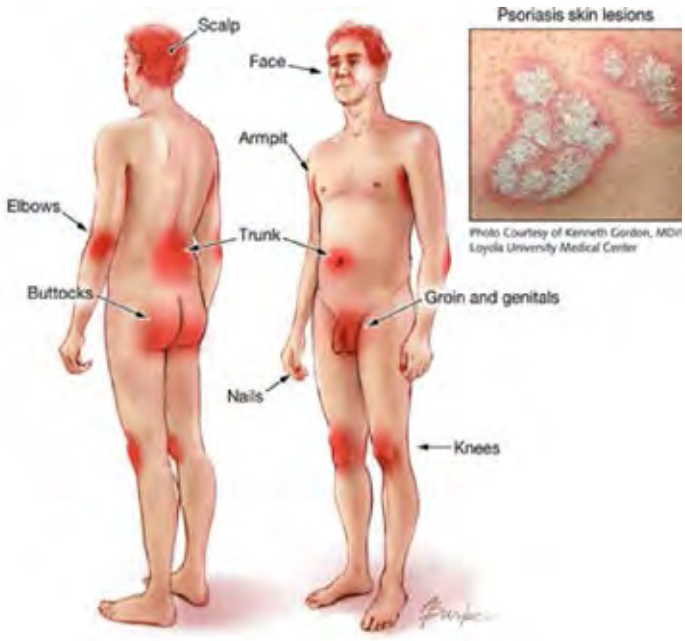
Table 1: Clinical forms of non-pustular and pustular psoriasis [55]

Non-pustular psoriasis	Pustular psoriasis
Type I, early onset	Generalized von Zumbusch type
Type II, late onset	Impetigo herpetiformis
Guttate psoriasis	Localized Palmo-plantar pustular psoriasis
Psoriatic erythroderma	Acrodermatitis continua
Drug-induced psoriasis	Annular pustular psoriasis

Chronic plaque psoriasis (Psoriasis vulgaris)

Chronic plaque-type psoriasis is the most common form of psoriasis that defined by well demarcated erythematous plaques, raised and covered by scaly skin lesion. The distribution is generally found at trunk, lower back, scalp, extensor surfaces (elbows and knees), surface of extremities and nails (Figure 4). The psoriatic nail changes comprise onycholysis (separation of the nail from its bed), pitting, oil spots (yellow and brown spots) and nail dystrophy. The psoriasis diagnosis typically is clinical, but infrequently a biopsy is necessary. Chronic plaque psoriasis typically is symmetric and bilateral. The lesional skins begin as papules and finally coalesce to plaque formation. Plaques reveal the Auspitz sign (bleeding after the removal of psoriatic scale) and the Koebner phenomenon (psoriatic lesions induced by trauma). Most patients with psoriasis are reported itching approximately 84% [52, 60, 61].

Figure 4: Common locations of chronic plaque-type psoriasis [62].



Psoriasis severity

Psoriasis Area and Severity Index is usually identified the severity of involvement, which takes into account the size of the involved area, redness, thickness and scaling [58]. The mild, moderate and severe of psoriasis is defined by scores of less than 10, 10 to 15 and greater than 15, respectively. A photograph of psoriasis patients who were defined by severity showed in Figure 5.

The calculation of Psoriasis Area and Severity Index (PASI)

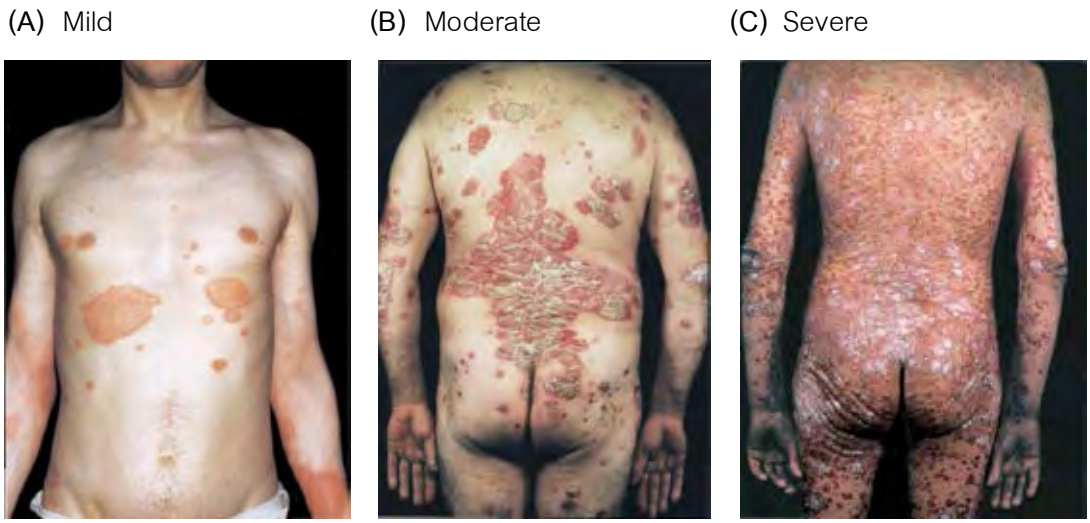
Method for calculating the Psoriasis Area and Severity Index (PASI), the original description of the PASI [63] which involves the assessment of erythema (E), infiltration (I), and desquamation (D), and body surface area involvement (A) are assessed on a scale of 0±4. And over 4 body regions (head (h), trunk (t), upper (u) and lower (l) extremities is given a numerical value from 0 to 6) (Table 2).

Table 2: The degree of severity and surface involvement

Degree of severity (per body region)	Value given	Surface involved (per body region)	Value given
No Symptoms	0	<10%	1
Slight	1	10-29%	2
Moderate	2	30-49%	3
Marked	3	50-69%	4
Very Marked	4	70-89%	5
		90-100%	6

For the reason that the head, upper extremities, trunk, and lower extremities correspond to approximately 10, 20, 30, and 40% of body surface area, respectively, the PASI score is calculated by the formula: PASI = 0.1(Eh + Ih +Dh)Ah + 0.2(Eu + Iu + Du)Au + 0.3(Et + It + Dt)At + 0.4(El +Il +Dl)Al [64]

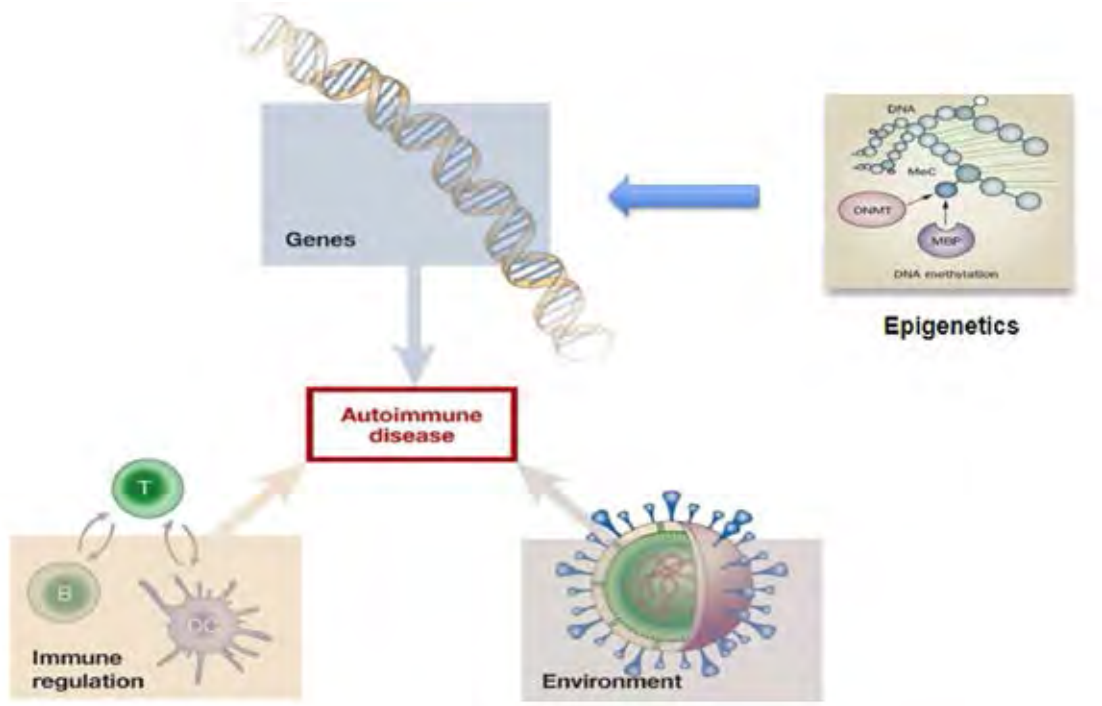
Figure 5: Photographs of patients with (A) Mild, (B) Moderate and (C) Severe psoriasis [58]



Etiology

Psoriasis describe as a complex and multifactorial autoimmune disease that is consistent with the involvement of multiple susceptibility genes, epigenetic dysregulation, immune disbalance as well as environmental risk factors [40-45, 50, 57, 65]. As shown in Figure 6.

Figure 6: Predisposing factors in psoriasis [66].



Evidence of a genetic predisposition to psoriasis

Plentiful evidence that psoriasis has an extraordinarily strong genetic trait comes from different population, family and twin studies, supporting the role of genetic component for susceptibility to psoriasis. The mainly evidence sustaining a genetic predisposition to psoriasis is supplied by a higher concordance rates in monozygotic twins than dizygotic

twins. Few studies of 61 twin pairs with at least one twin member affected, these studies establish a concordance rate of monozygotic twins 75% higher than that in 25% of dizygotic twins, 5-10 fold increased risks. Other information revealed that in twins who usually shared the same environments, the disease concordance rate is 15-23% for dizygotic twins and 72% for monozygotic twins. There 5-fold difference in the disease concordance rate between identical twins and fraternal twins [51]. These data support the role of genetics in psoriasis.

The degree of familial clustering, measured by comparing the risk of a sibling with the risk in the population as a whole(s), varies between 8-50% [57]. Several experiments have calculated the risk of sibling to develop psoriasis based on their family history. The risk is 14% if one parent was affected, 41% if both parents are affected, and 6% if one sibling affected (but not parents), compared to 2% when no parent or sibling is affected. In a great Swedish family data, the approximate risk of psoriasis was 28% if one parent was affected, 65% if both parents were affected and 24% if an affected sibling was present. Very interestingly, the offspring have a higher risk of increasing the disease and also might do at earlier age (genetic anticipation), when the father undergoes from psoriasis [67, 68].

The mode of inheritance of psoriasis is complex. The disease has been discovered in various pedigree analyses of psoriasis familial aggregation studies. Psoriasis is more prevalence among relatives of the affected patients. This information after studying large North Carolina kindred of British descent suggested a simple autosomal dominance pattern with reduced penetrance. Otherwise, some studies proposed a recessive mode of inheritance [67, 68]. Therefore, mode of inheritance of psoriasis is not followed by simple Mendelian inheritance patterns and has not been elucidate.

The mostly reported association has also been explained on the basis of genome-wide linkage studies. The genome-wide linkage analysis has identified at least nine

chromosomal loci with statistically significant linkage to psoriasis. These linkage loci are termed as psoriasis susceptibility 1 through 9 (*PSORS1-PSORS9*) [53]. *PSOR1* (6p) appears to be associated with up to 35-50% of psoriasis patients. Other susceptibility loci are located on chromosomes *PSOR2* (17q25), *PSOR3* (4q34), *PSOR4* (1q), *PSOR5* (3q21), *PSOR6* (19p13), *PSOR7* (1p) and *PSOR9* (4q31-4q34) (Table 3.1 and 3.2) [51, 53, 57].

Table 3: The Psoriasis Susceptibility Loci

Table 3.1: The PSORS loci reported to be associated with psoriasis [51, 53, 57]

PSORS locus*	Chromosome location	Associated gene‡	Study population
PSORS1§	6p21	MHC class I	Many
PSORS2	17q25	*SLC9A3R1* and *NAT9* *RAPTOR*	American American and British
PSORS3	4q	ND	–
PSORS4	1q21	Within EDC	Italian and American (unpublished)
PSORS5	3q21	*SLC12A8*	Swedish
PSORS6	19p13	ND	–
PSORS7	1p	ND	–
PSORS9	4q31–4q34	ND	–
PSORAS1	16q12	Possibly *NOD2*	–

*At least ten other linkages have also been reported, but these have not necessarily been replicated. ‡In many cases, the associated genes have not been identified or have not been confirmed in other populations. §Note that, in the case of the locus psoriasis susceptibility 1 (*PSORS1*), the predisposing gene has not been conclusively identified. EDC, epidermal differentiation complex; *NAT9*, *N*-acetyltransferase 9; ND, not determined; *NOD2*, nucleotide-binding oligomerization domain protein 2; *RAPTOR*, regulatory associated protein of mammalian target of rapamycin; *SLC9A3R1*, solute-carrier family 9 (sodium and hydrogen exchangers), isoform 3, regulator 1; *SLC12A8*, solute-carrier family 12 (potassium and chloride transporters), member 8.

Table 3.2: The major psoriatic gene variants and loci with independent replication [51, 53, 57]

Gene or Locus	Chromosomal Location	Odds Ratio for Disease	Comments	Other Disease Association	Reference
PSORS1	6p	6.4	Contains HLA-Cw6 (putative immune function) as major candidate gene and corneodesmosin	None	Trembath et al.,[9] Nair et al.,[9] Nair et al.[10]
PSORS2	17q	—	Putative role in immune synapse formation	None	Helms et al.[11]
IL12B	5q	1.4	T-cell differentiation	Crohn's disease	Cargill et al.,[12] Capon et al.,[13] Tsunemi et al.[14]
IL23R	1p	2.0	T-cell differentiation	Crohn's disease, ankylosing spondylitis, psoriatic arthritis	Nair et al.,[9] Cargill et al.,[12] Capon et al.,[13] Rahman et al.,[15] Rahman et al.,[16] Burton et al.[17]
ZNF313 (RNF114)	20q	1.25	Ubiquitin pathway	None	Nair et al.,[9] Capon et al.[13]
CDKAL1	6p	1.26	Unknown	Crohn's disease, type 2 diabetes mellitus	Wolf et al.,[19] Li et al.[20]
PTPN22	18p	1.3	T-cell signaling	Type 1 diabetes mellitus, juvenile idiopathic arthritis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune thyroid disease	Li et al.,[20] Hüffmeier et al.,[21] Smith et al.[22]
Interleukin-4–interleukin-13 cytokine-gene cluster	5q	1.27	T-cell differentiation	Crohn's disease (distinct variant)	Nair et al.,[9] Chang et al.[23]
LCE3B/3C	1q	1.31	Epidermal differentiation		de Cid et al.,[24] Zhang et al.[25]

The reported associations occupy a region in the major histocompatibility complex (MHC or HLA) region on the short arm (p) of chromosome 6p21.3 (Major susceptibility locus PSORS1) [68]. Many studies narrowed down in the area of MHC about 200 kb segment containing eight well-known genes, HLA-C, TCF19, OTF, HCR, CDSN, SEEK 1, SPR 1 and STG. The Three essentially candidate genes are HLA-Cw*0602 and the corneodesmosin (CDSN) gene as well as coiled-coil α-helical rod protein1 (HCR). HCR gene might negatively regulate the differentiation or proliferation of keratinocytes. Other genes included CDSN, a protein that is uniquely expressed in the granular and cornified layers of epidermis, are important for cell adhesions that are consecutively cleaved during skin desquamation [50, 51, 53].

Epigenetics

Epigenetics refer to an alteration in the genome, which has long-term effects on gene expression without changes in DNA sequence itself. The differentiation of cells are initiated and maintained by epigenetic mechanisms. Furthermore, epigenetic mechanism is found early during cellular development and differentiation [69, 70]. Epigenetic modifications of DNA molecule and chromatin have been known as important permissive and suppressive factors in regulating the expression of genome via transcriptional of gene (Figure 7). Recently, the importance of three major maternal epigenetic alterations is histone modification in chromatin, RNA-associated silencing as well as DNA methylation [71, 72].

Histone modifications in chromatin have been identified as epigenetic modifiers. Post translational modification of histones consist acethylation and methylation of lysine residues that is a conserved regions on the terminal tail domains. The acethylation of histones affect the transcriptional competent regions by activation to transcriptionally active chromatin, while hypoacethylated histones are initiated transcriptionally inactive chromatin or heterochromatic regions. Global histone H4 hypoacethylation in psoriatic PBMC has been reported [46, 72].

Epigenetically regulated by MicroRNAs, the role of RNA silencing has been associated with epigenetic controls by mitotically heritable transcriptional silencing, results in the formation of heterochromatin. Recent data showed how antisense transcription could lead to DNA methylation and stable silencing of a globin gene in a case of α-thalassaemia. Moreover, antisense RNAs are involved in the imprinted gene silencing [72].

Various evidences suggest a key important role for epigenetics in human pathologies such as inflammatory response and malignancies. Epigenetic dysregulation,

especially DNA methylation proposed as a potentially increasing in frequency of autoimmune and cancer (Table 4 and 5) [71].

Figure 7: Epigenetic regulation at human chromatin [73]. DNA strands are enfolded around histone octamers to form nucleosomes. Chromatin is organized by polynucleosome complex. (A) Histone is modified by various mechanisms including acetylation, methylation and phosphorylation. (B) The reversible changes in chromatin organization that alter gene expression by activation or silencing chromatin.

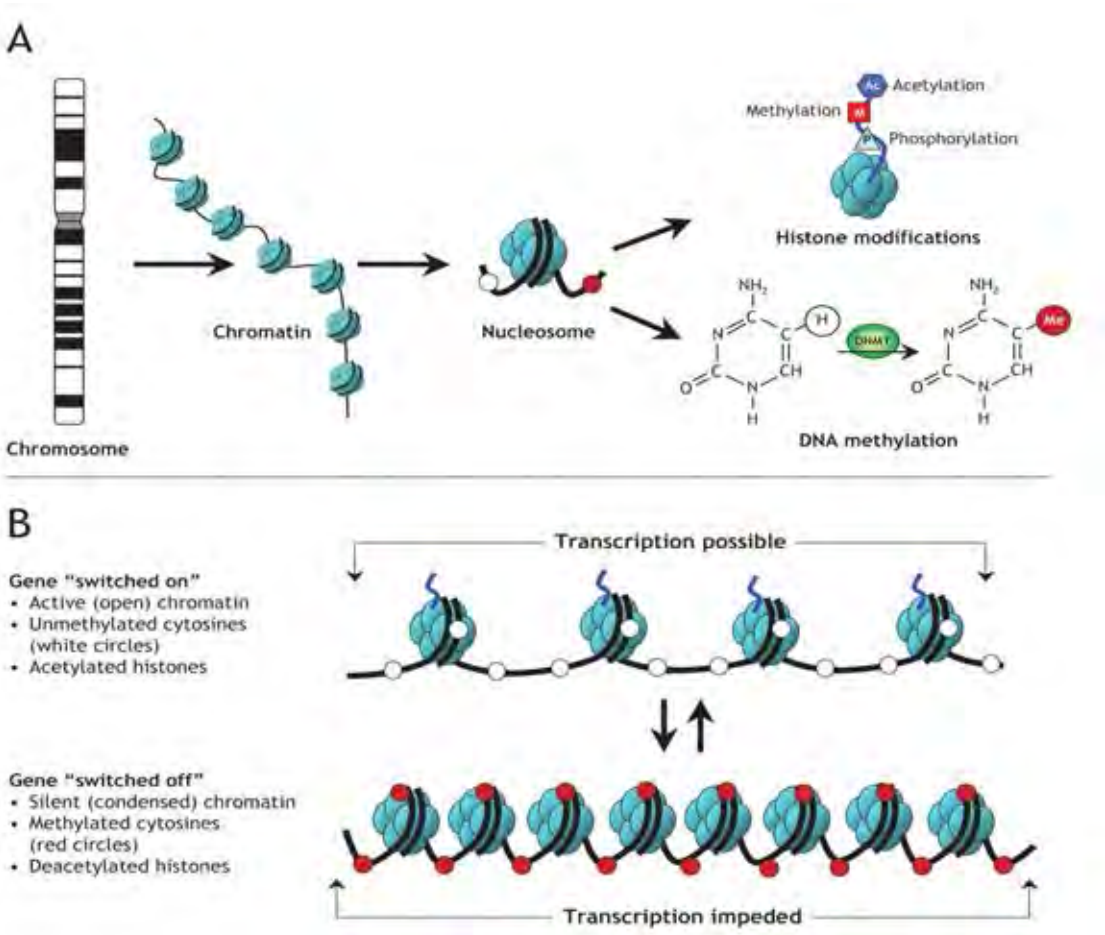


Table 4: Normal cellular functions regulated in part by epigenetic mechanisms and Molecular abnormalities caused by epigenetic errors [73]

Epigenetics and normal cellular functions	
Correct organization of chromatin	Controls active and inactive states of embryonic and somatic cells
Specific DNA methylation and histone modifications	Controls gene and tissue specific epigenetic patterns
Genomic imprinting	Is essential for development
X chromosome inactivation	Balances gene expression between males and females

Molecular abnormalities caused by epigenetic errors	
DNA hypermethylation	Results in chromatin condensation
DNA hypomethylation	Activates transposons
Mutations at methylated cytosines	Results in inappropriate gene expression
Imprinting defects	Results in loss of parental identity

Table 5: Associations between epigenetic modifications and human diseases [73]

Disease/condition	Gene	Biological process	Disease/condition	Gene	Biological process
Cancer			Neurologic		
Bladder	Multiple genes	Hypermethylation[20]	Schizophrenia	*RELN*	Hypermethylation[46,47]
Brain (glioma)	*RASSF1A*	Hypermethylation[28,29]	Bipolar disorder	*11p?*	Unknown[48]
Brain (glioblast)	*MGMT*	Hypermethylation[30]	Memory formation	Multiple genes	Hypo-, hypermethylation[49]
Breast	*BRCA1*	Hypermethylation[31]	Lupus	Retroviral DNA	Hypomethylation[50]
Breast	Multiple genes	Hypermethylation[32,33]	Cardiovascular		
Cervix	*p16*	Hypermethylation[34]	Atherosclerosis	Multiple genes	Hypo-, hypermethylation[19,51]
Colon	Multiple genes	Hypermethylation[20]	Homocysteinemia	Multiple genes	Hypomethylation[52]
Colorectal	L1 repeats	Hypomethylation[35]	Vascular endothelium	*eNOS*	Hypomethylation[53]
Esophagus	*CDH1*	Hypermethylation[20]	Imprinting and pediatric syndromes		
Head/neck	*p16, MGMT*	Hypermethylation[20]	PWS or AS	15q11-q13	Imprinting[54]
Kidney	*TIMP-3*	Hypermethylation[20]	BWS	11p15	Imprinting[55]
Leukemia	p15	Hypermethylation[20]	SRS	Chromosome 7	Imprinting[56]
Liver	Multiple genes	Hypermethylation[36]	UPD14	14q23-q32	Imprinting[57]
Lung	*p16, p73*	Hypermethylation[20]	PHP, AHO, MAS	20q13.2	Imprinting[58]
Lymphoma	*DAPK*	Hypermethylation[20]	Rett syndrome	*MECP2*	Mutation[59]
Myeloma	*DAPK*	Hypermethylation[37]	ICF syndrome	*DNMT3B*	Mutation[60]
Ovary	*BRCA1*	Hypermethylation[38]	ATRX	*ATRX*	Chromatin structure[61]
Ovary	*Sat2*	Hypomethylation[39]	FraX	Triplet repeat	Silencing[62]
Pancreas	*APC*	Hypermethylation[20]	FSHD	3.3 kb repeat	Chromatin structure[63]
Pancreas	Multiple genes	Hypomethylation[40]	Reproductive		
Prostate	*BRCA2*	Hypermethylation[20,41]	Ovarian teratoma	No paternal genome	Imprinting[64]
Rhabdomyosarcoma	*PAX3*	Hypermethylation[42]	CHM	No maternal genome	Imprinting[65]
Stomach	*Cyclin D2*	Hypomethylation[43]	BiCHM	Maternal genome	Imprinting[65]
Thymus	*POMC*	Hypomethylation[44]	Aging	Chromatin	Hypo-, hypermethylation[66]
Urothelial	Satellite DNA	Hypomethylation[45]			
Uterus	*hMLH1*	Hypermethylation[20]			

These data are revealed that DNA methylation play essential role in controlling the normal cellular function as well as caused diseases by epigenetic alterations.

DNA methylation

Chromosomal DNA is strongly associated with histone proteins and these interactions known as chromatin structure. DNA molecules can be modified through covalent addition of methyl groups by DNA methyltransferase enzymes. This regulation occurs at specific nucleotide 5' methyl cytosines or at CpG sites. In the genome, CpG-rich islands upstream from their transcriptional start site are found about 40% of genes and up to 80% of all CpG dinucleotides are methylated in the genome. The methylation likely silences by interfering transcription factors to the promoter region of the gene [71]. DNA methylation can silence transcription directly, by inhibition of specific transcription factor binding and indirectly, by recruiting methyl-CpG binding protein and their associated suppressive chromatin remodeling activities [74].

Several factors can effects on the expression of specific genes by epigenetic modification such as DNA methylation. Epigenetics is influenced by environmental factors such as cell-types, diets, medical treatments, severities and stage of diseases throughout living. For instance, folate is a crucial factor for the conversion of methionine to S-adenosylmethionine. Insufficiency in folate leads to genome-wide hypomethylation. Likewise, genomic DNA methyaltion is associated with methotrexate treatment, is a folate antagonist that has been used for treatment of inflammatory arthritis. Other nutritional components such as selenium, arsenic and polyphenols may also influence the DNA methylation status with potential effects for several diseases including malignancies and autoimmune disorder [71, 75].

Repetitive elements

In the human genome content, approximately 45% of the genome consists of transposable elements (TEs). The majority of TEs are non-long terminal repeat (LTR)

retrotransposons including long interspersed transposable elements (LINEs) and short interspersed transposable elements (SINEs or Alu) (Figure 8). DNA methylation occurs principally in repetitive elements of genomic regions such as long interspersed transposable elements (LINEs) and short interspersed transposable elements (SINEs) [74]. LINE-1s are broadly interspersed human retrotransposon sequences. In human genome consists of 17% of the human LINE-1s up to 600000 copies present. The human SINE-1 (Alu) is a non-autonomous LTR retrotransposon of about 80-400 bp that required activities of enzymatic assistance from LINE-1 or the host for their mobility. Alu contribute to approximately 11% of human genome with up to 1 million copies present. LINE-1 and Alu are the majority of an abundant human non-LTR retrotransposons [9, 76-78]. The structure of LINEs and SINEs (Alu) was showed in figure 9.

Figure 8: The genome content of transposable elements

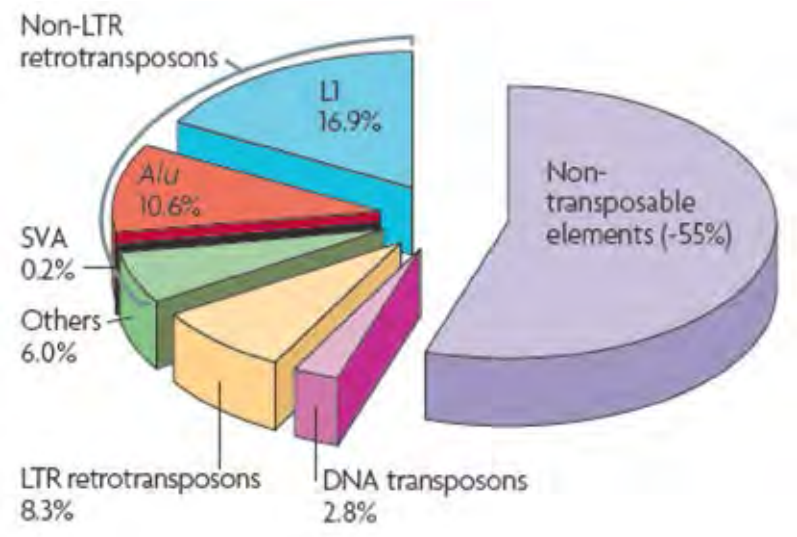
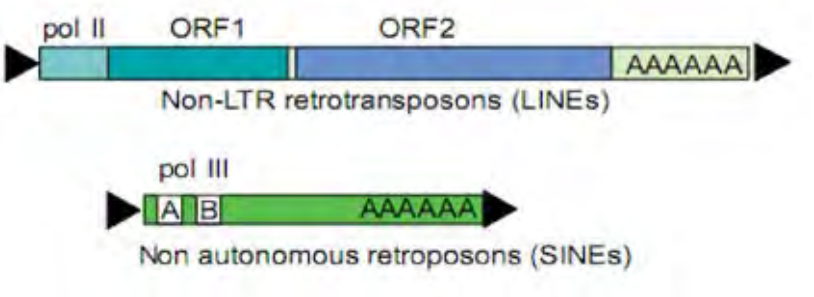


Figure 9: Structures of LINEs and SINEs [77]



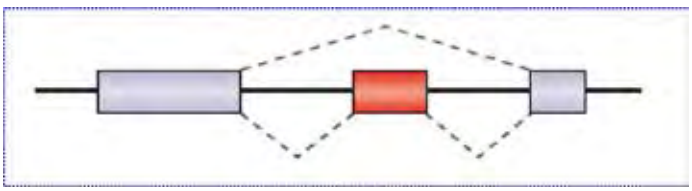
The impact of interspersed repetitive sequences on human transcriptomes

Repetitive sequences (LINE-1 and Alu) can integrate into the human genome and affect human DNA resulting in alters human gene transcription via various mechanisms such as Exonization or alternative splicing, altered transcription elongation, alternative promoter effect, gene expression modulation, RNA editing and epigenetic control [1].

Figure 10: The impact of repetitive sequences on human gene expression [1]. Repetitive sequence (Red box) and Exon (Grey box)

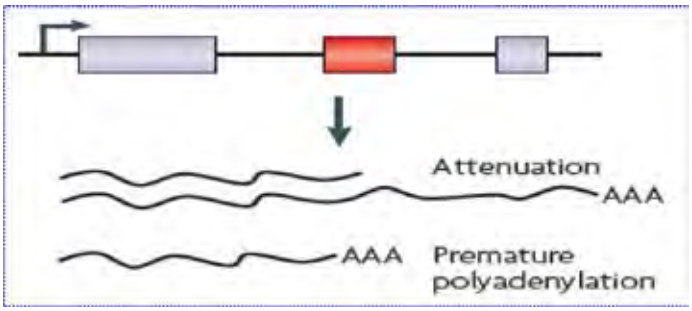
(A) Alternative splicing

This mechanism arises in 40-60% of genes in human genome. Repetitive sequences such as LINE-1 can integrate in the gene act as exons. A single gene produces variety types of mRNA and contributes to a variation of human proteome by promoting a new splice sites.



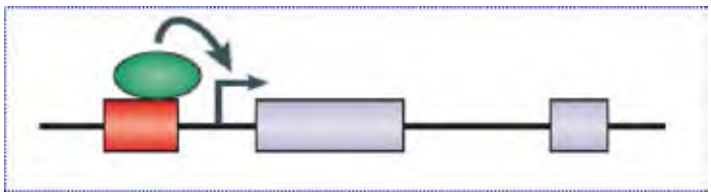
(B) Altered transcription elongation

The repetitive sequences such as LINE-1 affect genes by interfering with transcriptional elongation. It can attenuate the ability of transcriptional reading by promoting polyadenylation signals.



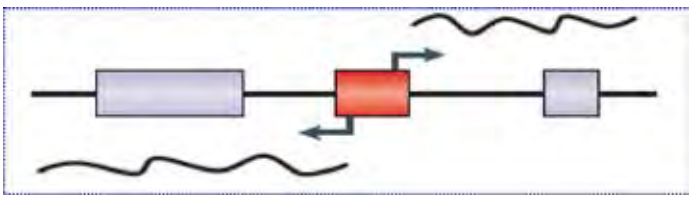
(C) Mudulation of expression in various genes

Some repetitive sequences such as Alu have transcriptional binding sites that might modify gene expression by modulation.



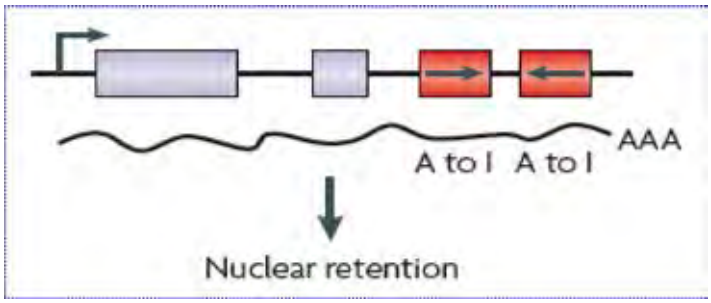
(D) Alternative promoter

LINE-1 and Alu promoter sequences have a function to initiate sense or antisense transcription through nearby genes.



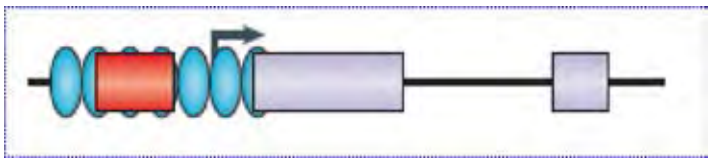
(E) RNA editing

The co- or post transcriptional modification of RNA sequences by conversion of adenosine (A) to inosine (I) is called RNA editing. This phenomenon is found in Alu sequences more than 90% of all A to I replacement. The RNA editing might influence the alternative splicing and also suppress transcription through nuclear retention.



(F) Epigenetic controls

Epigenetic regulation by DNA methylation control repetitive sequence (LINE-1 and Alu) activity via highly methylated at CpG sites.



DNA methylation on repetitive elements and human diseases

A relation between DNA methylation and cancer was first exhibited in 1983. It was shown that the cancerous genomes are hypomethylated relative to their normal counterparts. Hypomethylation of malignancies is due to the loss of DNA methylation from repetitive regions in the genome. Reactivation of the repetitive transposon promoters

following demethylation might also supply to aberrant gene regulation in cancer by transcriptional interference or the generation of antisense transcripts. For occurrence, repetitive element LINE-1 was showed global hypomethylation in oral rinses of oral squamous cell carcinoma patients [25]. Differential DNA hypermethylation and hypomethylation are independent processes and appear to play different roles in colorectal tumor progression [35]. Irreversible genome-wide DNA methylation is a key step in hepatocarcinogenesis and to promote clonal expansion of initiated cells induced by dietary methyl deficiency [79]. Moreover, the serum level of LINE-1 hypomethylation correlated with the presence of HBsAg, large tumor sizes, and advanced tumor stages for hepatocellular carcinoma [15]. Other data indicate that genome-wide DNA hypomethylation at LINE-1 correlated to an early change in urothelial carcinoma [80].

Hypomethylation of LINE-1 and Alu were found in well-differentiated neuroendocrine tumors (pancreatic endocrine tumors and carcinoid tumors). The tumor samples were less methylated of LINE-1 and Alu than normal tissue [27]. DNA repetitive elements (LINE1, Alu, Satellite-alpha and Satellite-2) were significantly more hypomethylated in chronic myeloid leukemia (CML) progression [28]. Long interspersed nuclear element 1 (LINE-1), Alu and satellite alpha (SAT-alpha) sequences has been associated with tumor progression in Multiple myeloma (MM) [81]. LINE-1 or Alu hypomethylation was discovered along the multistep hepatocarcinogenesis.

Moreover, Hypomethylation of LINE-1 but not Alu in lymphocyte subsets of systemic lupus erythematosus (SLE) patients has also been reported [26]. Several reports of DNA methylation at LINE-1 and Alu sequence were showed in Table 6 and 7.

Table 6: Several evidence of genome-wide LINE-1 methylation in human diseases [14, 15, 25, 80, 82]

Disease and condition	Gene	Biological process
Human ovarian carcinomas	LINE-1	Hypomethylation
Oral squamous cell carcinomas	LINE-1	Hypomethylation
Urothelial carcinomas	LINE-1	Hypomethylation
Hepatocellular carcinomas	LINE-1	Hypomethylation
Colorectal carcinomas	LINE-1	Hypomethylation

Table 7: Several evidence of ALU methylation in human diseases [27-29, 81]

Disease and condition	Gene	Biological process
Multiple Myeloma / tumor progression	LINE-1/Alu	Hypomethylation
Hepatocellular carcinomas	LINE-1/Alu	Hypomethylation
Chronic myeloid leukemia	LINE-1/Alu	Hypomethylation
Neuroendocrine tumors	LINE-1/Alu	Hypomethylation

DNA methylation and autoimmune diseases

Several studies have revealed the likely important of DNA methylation, especially hypomethylation, in systemic lupus erythematosus (SLE) pathogenesis [83, 84]. DNA hypomethylation is correlated with impaired T cell DNA methylation in systemic lupus erythematosus [85]. $CD4^+$ T lymphocyte DNA from subcutaneous lupus erythematosus was hypomethylated comparative to healthy volunteers [86]. In addition, DNA hypomethylation is important to initiate apoptotic DNA to induce SLE-like autoimmune disease in non-susceptible mice, which may provide in elucidating the SLE pathogenesis [87].

Moreover, some data shown genome-wide DNA hypomethylation in inflammatory arthritis [75]. Other studies showed predominantly methylation at CpG in the IL16 promoter region may influence IL6 gene regulation and involved in rheumatoid arthritis pathogenesis [88]. Furthermore, preceding study found that inflammatory arthritis is associated with genomic DNA hypomethylation and reversed with methotrexate [75].

DNA methylation and psoriasis

Previous studies shown as demethylation at SHP-1 promoter correlated with up-regulation of SHP-1 mRNA in psoriasis [42]. Moreover, down-regulation of inhibitor of differentiation 4 is associated with promoter methylation in psoriasis parakeratosis [44]. Other studies have been established that DNA demethylated at p16 and p16INK4a gene correlated with up-regulated mRNA transcriptional levels. It has been suggested that plaque formation is linked to keratinocytes that have unsuccessful to undergo programmed cell death and a state of arrested senescence. Likewise, Abnormal expression of $p16^{INK4a}$, an anti-apoptotic protein is demonstrated in psoriasis [40, 41, 43]. Moreover, significantly loss of positive frequencies of promoter methylation and elevated transcription level for p15 and p21, cell cycle proteins, genes were discovered in psoriasis [65]. DNA demethylation has been found to associate with skin pathogenesis, particularly in psoriasis. The promoter demethylation may play an essential role in skin disorder. However, there is no any information about DNA methylation study on repetitive elements in chronic plaque-type psoriasis.

Immunopathologic features of psoriasis

The epidermal changes in lesion of psoriatic skin occur in response to cellular immunity. Currently, psoriasis is a T-lymphocyte-mediated dermal immune response with T-type 1 profiles including Interferon-γ (IFN-γ), Tumor necrosis factor-α (TNF-α), Interleukin-2 (IL-2), Interleukin-12 (IL-12), Interleukin-22 (IL-22) and Interleukin-23 (IL-23). Recently, the study of psoriasis focused on interleukin-17, producing by type 17 helper T lymphocytes (Th17). This cell-type is specialized in epithelial immunosurveillance. It also secretes interleukin-22, a key cytokine play important role in adaptive immune effectors and epithelial dysregulation in psoriasis [49-53].

In psoriatic lesions, there is a mixture of innate immune cells (neutrophils, dendritic antigen-presenting (APCs) cells and natural killer T (NKT) cells), adaptive immune cells (T-cells) and inflammatory infiltrates. $CD4^+$ and $CD8^+$ T-lymphocytes are present in psoriatic skin while $CD4^+$ T-lymphocytes are present generally in the dermis. The mature peripheral T-lymphocytes that present in psoriatic skin lesions are skin-homing memory T-cells. The specific antigen of skin T-lymphocytes has not been identified. But, the possibility of antigen recognition consists of self-epidermal antigens or even keratinocyte (KC)-derived polypeptides, those derived from microbial antigens or superantigens [56, 89-91].

Immunopathogenesis and cytokine network of psoriasis, environmental and genetic factors initiate the disease occurrence. A cascade of events is started that include the formation of LL-37-DNA complexes, plasmacytoid dendritric cell activation and secretion of IFN-α. The key cytokines are produced by innate immune cells such as tumor necrosis factor α (TNF-α), interferon-α, interferon-γ, interleukin-1β, and interleukin-6 also activate plasmacytoid dendritic cells. Activated myeloid dendritic cells transmigrate to draining lymph nodes, presenting antigens and secreting interleukin-12 and interleukin-23 that stimulate T cell differentiation such as type 17 and type 1 helper T cell (Th17 and Th1) and

type 17 and type 1 cytotoxic T cell (Tc17 and Tc1). Key processes during the disease maintenance are the presentation of putative autoantigens to T cells and secretion of interleukin-23 by dermal dendritic cells. Activated T cells produce cytokines including interleukin-17A, interleukin-17F and interleukin-22 that can activate keratinocyte proliferation and influence the secretion of antimicrobial peptides (LL-37 cathelicidin and β-defensins), proinflammatory cytokines (TNF-α, interleukin-1β and interleukin-6), chemokines (CXCL8-11 and CCL20) and S100 proteins. These mediators can drive proimflammatory processes by feedback loops to inflammatory cycles (Figure 11 and 12) [53].

Figure 11: Key immune cells and mediators in the transition from innate to adaptive immunity in psoriasis [53]

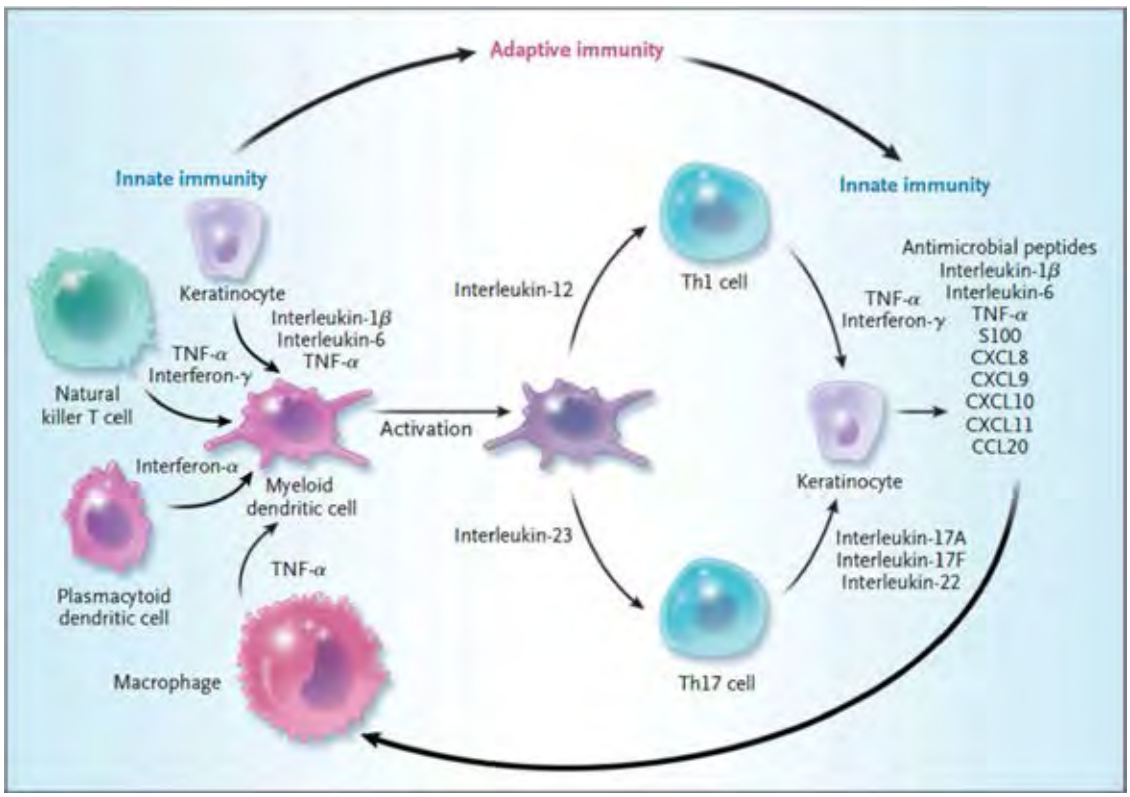
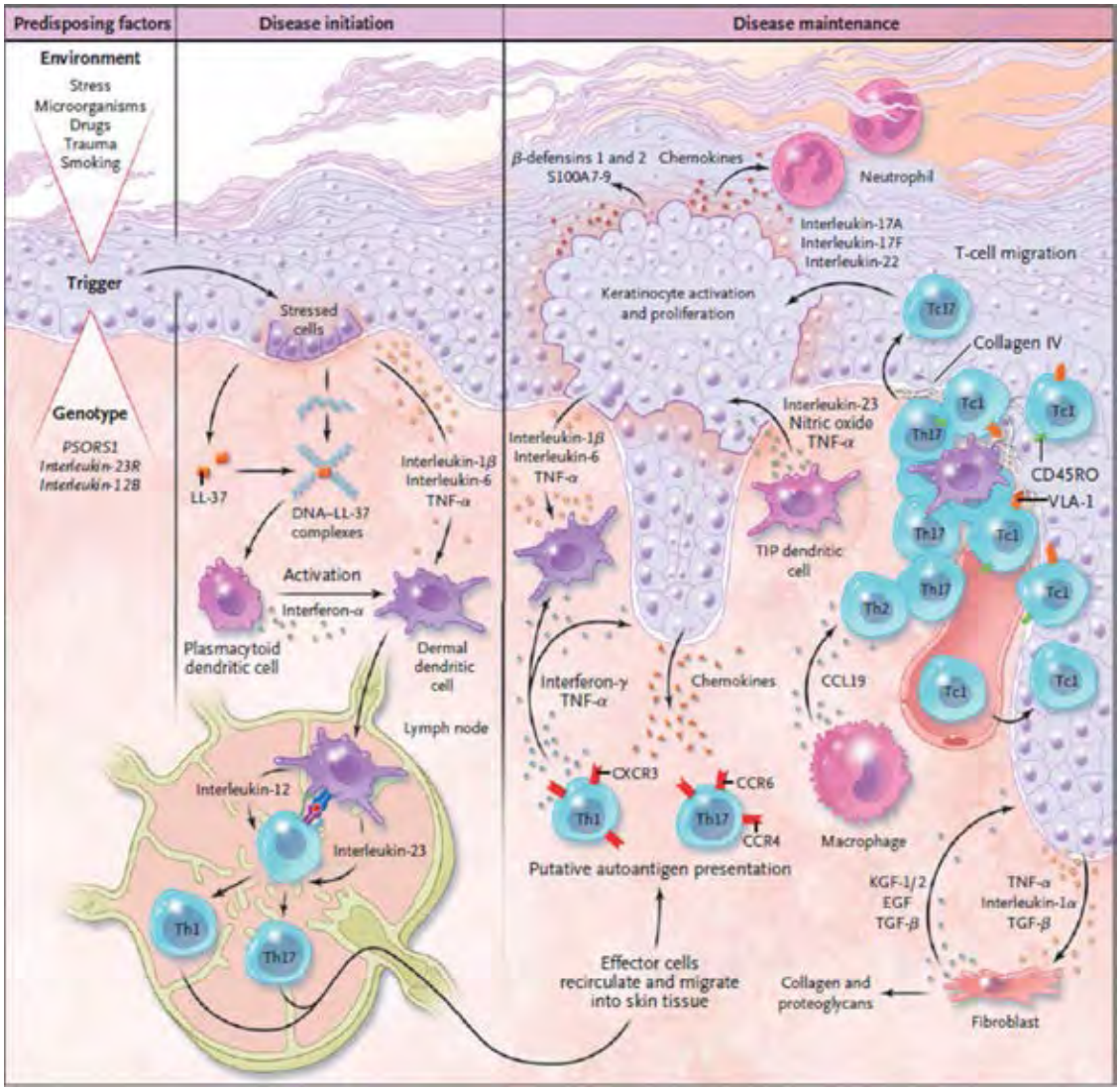


Figure 12: The schema of the evolution of a psoriatic lesion from beginning to maintenance of disease [53].



Environmental factors

Psoriasis pathogenesis is influenced by various environmental factors such as stress, infections, drugs, smoking and trauma. Physical trauma may trigger to psoriasis at the sites of injury called Koebner's phenomenon. Some treatments of psoriasis also trigger psoriasis if used too high doses because they have proimflammatory potential such as anthralin and phototherapy. Beta-adrenergic blockers may influence epidermal hyperproliferation. Lithium may increase proinflammatory cytokines, thereby inducing leukocyte recruitment in skin. And chloroquine blocks transglutaminase in epidermal skin that affects the terminal differentiation of keratinocytes. For infections by human immunodeficiency virus (HIV) or Group A streptococcus, predominantly streptococcal infections may also trigger psoriasis pathogenesis [57].

CHAPTER III

MATERIALS AND METHODS

Subjects

Patients and healthy controls

White blood cells of 36 subjects (20 healthy volunteers and 16 patients with chronic plaque-type psoriasis) and skin tissues biopsies of 44 subjects (13 healthy volunteers, 13 patients with chronic plaque psoriasis, 10 patients with eczema, and 8 patients with squamous cell carcinoma (SCC)) were registered in the studied. All of patients with psoriasis, eczema, and SCC were recruited from King Chulalongkorn Memorial Hospital. All patients included in the study were diagnosed as chronic plaque type psoriasis, eczema, and SCC by a dermatologist at King Chulalongkorn Memorial Hospital. Patients with psoriatic arthritis or other autoimmune diseases were excluded from the study. All patients were free from systemic skin therapies for at least 4 weeks or topical skin therapies for at least 2 weeks prior to sample collection. Each psoriatic patient was classified by the Psoriasis area and severity index (PASI) (<15= mild and $\geq$ 15= severe). Thirty-three healthy volunteers with no history of autoimmune disease and cancer served as normal controls. Normal skin was recruited from elective plastic surgery. Normal skin was recruited from healthy subjects from elective plastic surgery cases at King Chulalongkorn Memorial Hospital. The ethical committee of the King Chulalongkorn University approved the study and the subjects gave informed consent. The Institutional Review Board of Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand approved the trial. The demographic data was shown in Table 8.

Method for calculating the Psoriasis Area and Severity Index (PASI), the original description of the PASI [63]. The formula of PASI score was explaned in the part of the calculation of Psoriasis Area and Severity Index (PASI) in literature review (Table 2).

Patient criteria

Inclusion criteria:

- All patients included in the study were diagnosed as chronic plaque-type psoriasis.
- All patients included both male and female.
- All patients included Thai or Thai Chinese.
- All patients included all severity.
- All patients included all age.

Exclusion criteria:

- All patients were free from systemic skin therapies for at least 4 weeks or topical skin therapies for at least 2 weeks prior to sample collection.
- All patients with psoriatic arthritis or other autoimmune diseases were excluded from the study.
- All patients with malignancies were excluded from the study.

Sample sizes

We calculated a sample sizes (two independent groups) by this formula for DNA methylation study.

$$N/group = 2(Z_{\alpha/2} + Z_{\beta})^2 \sigma^2 / (\overline{x}_1 - \overline{x}_2)^2$$

$$\sigma^2 = \text{Pooled variance} = \frac{(n_1 - 1) S_1^2 + (n_2 - 1) S_2^2}{n_1 + n_2 - 2}$$

$\alpha = 0.05$, $\beta = 0.10$

$Z_{\alpha/2} = Z_{0.05/2} = 1.96$ (two tail)

$Z_{\beta} = Z_{0.10} = 1.28$

Skin biopsy

Normal tissues $n_1 = 6$

Psoriasis tissue $n_2 = 8$

n_1 mean ± SD = 39.84 ± 1.51

n_2 mean ± SD = 33.23 ± 3.12

$\sigma^2 = (15.96 + 68.11)/ 12 = 7$

N/group = $(2 \times (3.24^2) \times 7)/ 6.61^2$

= $(2 \times 10.50 \times 7)/ 43.69$

= 147/43.69 = 3.36 ~ 4 samples/group

White blood cells

Normal White Blood Cells $n_1 = 6$

Psoriasis White Blood Cells $n_2 = 13$

n_1 mean ± SD = 40.79 ± 1.55

n_2 mean ± SD = 38.09 ± 2.78

$\sigma^2 = (92.76 + 12)/ 17 = 6.16$

N/group = $(2 \times (3.24^2) \times 6.16)/ 7.29$

= $(2 \times 10.50 \times 6.16)/ 7.29$

= 129.36/7.29 = 17.74 ~ 18 samples/group

Table 8: Demographic data of the participants

Sample group	Sex (Male/Female)	Age (Mean ± SD) Year	Onset (Mean ± SD) Year	Disease duration (Mean ± SD) Year	WBC (Mean ± SD) Cells/mm^3	% Lymphocyte (Mean ± SD)	% Neutrophil (Mean ± SD)	Nail involvement (Involved/non-involved)
Normal controls (n = 33)	8/25	36.21 ± 13.33						
Squamous cell carcinoma (n = 8)	3/5	53.83 ± 20.77						
Psoriasis patients (n = 29)	16/13	38.93 ± 12.85	30.69 ±11.77	8.38 ± 9.33	8685.00 ± 2875.58	25.63 ± 8.32	64.15 ± 10.11	17/12

P-value = 0.010 (Age of normal controls compared to SCC)

No association between normal controls compared to psoriasis patients

Cell isolation

The epidermal skin from paraffin-embedded and frozen tissues of 13 normal subjects, 13 psoriatic patients, 10 eczema, and 8 squamous cell carcinomas (SCC) were isolated using The PALM Micro Laser Microdissection System (P.A.L.M. MicroLaser Technologies AG, Burnried, Germany) (Figure 13). All specimens were cut into 4-µm-thick sections on glass slides. The principle of the PALM Microsystems is based on a cutter pulsed by UVA laser that is focused through the microscope to allow laser ablation of cells and tissue on a tissue section. The area of Munro's microabscess and psoriatic dermis was eliminated. Briefly, a microcentrifuge cap moistened with lysis buffer was mounted upside down just above the tissue section. Area of interest was performed by cutting with a fine focused laser beam and cutter pulse them into the microcentrifuge cap. Cells were

collected for each sample approximately 8-10 x 10^6 μm^2. After that, lysis buffer was added into the microcentrifuge tube, vortexed briefly, and centrifuged for 5 min to spin down cells from the lid to later perform DNA or RNA extraction or keep at 80 °C until procedure. The schematic of dissected tissue was revealed before and after catapulting (Figure 14).

Figure 13: PALM Micro Laser Microdissection

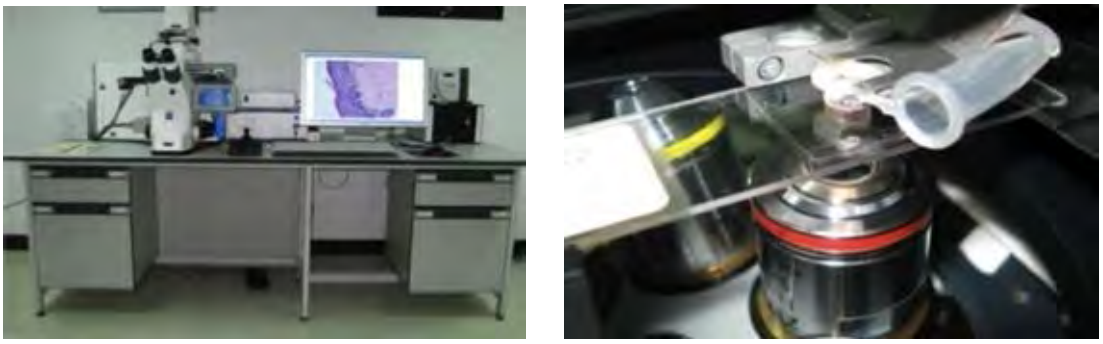
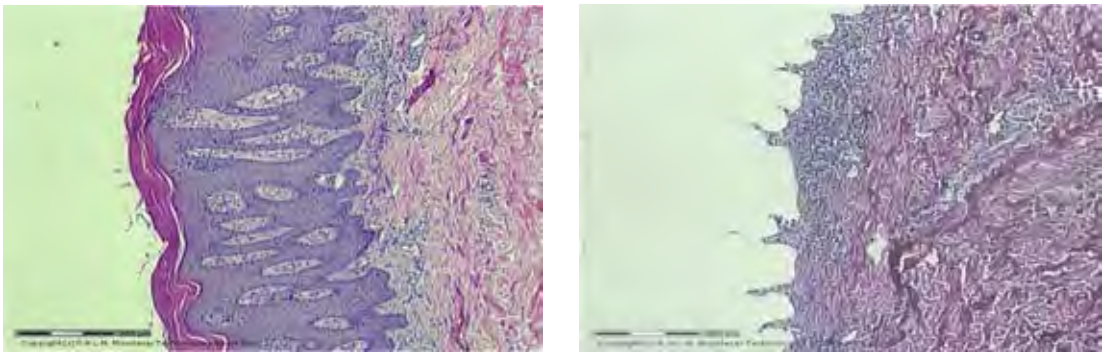


Figure 14: Microdissected tissue, (A) Tissue before section (B) Tissue after section.

(A) Before section **(B) After section**



PBMC were isolated from heparinized venous blood by gradient centrifugation using Ficoll-Hypaque (Amersham-Pharmacia Biotech, Uppsala, Sweden). In brief, the collected heparinized blood was diluted in RPMI by ratio 1:1. The diluted blood was layered on Ficoll-Hypaque. The mononuclear cell fraction was obtained from gradient centrifugation, which was collected and washed twice with RPMI. PBMCs were counted using a hemacytometer. Then, various populations of cell types (B cell, T cell and non-T non-B cell) were isolated from PBMCs by antibody-coated magnetic beads (Dynal Biotech; Invitrogen). First, B cells were isolated from PBMCs by positive selection using Dynabeads CD19 (Pan B) (Dynal Biotech; Invitrogen). After B cells were collected. Dynal T cell negative Isolation Kit Ver II (Dynal Biotech; Invitrogen) was used to isolate T cell from negative flow through by negative selection. Dynal CD4 Positive Isolation Kit (Dynal Biotech; Invitrogen) was used to isolate CD4+ T cells from the second negative flow through by positive selection. Cells from the third negative flow through, non-CD4+ T cells were collected. The purity of the difference cell types was verified by flow cytometry. All cell-types were stored at -80 °C until procedure. For RNA preparation, all cells were stabilized and protected in RNA later and keep at 80 °C.

DNA preparation and Bisulfite-modification

Heparinized blood samples were collected. All white blood cells and keratinocytes were isolated as previous described. All samples were extracted DNA using 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. Briefly, 20 µl of 500 ng of DNA was dissolved in 130 µl of the reagent mixture of bisulfite solution and then was performed in thermal cycler under the following conditions: 98 °C for 10 min, 64 °C for 2.5 hours, and 4 °C storage up to 20 hours. The

Zymo-Spin™ IC Column was used to isolate the bisulfite-treated DNA. The DNA was eluted with 10 µl of M-Elution Buffer. The bisulfite-treated DNA samples were stored at -20°C until procedure.

Combined Bisulfite -Restriction analysis (COBRA) LINE-1 and Alu

DNA methylation of LINE-1 and Alu repetitive element was performed by the Combined Bisulfite -Restriction analysis (COBRA) of interspersed repetitive elements as previous described [12, 38, 92, 93]. To compare the methylation of LINE-1 and Alu repetitive elements, bisulfited DNA were amplified by using primers sequence as follows: LINE-1 (GenBank: M80343) forward 5'- ccg taa ggg gtt agg gag tttt -3' and LINE-1 reward 5'- (ag)ta aaa ccc tcc (ag)aa cca aat ata aa -3'. The Alu sequence primers, which related to the nucleotides of the Alu Sx subfamily sequence [92]. Alu forward 5'- gg(t/c) g(c/t)g gtg gtt ta(c/t) gtt tgt aa -3' and Alu reward 5'- cac cat att aac caa act aat ccc ga -3'. For LINE-1 COBRA, 2µL of modified DNA was amplified by the condition of Hot-start PCR was carried out as follows. After a hot start at 95 °C for 15 min, the cycling parameters were: 95 °C for 45 sec, 50 °C for 45 sec, and 72 °C for 45 sec for 30 cycles and final extension at 72 °C for 7 min. For Alu COBRA, 2µL of modified DNA was amplified by the condition of Hot-start PCR was carried out as follows. After a hot start at 95 °C for 15 min, the cycling parameters were: 95 °C for 1 min, 50 °C for 1 min, and 72 °C for 1 min for 35 cycles and final extension at 72 °C for 7 min. After amplification, 8µL of PCR products (160bp in length) were digested with the restriction enzyme TaqI and TasI (MBI Fermentas). Each reaction will be incubated overnight at 65 °C and then DNA fragments will be separated in 8% polyacrylamide gels. Then, the gel was stained using the SYBR green nucleic-acid stain. The intensity of DNA fragments was measured with PhosphoImagerI using Image Quant software (Molecular Dynamics). COBRA LINE-1 and Alu amplicons were 160 and 99

bp, respectively. After digestion with restriction enzyme TaqI and TasI (MBI Fermentas), the LINE-1 and Alu methylated bands were 80 and 57 bp, respectively. The unmethylated bands of LINE-1 were 98 and 62 bp, and the unmethylated band of Alu was 78 bp. For interassay-variation normalization, we used DNA templates from HeLa, Jurkat and Daudi cell line as positive controls in all experiment. For Alu, after digestion with restriction enzyme TaqI and TasI (MBI fermentas), the Alu methylated and unmethylated bands were 57 bp and 78 bp. The %intensity of Alu methylated band were represented %methylation of Alu.

LINE-1 methylation analysis

As identified by COBRA, LINE-1 loci were categorized into four groups based on the status of methylation at 2 CpG dinucleotides in the 5' and 3' of the sequence. These four groups consisted of: (1) 2 unmethylated CpGs ($^{u}C^{u}C$) at LINE-1 loci; (2) 2 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. We calculate the LINE-1 methylation level and percentage of LINE-1 loci from each group by using the percentage of intensity of COBRA-digested LINE-1 products. Phosphorimager and the ImageQuant Software (Molecular Dynamics, GE Healthcare, Slough, UK) were used to quantitate the intensities of the COBRA-LINE-1 bands. DNA fragments from enzymatic digestion for COBRA LINE-1 were separated into 5 fragments including 160, 98, 80, 62 and 18 bp. $^{m}C^{u}C$ was represented the methylation state of the 160 bp fragments. $^{u}C^{u}C$ was represented the methylation state of the 98 bp fragment. $^{m}C^{m}C$ and $^{u}C^{m}C$ mixture was represented the methylation states of the 80 bp fragments. Eventually, $^{u}C^{u}C$ and $^{u}C^{m}C$ mixture was represented the methylation states of the 62 bp fragments. The number of CpG dinucleotides was calculated from the band intensity and dividing the intensity of each

band by the number of double-stranded bp of DNA sequence 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. After calculating the number of CpG dinucleotides, LINE-1 methylation levels were calculated according to the following formulas: LINE-1 methylation level percentage = 100×(C+A)/(C+A+A+B+D); percentage of $^{m}C^{u}C$ loci ($\%^{m}C^{u}C$) = 100×(A)/(((C-D+B)/2)+A+D); percentage of $^{u}C^{m}C$ loci ($\%^{u}C^{m}C$) = 100×(D-B)/((C-D+B)/2)+A+D; percentage of $^{u}C^{u}C$ loci ($\%^{u}C^{u}C$) = 100×B/(((C-D+B)/2)+A+D); and percentage of $^{m}C^{m}C$ loci ($\%^{m}C^{m}C$) = 100×((C-D+B)/2)/(((C- D+B)/2)+D+A) [38, 94].

5' LINE-1 real-time quantitative polymerase chain reaction (5' LINE-1 real-time PCR)

Total RNA was extracted from keratinocytes of psoriasis and healthy volunteers by using the Trizol reagent (Life technologies, Inc.). RNase-free DNase (Promega, Southampton, UK) following the manufacturer's instructions was used to treat DNA contamination. Subsequently, all treated samples were reverse transcribed into cDNA by AMV Reverse Transcriptase (AMV RT) (Promega Corporation). 5' LINE-1 cDNA was amplified by using primers as forward primer; 5'- ggc cag tgt gtg tgc gca ccg -3' and reverse primer, 5'- cca ggt gtg gga tat agt ctc -3'. 3' LINE-1 cDNA was amplified by using primers as forward primer; 5'- cag gaa ggg gaa tat cac actc -3' and reverse primer, 5'- tgc gct gca ccc act aac tc -3'. The 5' and 3' LINE-1 mRNA levels was quantitated relative to 18S rRNA transcripts. Real-time RT-PCR was performed in a Light Cycler machine (Roche Molecular Biochemicals, Indianapolis, IN, USA) using QuantiTect SYBR Green I (Qiagen, Hilden, Germany), according to the manufacturer's instructions.

Differential expression of genes in psoriasis keratinocytes

Connection Up- or Down- Regulation Expression Analysis of Microarrays (CU-DREAM) was used to identify differentially expressed genes in keratinocyte of psoriasis patients [8, 95]. The up- or down regulated genes were identified by fold change of genes between the two expression microarray data available from Gene Expression Omnibus (GEO), a public repository that archives and freely distributes microarray data submitted by the scientific community [96, 97]. The differential expression genes from comparing two expression microarray data were classified as up- or down-regulated and not up- or not down-regulated genes. The chi-square test was used to compare the numbers of genes in each subcategory as shown in table 9. The specific association between two independent studies than random chance was indicated by significant *P*-value. The odds ratios > 1 reveals the regulated genes in one study were also regulated in another study. This result established that both of studies involved a common mechanism of gene regulation. In a different way, odds ratios < 1 indicate that the event of interest (up or down) inhibit the mechanism (s) that altered gene expression in another experiment [95].

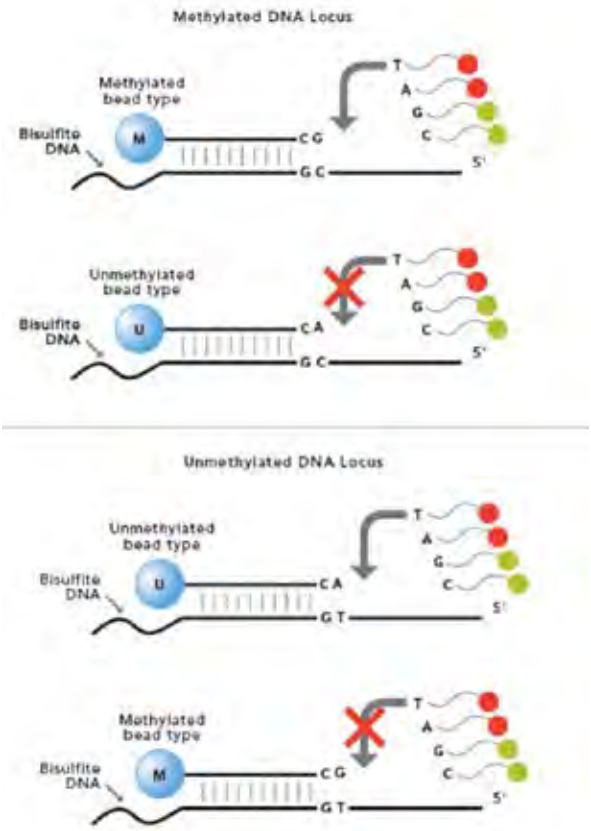
Table 9: 2 x 2 table of chi-square test

	Up- or Down-regulated genes of microarray experiment 1	Not up- or Not down-regulated genes of microarray experiment 1
Up- or Down-regulated genes of microarray experiment 2	Numbers of genes that regulated in both experiments	Numbers of genes that not regulated only in microarray experiment
Not up- or Not down-regulated genes of microarray experiment 2	Numbers of genes that not regulated only in RNA-Seq experiment	Numbers of genes not regulated in both experiments

Methylation microarray

Ruchusatsawat, K. et al. investigated the DNA methylation at CpG site of gene promoters in the genome by Infinium HumanMethylation27 BeadChip Kit. In Infinium Methylation Assay, this BeadChips interrogate 27,578 highly informative CpG sites per sample at single-nucleotide resolution. The HumanMethylation27 panel targets CpG sites positioned within the proximal promoter regions of transcription start sites of 14,475 consensus coding sequencing (CCDS) from the NCBI Database. Briefly, DNA samples were treated with bisulfite reagents. The Infinium Methylation Assay distinguishes cytosine methylation at CpG islands based on highly multiplexed bisulfite-converted genomic DNA genotyping. Upon treatment with bisulfite, unmethylated cytosine bases are converted to uracil, whereas methylated cytosine bases remain unaffected. The assay is explored by chemically differentiated loci using two site-specific probes, designed for the methylated locus (M bead type) and the unmethylated locus (U bead type). For single-base extension analysis of the probes, a labeled ddNTP is incorporated. Then, the amplicons is stained with a fluorescence reagent. The methylation level of interrogated locus can be calculated by ratio of the fluorescent signals from the methylated vs. unmethylated sites, following to evaluate the fluorescent signals for data analysis by iScan System (Figure 15) (http://www.illumina.com/technology/infinium_methylation_assay.ilmn).

Figure 15: The assay of Infinium Methylation Array (Illumina Inc.)



CU-DREAMX analysis

LINE-1 data were reported in the L1base (http://l1base.molgen.mpg.de) [98]. We extracted all microarray data from GEO dataset (http://www.ncbi.nlm.nih.gov/geo). The expression libraries from GEO were GSE13355 [99] and GSE14905 [100] psoriasis expression microarray for psoriasis keratinocytes. CU-DREAMX program, available at http://pioneer.netserv.chula.ac.th/~achatcha/cu-dream/, was used to measure the correlation between gene-containing LINE-1 and differential expression in psoriasis expression array. Moreover, we performed the correlation between genes containing LINE-1 with hypo- or hyper methylation, which is methylation microarray data from previous study

of Ruchusatsawat et al. (Unpublished data) and differential expression in psoriasis expression array. The CU-DREAMX program was used to identify regulated gene at p-value <0.01 [8].

Functional analysis

We performed functional classification analysis by using DAVID Bioinformatics' tools Resources (http://david.abcc.ncifcrf.gov/home.jsp) [101, 102]. Gene list was performed with DAVID functional annotation chart to get EASE scores, a modified Fisher Exact *P*-Value, for each enriched annotation terms and calculate geometric mean of EASE scores of those terms involved in this gene group. Minus log transformation is applied on the geometric mean to emphasize that the geometric mean is a relative score instead of an absolute *P* value. For example, enrichment scores 0.05 is equivalent to 1.3 in minus log scale. Ranks the biological significant of gene groups are based on overall EASE scores (enrichment score) of all enriched annotation terms. Therefore, a higher enrichment score for a group indicates that the group members are involved in more important roles [101]. Furthermore, pathway analysis was performed by Ingenuity Pathway Analysis (IPA) system (http://www.ingenuity.com/index.html).

Statistical analysis

The methylation status was compared between groups by Independent or dependent sample T-test (sig 2-tailed) using the SPSS software for windows 15.0 (SPSS Inc., Chicago, IL, USA). A P value of < 0.05 was considered to be significant. A receiver-operating characteristic (ROC) curve analysis was performed to verify the possibility of COBRA-LINE-1 methylation status to discriminate psoriasis and disease severity.

CHAPTER IV

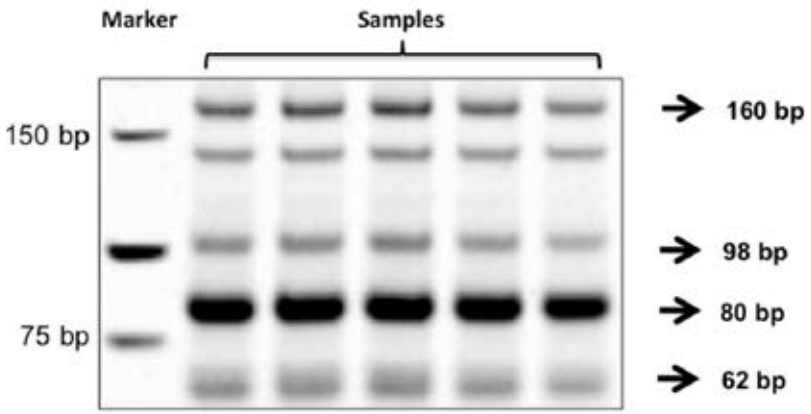
RESULTS

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

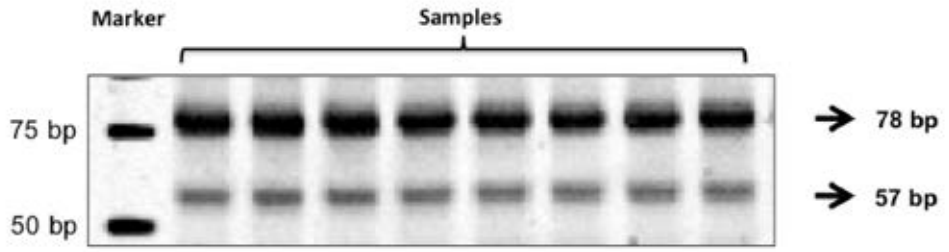
We performed COBRA for LINE-1 and Alu as shown in Figure 16. The PCR product size of LINE-1 and Alu were 160 and 99 bp, respectively. The percentage of the intensity of the digested bands were measured and calculated to methylation levels.

Figure 16: COBRA for LINE-1 and Alu. (A) LINE-1 (B) Alu amplicon sizes are 160 and 99 bp, respectively. All products were digested by restriction enzymes (TaqI and TasI). The LINE-1 amplicons were 160, 98, 80, and 62 bp. Alu amplicons were 78 and 57 bp. The methylation level was calculated as a percentage of the intensity of the digested methylation fragment divided by the sum of the undigested and digested products. Marker is Low range DNA ladder.

(A) COBRA analysis of repetitive element LINE-1



(B) COBRA analysis of repetitive element Alu



Alterations in DNA methylation levels of interspersed repetitive elements LINE-1 between psoriasis patients versus normal individuals

The micro-dissected epidermal skin from psoriatic lesion, SCC, eczema and normal subjects were examined. By COBRA-LINE-1 analysis, there were different in LINE-1 methylation level and pattern of epidermis from psoriasis (n=13), SCC (n=8) and normal controls (n=13). We found a significant difference of methylation level between psoriasis and normal controls. The precise %methylation (^{m}C) of LINE-1 of microdissected epidermal keratinocytes from psoriatic patients (n=13) was significantly lower than epidermal keratinocytes from healthy subjects (n=13) (*p*-value=0.044, % methylation in psoriatic skin vs. normal skin = 41.64 VS. 45.15). Moreover, the number of hypomethylated loci ($^{u}C^{u}C$) of LINE-1 from psoriatic patients (n=13) was significantly higher than epidermal skin from healthy subjects (n=13) (*p*-value=0.045, % methylation in psoriatic skin vs. normal skin = 41.11VS. 37.40).

We analyzed the association of LINE-1 methylation in SCC compared to normal controls as a positive LINE-1 methylation control in skin cancer. We found that the keratinocyte of SCC patients have hypomethylation of LINE-1 in comparing with normal individuals contrasting with eczema (*p*-value=0.024, % ^{m}C methylation in SCC VS. normal

skin = 41.09 VS. 45.15). The alteration of LINE-1 methylation between this psoriatic diseases and controls were correlated with skin malignancy including SCC (Table 10).

We explored the LINE-1 methylation level in various hematopoietic cell types (B, $CD4^+$, $CD8^+$ T lymphocytes, and non-TB). Unlike epidermis, the LINE-1 methylation levels of all of hematopoietic cell subsets from psoriatic patients were no significantly different compared as healthy subjects. The data were shown in Table 10.

Alterations in DNA methylation of interspersed repetitive sequences Alu in psoriatic epidermis and various hematopoietic cells versus normal individuals

The micro-dissected epidermal skin from psoriatic lesion, SCC, eczema and normal subjects were examined. By COBRA-Alu analysis, there were no significantly different in Alu methylation level of epidermis from psoriasis (n=10), SCC (n=8), eczema (n=10) and normal controls (n=10). We analyzed 10 epidermal keratinocytes from psoriatic patients compared to 10 healthy subjects. In addition, 16 hematopoietic cells from psoriasis subjects and 20 healthy donors were determined. However, hypomethylation of Alu was not revealed in epidermal keratinocytes. But, Alu hypomethylation was found in non-TB from psoriatic patients (n=16) when compared to healthy donors (n=20) (*p*-value=0.022) (% methylation in psoriasis vs. normal non-TB lymphocytes = 30.26 VS. 32.02). However, Alu methylation has trend to alteration in methylation in lymphocyte subset in Non-TB of psoriasis patients. The data was shown in Table 11.

Table 10: Changes in DNA methylation of LINE-1

LINE-1 methylation	Normal					Psoriasis					SCC	Eczema
	Keratinocyte (n= 13)	B lymphocyte (n= 20)	CD4+ lymphocyte (n= 20)	CD8+ lymphocyte (n= 20)	Non-TB (n= 20)	Keratinocyte (n= 13)	B lymphocyte (n= 16)	CD4+ lymphocyte (n= 16)	CD8+ lymphocyte (n= 16)	Non-TB (n= 16)	Keratinocyte (n= 8)	Keratinocyte (n= 10)
^{m}C	45.15 ± 3.67	43.07 ± 4.71	43.69 ± 2.41	43.40 ± 3.64	44.80 ± 2.13	41.64 ± 4.67[a]	43.34 ± 3.97	42.86 ± 3.80	42.36 ± 4.33	44.55 ± 2.31	41.09 ± 3.72[c]	42.90 ± 1.15
$^{m}C^{m}C$	27.71 ± 4.61	21.92 ± 11.65	23.09 ± 8.04	21.94 ± 10.71	29.43 ± 2.09	24.39 ± 6.78	19.19 ± 10.57	19.13 ± 10.68	19.48 ± 11.71	30.22 ± 3.65	22.15 ± 2.67[d]	23.26 ± 2.38[g]
$^{u}C^{m}C$	15.31 ± 3.79	24.22 ± 18.72	21.85 ± 15.03	23.89 ± 17.91	11.26 ± 4.07	14.33 ± 8.03	30.91 ± 16.03	29.53 ± 16.06	27.78 ± 16.89	8.99 ± 5.60	19.59 ± 3.54[e]	21.13 ± 4.79[h]
$^{m}C^{u}C$	19.58 ± 1.38	18.08 ± 3.16	19.34 ± 3.47	19.04 ± 3.35	19.48 ± 2.21	20.17 ± 2.00	17.41 ± 3.05	17.92 ± 2.78	17.97 ± 2.59	19.67 ± 2.42	18.31 ± 1.13[f]	18.13 ± 1.58[i]
$^{u}C^{u}C$	37.40 ± 3.41	35.78 ± 6.55	35.71 ± 4.91	35.13 ± 5.62	39.83 ± 3.42	41.11 ± 5.30[b]	32.50 ± 3.82	33.42 ± 3.57	34.77 ± 4.24	41.12 ± 4.11	39.96 ± 5.25	37.47 ± 2.51
Partial	34.89 ± 3.46	42.30 ± 16.39	41.19 ± 12.43	42.93 ± 15.48	30.73 ± 3.74	34.50 ± 7.80	48.32 ± 13.77	47.45 ± 13.99	45.75 ± 15.33	28.66 ± 6.25	37.89 ± 3.73	39.27 ± 4.31[j]

P-value = 0.044 [a] (^{m}C normal keratinocyte compared to psoriasis keratinocyte),

P-value = 0.045 [b] ($^{u}C^{u}C$ normal keratinocyte compared to psoriasis keratinocyte),

P-value = 0.024 [c] (^{m}C normal keratinocyte compared to squamous cell carcinoma),

P-value = 0.006 [d] ($^{m}C^{m}C$ normal keratinocyte compared to squamous cell carcinoma),

P-value = 0.019 [e] ($^{u}C^{m}C$ normal keratinocyte compared to squamous cell carcinoma),

P-value = 0.041 [f] ($^{m}C^{u}C$ normal keratinocyte compared to squamous cell carcinoma)

P-value = 0.012 [g] ($^{m}C^{m}C$ normal keratinocyte compared to Eczema)

P-value = 0.004 [h] ($^{u}C^{m}C$ normal keratinocyte compared to Eczema)

P-value = 0.029 [i] ($^{m}C^{u}C$ normal keratinocyte compared to Eczema)

P-value = 0.013 [j] (Partial methylation normal keratinocyte compared to Eczema)

Table 11: Changes in DNA methylation of Alu

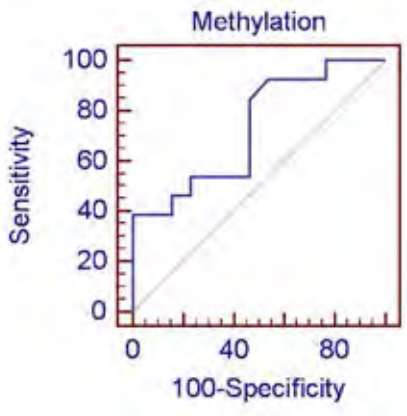
Alu methylation	Normal					Psoriasis					SCC	Eczema
	Keratinocyte (n= 10)	B lymphocyte (n= 20)	CD4+ lymphocyte (n= 20)	CD8+ lymphocyte (n= 20)	Non-TB (n= 20)	Keratinocyte (n= 10)	B lymphocyte (n= 16)	CD4+ lymphocyte (n= 16)	CD8+ lymphocyte (n= 16)	Non-TB (n= 16)	Keratinocyte (n= 8)	Keratinocyte (n= 10)
% Methylation	30.99 ± 3.17	36.98 ± 6.28	36.21 ± 6.34	35.63 ± 7.34	32.02 ± 2.23	31.19 ± 1.84	35.72 ± 9.30	35.60 ± 8.93	36.96 ± 9.52	30.26 ± 2.10[a]	30.92 ± 2.12	30.01 ± 1.76

P-value = 0.022 [a] (Normal non-TB compared to psoriasis non-TB)

ROC curve analysis of LINE-1 methylation in psoriasis keratinocytes

We performed ROC curve analysis to explore the potential use of LINE-1 analysis as a biomarker for psoriasis. The ROC curve analysis was performed to explore the potential use of LINE-1 methylation pattern for discrimination psoriasis from normal controls. Based on previous data, we determined a significant test of the ^{m}C and $^{u}C^{u}C$ pattern and we indicated the various cut-off values, sensitivities and specificities. The optimal cut-off value, sensitivity and specificity were selected. The LINE-1 methylation level (^{m}C) was revealed moderate sensitivity with a cut-off value of $\leq$ 43.23% (sensitivity = 53.85% and specificity = 76.92%). Nevertheless, the LINE-1 methylation pattern ($^{u}C^{u}C$) was appropriated for psoriasis detection by moderate levels of sensitivity and specificity with a cut-off value of >39.67% (sensitivity = 69.23% and specificity = 69.23%) as shown in Figure 17 and 18. Specificity, sensitivity and predictive value of each cut-off were shown in table 12.

Figure 17: ROC curve analysis of the ^{m}C pattern between psoriasis keratinocytes versus normal individuals.

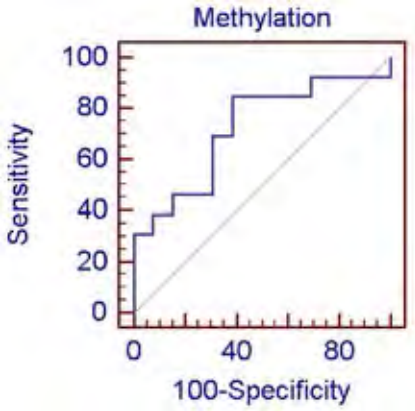


Methylation with % ^{m}C

Normal & Psoriasis keratinocytes

Area under curve	0.731
Cut off value	≤ 43.23 %
Sensitivity	53.85 %
Specificity	76.92 %
Normal	n = 13
Psoriasis	n = 13

Figure 18: ROC curve analysis of the $^{u}C^{u}C$ pattern between psoriasis keratinocytes versus normal individuals



Methylation with % $^{u}C^{u}C$

Normal & Psoriasis keratinocytes

Area under curve	0.722
Cut off value	> 39.67 %
Sensitivity	69.23 %
Specificity	69.23 %
Normal	n = 13
Psoriasis	n = 13

Table 12: ROC analysis of the $^{u}C^{u}C$ methylation pattern in psoriatic keratinocytes was showed varies cut-off criterions in $^{u}C^{u}C$ methylation pattern. The suitable cut-off value is > 39.67% for detecting psoriasis with optimal sensitivity and specificity.

Disease	Criterion values	Total number of tests	Number of positive tests	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
$^{u}C^{u}C$ Psoriasis	> 38.00	13	11	84.62	61.54	68.75	80.00
	>39.00	13	9	69.23	61.54	64.29	66.67
	> 39.67*	**13**	**9**	**69.23**	**69.23**	**69.23**	**69.23**
	>39.90	13	8	61.54	69.23	66.67	64.29
	>40.00	13	7	53.85	69.23	63.64	60.00
	> 40.92	13	6	46.15	84.62	66.67	58.82

* The best of sensitivity and specificity of psoriasis compared to normal keratinocytes when used criteria > 39.67 for the $^{u}C^{u}C$ pattern.

Alterations in DNA methylation of interspersed repetitive sequences LINE-1 in chronic plaque psoriasis according to disease severity

In addition, we analyzed the association of LINE-1 methylation pattern and severity of diseases as shown in Table 13. Psoriatic patients were classified according to severity of the disease with Psoriasis area and severity index (PASI) < 15 = mild and PASI ≥ 15 = severe. Interestingly, the % $^{u}C^{m}C$ pattern and % partial methylation of LINE-1 were significant lower in severe cases of psoriasis compared to mild cases of psoriasis (*p*-value=0.022 and *p*-value=0.043) (% methylation in severe vs. mild psoriasis = 9.10 vs. 18.80 and 29.91 vs. 38.43, respectively).

Furthermore, we explored ROC curve analysis by comparing between mild and severe psoriasis patients and reported the optimal cut-off value, sensitivity and specificity. As shown in Figure 19, the LINE-1 methylation pattern ($^{u}C^{m}C$) was suitable as biomarker for detecting the severity of psoriasis with a cut-off value of ≤ 10.87% (sensitivity = 83.33%; specificity = 100.00%).

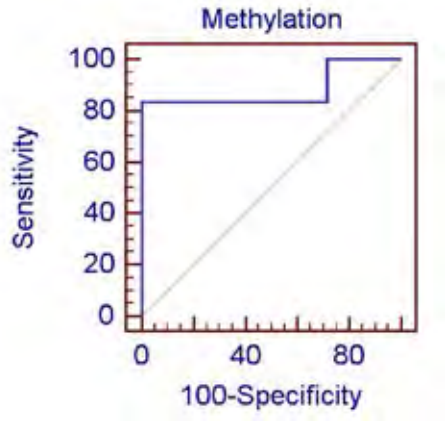
Table 13: Alteration of LINE-1 methylation in psoriasis severity (Mild, PASI <15 and Severe, PASI ≥ 15)

LINE-1 methylation	Mild psoriasis (n= 7)	Severe psoriasis (n= 6)
^{m}C	40.72 ± 4.96	42.72 ± 4.51
$^{m}C^{m}C$	21.51 ± 7.32	27.76 ± 4.55
$^{u}C^{m}C$	18.80 ± 5.99	9.10 ± 7.12[a]
$^{m}C^{u}C$	19.63 ± 1.55	20.81 ± 2.41
$^{u}C^{u}C$	40.06 ± 4.19	42.33 ± 6.56
Partial	38.43 ± 6.65	29.91 ± 6.79[b]

P-value = 0.022 [a] ($^{u}C^{m}C$ mild psoriasis compared to severe psoriasis keratinocyte)

P-value = 0.043 [b] (Partial methylation mild psoriasis compared to severe psoriasis keratinocyte)

Figure 19: ROC curve analysis of LINE-1 methylation pattern and psoriasis severity. The methylation level of $^{u}C^{m}C$ pattern of LINE-1 and psoriasis severity compared between mild and severe psoriasis with criterion value ≤ 10.87% (Sensitivity = 83.33% and specificity = 100.00).



Methylation with % $^{u}C^{m}C$

Mild & Severe psoriasis keratinocytes

Area under curve	0.881
Cut off value	≤ 10.87%
Sensitivity	83.33%
Specificity	100.00%
Mild psoriasis	n = 7
Severe psoriasis	n = 6

Changes in DNA methylation of interspersed repetitive sequences LINE-1 in chronic plaque psoriasis according to demographic data, diseases and leukocyte counts

We analyzed the association between LINE-1 DNA methylation patterns with demographic data, disease, and clinical parameters such as leukocyte counts. The precise methylation of LINE-1 was discovered to associated with sex in non-TB lymphocytes from psoriatic patients when compared between male (n=11) and female (n=5) (*p*-value=0.017) (% methylation in male vs. female = 45.43 VS. 42.60). In contrast, when compared between psoriasis and normal controls (male + female) or individual, no significant associations were found in B, CD4+, CD8+ and non-TB lymphocytes (*p*-value=0.852, 0.453, 0.436 and 0.736, respectively).

However, we also found correlation between LINE-1 DNA methylation patterns with age and clinical features. We found $^{u}C^{u}C$ inversely correlation with age in psoriatic B-lymphocytes (n=16) (r=-0.511 and *p*-value=0.043). On the contrary, No correlation between the $^{u}C^{u}C$ patterns with age in B-lymphocytes (n=20) from normal controls were investigated (r=0.376 and *p*-value=0.103). LINE-1 association with psoriatic nail pathology was also revealed in CD8+ T lymphocytes form psoriatic patients when compared between involved (n=11) and non-involved nails (n=5) (*p*-value=0.031) (% methylation in involved vs. uninvolved nails = 40.83 VS. 45.70). Surprisingly, $^{m}C^{u}C$ was strongly correlated with white blood cell count in B-lymphocytes (n=12) (r=0.894 and *p*-value<0.001), CD4+ (n=12) (r=0.829 and *p*-value=0.001) and CD8+ T lymphocytes (n=12) (r=0.804 and *p*-value=0.002). Likewise, the $^{u}C^{m}C$ also correlated with % Neutrophils (n=7) (r=0.772 and *p*-value=0.042) in psoriatic keratinocytes. The data were shown in Table 14-16.

Changes in DNA methylation of interspersed repetitive sequences Alu in chronic plaque psoriasis according to demographic data, diseases and leukocyte counts

We analyzed the association between Alu methylation levels with demographic data, disease, and clinical parameters. We only found Alu association with sex in CD8+ lymphocytes from psoriatic patients when compared between male (n=11) and female (n=5) (*p*-value=0.016) (% methylation in male vs. female = 33.29 VS. 45.05). The data were shown in Table 17-19.

Table 14: The association and correlation between LINE-1 methylation patterns with psoriasis demographic data

Table 14.1: The association and correlation between LINE-1 methylation patterns with Sex

LINE-1 Methylation Pattern	Demographic Data				
	Sex				
	Keratinocytes	B	CD4+	CD8+	Non-T non B
	t-test *p*-value (male vs. female)				
^{m}C	0.61	0.05	0.19	0.68	0.02
$^{m}C^{m}C$	0.27	0.27	0.18	0.33	0.12
$^{u}C^{m}C$	0.43	0.56	0.31	0.28	0.85
$^{m}C^{u}C$	0.16	0.80	0.93	0.74	0.40
$^{u}C^{u}C$	0.71	0.74	0.37	0.10	0.26
Partial	0.20	0.46	0.24	0.26	0.88

The association was calculated by student's t-test.

Table 14.2: The correlation between LINE-1 methylation patterns with Age

LINE-1Methylation Pattern	Demographic Data									
	Age									
	Keratinocytes		B		CD4+		CD8+		Non-T non B	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
^{m}C	-0.23	0.53	0.02	0.94	-0.12	0.65	-0.25	0.36	0.21	0.43
$^{m}C^{m}C$	-0.18	0.61	-0.17	0.53	-0.15	0.58	-0.20	0.47	-0.07	0.80
$^{u}C^{m}C$	-0.08	0.84	0.26	0.34	0.14	0.60	0.11	0.68	0.15	0.58
$^{m}C^{u}C$	0.35	0.33	-0.13	0.64	0.01	0.98	0.21	0.43	0.27	0.32
$^{u}C^{u}C$	0.18	0.62	-0.51	0.04	-0.19	0.48	-0.03	0.90	-0.30	0.26
Partial	0.03	0.94	0.27	0.31	0.16	0.54	0.16	0.56	0.24	0.38

The relationship was calculated by Pearson's correlation (r) coefficient test

Table 15: The association and correlation between LINE-1 methylation patterns with Diseases

Table 15.1: The correlation between LINE-1 methylation patterns with Onset

LINE-1 Methylation Pattern	Disease									
	Onset									
	Keratinocytes		B		CD4+		CD8+		Non-T non B	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
^{m}C	-0.36	0.31	-0.13	0.62	-0.25	0.35	-0.16	0.56	0.15	0.58
$^{m}C^{m}C$	-0.43	0.21	-0.16	0.56	-0.19	0.49	-0.23	0.40	-0.12	0.66
$^{u}C^{m}C$	0.29	0.41	0.11	0.70	0.10	0.71	0.20	0.46	0.26	0.32
$^{m}C^{u}C$	0.09	0.81	0.19	0.47	0.18	0.52	0.22	0.41	0.03	0.90
$^{u}C^{u}C$	0.11	0.76	-0.16	0.55	-0.03	0.91	-0.30	0.26	-0.27	0.31
Partial	0.30	0.40	0.17	0.54	0.15	0.58	0.26	0.34	0.25	0.35

The relationship was calculated by Pearson's correlation (r) coefficient test

Table 15.2: The correlation between LINE-1 methylation patterns with Disease duration

LINE-1 Methylation Pattern	Disease									
	Disease duration									
	Keratinocytes		B		CD4+		CD8+		Non-T non B	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
^{m}C	0.11	0.761	0.18	0.52	0.13	0.63	-0.11	0.69	0.09	0.75
$^{m}C^{m}C$	0.31	0.39	-0.02	0.94	0.03	0.90	0.02	0.93	0.05	0.85
$^{u}C^{m}C$	-0.59	0.08	0.18	0.50	0.05	0.85	-0.09	0.74	-0.11	0.68
$^{m}C^{u}C$	0.52	0.12	-0.36	0.17	-0.18	0.50	0.01	0.98	0.27	0.31
$^{u}C^{u}C$	0.17	0.64	-0.42	0.11	-0.19	0.49	0.29	0.28	-0.05	0.85
Partial	-0.40	0.25	0.13	0.63	0.02	0.94	-0.10	0.72	0.01	0.99

The relationship was calculated by Pearson's correlation (r) coefficient test

Table 15.3: The association between LINE-1 methylation patterns with Nail involvements

LINE-1 Methylation Pattern	Disease				
	Nail involvements				
	Keratinocytes	B	CD4+	CD8+	Non-T non B
	t-test *p*-value (male vs. female)				
^{m}C	0.31	0.12	0.06	0.03	0.89
$^{m}C^{m}C$	0.48	0.08	0.06	0.07	0.98
$^{u}C^{m}C$	0.64	0.06	0.05	0.08	0.71
$^{m}C^{u}C$	0.12	0.20	0.18	0.16	0.36
$^{u}C^{u}C$	0.37	0.12	0.11	0.46	0.89
Partial	0.98	0.07	0.06	0.11	0.94

The association was calculated by student's t-test.

Table 16: The correlation between LINE-1 methylation patterns with leukocyte counts

Table 16.1: The correlation between LINE-1 methylation patterns with % White blood cells

LINE-1 Methylation Pattern	Leukocyte counts									
	% White blood cells									
	Keratinocytes		B		CD4+		CD8+		Non-T non B	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
^{m}C	0.08	0.87	0.52	0.08	0.40	0.19	0.57	0.05	0.36	0.25
$^{m}C^{m}C$	-0.32	0.49	0.50	0.10	0.47	0.12	0.46	0.13	0.21	0.52
$^{u}C^{m}C$	0.45	0.31	-0.57	0.05	-0.58	0.05	-0.49	0.11	0.18	0.57
$^{m}C^{u}C$	0.36	0.43	0.89	<0.001	0.83	0.001	0.80	0.002	-0.20	0.53
$^{u}C^{u}C$	-0.50	0.26	0.34	0.28	0.57	0.05	0.08	0.79	-0.29	0.37
Partial	0.52	0.23	-0.47	0.12	-0.50	0.10	-0.38	0.22	0.06	0.85

The relationship was calculated by Pearson's correlation (r) coefficient test

Table 16.2: The correlation between LINE-1 methylation patterns with % Lymphocytes

LINE-1 Methylation Pattern	Leukocyte counts									
	% Lymphocytes									
	Keratinocytes		B		CD4+		CD8+		Non-T non B	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
^{m}C	0.41	0.37	-0.30	0.34	-0.08	0.81	-0.10	0.77	-0.50	0.10
$^{m}C^{m}C$	0.64	0.12	-0.21	0.51	-0.18	0.58	-0.17	0.60	-0.29	0.36
$^{u}C^{m}C$	-0.73	0.06	0.17	0.61	0.24	0.45	0.22	0.50	-0.17	0.60
$^{m}C^{u}C$	0.43	0.34	-0.18	0.59	-0.26	0.43	-0.16	0.61	0.14	0.67
$^{u}C^{u}C$	0.19	0.68	0.03	0.93	-0.37	0.24	-0.27	0.40	0.39	0.21
Partial	-0.57	0.18	0.15	0.63	0.23	0.47	0.21	0.52	-0.08	0.80

The relationship was calculated by Pearson's correlation (r) coefficient test

Table 16.3: The correlation between LINE-1 methylation patterns with % Neutrophils

LINE-1 Methylation Pattern	Leukocyte counts									
	% Neutrophils									
	Keratinocytes		B		CD4+		CD8+		Non-T non B	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
^{m}C	-0.55	0.21	0.39	0.21	0.20	0.52	0.17	0.60	0.34	0.28
$^{m}C^{m}C$	-0.76	0.05	0.32	0.31	0.25	0.43	0.22	0.49	0.16	0.63
$^{u}C^{m}C$	0.77	0.04	-0.29	0.37	-0.29	0.36	-0.26	0.41	0.28	0.38
$^{m}C^{u}C$	-0.39	0.39	0.24	0.46	0.29	0.35	0.25	0.43	-0.28	0.37
$^{u}C^{u}C$	-0.13	0.78	0.12	0.71	0.33	0.30	0.26	0.42	-0.31	0.34
Partial	0.62	0.14	-0.28	0.38	-0.27	0.39	-0.24	0.45	0.11	0.74

The relationship was calculated by Pearson's correlation (r) coefficient test

Table 17: The association and correlation between Alu methylation with psoriasis demographic data

Table 17.1: The association and correlation between Alu methylation with Sex

% Alu Methylation	Demographic Data				
	Sex				
	Keratinocytes	B	CD4+	CD8+	Non-T non B
	t-test *p*-value (male vs. female)				
% Methylation	0.22	0.20	0.14	0.02	0.28

The association was calculated by student's t-test.

Table 17.2: The correlation between Alu methylation with Age

% Alu Methylation	Demographic Data									
	Age									
	Keratinocytes		B		CD4+		CD8+		Non-T non B	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
% Methylation	0.45	0.19	0.37	0.15	0.34	0.20	0.21	0.45	0.12	0.66

The relationship was calculated by Pearson's correlation (r) coefficient test

Table 18: The association and correlation between Alu methylation with Diseases

Table 18.1: The correlation between Alu methylation with Onset

% Alu Methylation	Disease									
	Onset									
	Keratinocytes		B		CD4+		CD8+		Non-T non B	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
% Methylation	0.30	0.41	0.35	0.19	0.33	0.21	0.17	0.53	-0.20	0.46

The relationship was calculated by Pearson's correlation (r) coefficient test

Table 18.2: The correlation between Alu methylation with Disease duration

% Alu Methylation	Disease									
	Disease duration									
	Keratinocytes		B		CD4+		CD8+		Non-T non B	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
% Methylation	0.41	0.23	0.06	0.83	0.03	0.91	0.05	0.85	0.35	0.18

The relationship was calculated by Pearson's correlation (r) coefficient test

Table 18.3: The association between Alu methylation with Nail involvements

% Alu Methylation	Disease				
	Nail involvements				
	Keratinocytes	B	CD4+	CD8+	Non-T non B
	t-test *p*-value (male vs. female)				
% Methylation	0.26	0.11	0.41	0.61	0.27

The association was calculated by student's t-test.

Table 19: The correlation between Alu methylation with leukocyte counts

Table 19.1: The correlation between Alu methylation with % White blood cells

% Alu Methylation	Leukocyte counts									
	% White blood cells									
	Keratinocytes		B		CD4+		CD8+		Non-T non B	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
% Methylation	-0.34	0.46	-0.19	0.56	-0.19	0.56	-0.24	0.46	-0.45	0.14

The relationship was calculated by Pearson's correlation (r) coefficient test

Table 19.2: The correlation between Alu methylation with % Lymphocytes

% Alu Methylation	Leukocyte counts									
	% Lymphocytes									
	Keratinocytes		B		CD4+		CD8+		Non-T non B	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
% Methylation	-0.18	0.71	0.20	0.53	0.30	0.35	0.56	0.06	0.25	0.44

The relationship was calculated by Pearson's correlation (r) coefficient test

Table 19.3: The correlation between Alu methylation with % Neutrophils

% Alu Methylation	Leukocyte counts									
	% Neutrophils									
	Keratinocytes		B		CD4+		CD8+		Non-T non B	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
% Methylation	-0.09	0.85	-0.08	0.80	-0.15	0.64	-0.39	0.21	-0.33	0.30

The relationship was calculated by Pearson's correlation (r) coefficient test

Alterations in DNA methylation levels of interspersed repetitive elements LINE-1 between psoriasis patients before treatment with methotrexate versus psoriasis patients after treatment with methotrexate

Methylation levels in lymphocyte subsets from psoriasis patients with methotrexate before and after treatment were compared. No significant association between before and after treatment with methotrexate in psoriasis keratinocytes. Interestingly, the increasing of LINE-1 methylation was significantly found in $CD4^+$ lymphocytes after treatment with methotrexate (*p*-value=0.047, % ^mC methylation in before vs. after treatment = 42.03 VS. 45.33, respectively). Moreover, the hypermethylated loci of LINE-1 in B, CD4+, and CD8+ lymphocytes after treatment with methotrexate were significantly increased the number of methylation levels (*p*-value=0.017, *p*-value=0.010, and *p*-value=0.043, % $^mC^mC$ methylation in before vs. after treatment = 18.82 VS. 27.48, 17.45 VS. 27.12, and 17.14 VS. 26.36, respectively) (Table 20).

Table 20: Changes in DNA methylation of LINE-1

LINE-1 methylation	Psoriasis before treatment with methotrexate					Psoriasis after treatment with methotrexate				
	Keratinocyte (n= 6)	B lymphocyte (n= 11)	CD4+ lymphocyte (n= 11)	CD8+ lymphocyte (n= 11)	Non-TB (n= 11)	Keratinocyte (n= 6)	B lymphocyte (n= 11)	CD4+ lymphocyte (n= 11)	CD8+ lymphocyte (n= 11)	Non-TB (n= 11)
^{m}C	45.20 ± 2.89	43.38 ± 3.51	42.03 ± 3.05	41.70 ± 4.30	44.25 ± 2.51	44.76 ± 2.45	45.73 ± 1.89	45.33 ± 3.26[f]	43.89 ± 1.96	46.56 ± 2.06
$^{m}C^{m}C$	29.46 ± 8.07	18.82 ± 9.73	17.45 ± 9.57	17.14 ± 10.34	28.38 ± 3.22	32.85 ± 3.08	27.48 ± 4.42[a]	27.12 ± 4.90[g]	26.36 ± 4.97[k]	28.98 ± 2.84
$^{u}C^{m}C$	12.82 ± 7.95	32.19 ± 14.54	31.47 ± 15.07	31.19 ± 14.22	11.78 ± 6.12	6.87 ± 4.33	16.75 ± 6.85[b]	15.70 ± 8.59[h]	15.30 ± 7.27[l]	17.04 ± 4.30[o]
$^{m}C^{u}C$	18.68 ± 4.97	16.94 ± 2.68	17.71 ± 2.60	17.93 ± 2.67	19.96 ± 2.61	16.95 ± 1.17	19.74 ± 1.82[c]	20.72 ± 2.81[i]	19.78 ± 2.65	18.12 ± 3.09
$^{u}C^{u}C$	39.05 ± 3.52	32.06 ± 3.45	33.38 ± 3.64	33.75 ± 3.41	39.88 ± 4.30	43.33 ± 2.92	36.03 ± 2.62[d]	36.46 ± 5.51	38.57 ± 4.21[m]	35.86 ± 2.59[p]
Partial	31.49 ± 11.03	49.12 ± 12.79	49.18 ± 13.13	49.12 ± 12.78	31.74 ± 5.71	23.82 ± 3.47	36.48 ± 6.21[e]	36.42 ± 8.13[j]	35.08 ± 8.33[n]	35.16 ± 3.54

P-value= 0.017 [a] ($^{m}C^{m}C$, B-cell of Psoriasis before treatment compared to Psoriasis after treatment with methotrexate)

P-value= 0.006 [b] ($^{u}C^{m}C$, B-cell of Psoriasis before treatment compared to Psoriasis after treatment with methotrexate)

P-value= 0.021 [c] ($^{m}C^{u}C$, B-cell of Psoriasis before treatment compared to Psoriasis after treatment with methotrexate)

P-value= 0.003 [d] ($^{u}C^{u}C$, B-cell of Psoriasis before treatment compared to Psoriasis after treatment with methotrexate)

P-value= 0.008 [e] (Partial, B-cell of Psoriasis before treatment compared to Psoriasis after treatment with methotrexate)

P-value= 0.047 [f] (^{m}C, $CD4^{+}$ of Psoriasis before treatment compared to Psoriasis after treatment with methotrexate)

P-value= 0.010 [g] ($^{m}C^{m}C$, $CD4^{+}$ of Psoriasis before treatment compared to Psoriasis after treatment with methotrexate)

P-value= 0.005 [h] ($^{u}C^{m}C$, $CD4^{+}$ of Psoriasis before treatment compared to Psoriasis after treatment with methotrexate)

P-value= 0.006 [i] ($^{m}C^{u}C$, $CD4^{+}$ of Psoriasis before treatment compared to Psoriasis after treatment with methotrexate)

P-value= 0.010 [j] (Partial, $CD4^{+}$ of Psoriasis before treatment compared to Psoriasis after treatment with methotrexate)

P-value= 0.043 [k] ($^{m}C^{m}C$, $CD8^{+}$ of Psoriasis before treatment compared to Psoriasis after treatment with methotrexate)

P-value= 0.014 [l] ($^{u}C^{m}C$, $CD8^{+}$ of Psoriasis before treatment compared to Psoriasis after treatment with methotrexate)

P-value= 0.017 [m] ($^{u}C^{u}C$, $CD8^{+}$ of Psoriasis before treatment compared to Psoriasis after treatment with methotrexate)

P-value= 0.021 [n] (Partial, $CD8^{+}$ of Psoriasis before treatment compared to Psoriasis after treatment with methotrexate)

P-value= 0.024 [o] ($^{u}C^{m}C$, Non-TB of Psoriasis before treatment compared to Psoriasis after treatment with methotrexate)

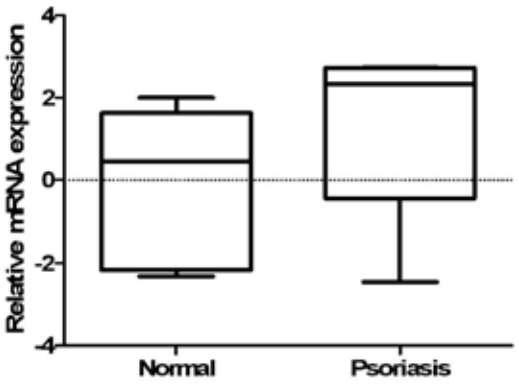
P-value= 0.007 [p] ($^{u}C^{u}C$, Non-TB of Psoriasis before treatment compared to Psoriasis after treatment with methotrexate)

Analysis of 5' and 3' LINE-1 expression in keratinocytes of psoriasis patients versus healthy individuals

This study was performed to investigate whether expression of 5' and 3' orientation of LINE-1 differ among keratinocytes of psoriasis and healthy controls. Expression was measured as of 5' or 3' LINE-1 mRNA relative to 18S rRNA. As shown in Figure 20, we found no significant association of 5' and 3' orientation of LINE-1 between psoriasis and healthy volunteers (*p*-value=0.269 and *p*-value=0.156, respectively) but they have trended to increase 5' and 3' LINE-1 expression in keratinocytes of psoriasis patients.

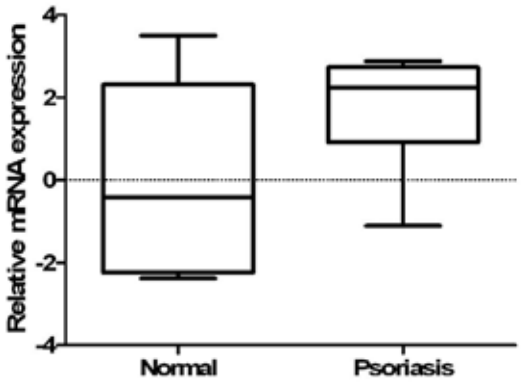
Figure 20: 5' and 3' LINE-1 expression in keratinocytes of psoriasis patients. (A) 5' LINE-1 expression by real-time PCR (B) 3' LINE-1 expression by real-time PCR

(A) 5' LINE-1 expression



No significant association (*P-value* = 0.269)

(B) 3' LINE-1 expression



No significant association (*P-value* = 0.156)

The identification of up- or down-regulated genes in psoriasis keratinocytes

The correlation between differential expressions in psoriasis expression array was studied. CU-DREAM program was used to identify up- or down-regulated genes in psoriasis keratinocytes. We performed a chi-square to validate the results with expression microarray data available from GEO, GSE14905 and GSE13355. When compared between two microarray data (GSE14905 and GSE13355), up-or down-regulated genes in expression microarray data were significantly found (p-value<0.0001; OR = 13.40; 95% CI = 12.25-14.66 and p-value<0.0001; OR = 21.63; 95% CI = 19.63-23.82, respectively (Table 21). The differentially expressed genes were found between the two libraries, which 4,106 genes were up- regulated and 3,652 genes were down regulated in keratinocytes of psoriasis compared with normal controls.

To identify functional networks, genes were described as up-or down-regulated genes in psoriasis. Keratinocytes of psoriasis were performed gene set enrichment analysis using the online available DAVID Bioinformatics Resources. The annotation cluster with a group enrichment score less than or equal to 0.05 (1.3 in minus log scale) were analyzed. From 4,106 up-regulation genes, the higher-enrichment score was cell-cycle progression cluster (Enrichment score = 12.78 and 9.57) (Table 22). Interestingly, various genes are clearly involved in pathogenesis of psoriasis through proliferative pathway including mitotic cell division or DNA replication.

Table 21: The intersections between differential expressions of expression microarray data from psoriasis keratinocytes (GSE14905 and GSE13355). The down-or up-regulation of a gene (denoted by "Up" and "Down") was evaluated by paired t-test (P-value threshold is set at 0.01). The p-values of 2x2 tables were obtained from Chi-square distribution.

		GSE14905 Psoriasis skin			
		Up (P<0.01)	Not-Up	Down (P<0.01)	Not-Down
GSE13355 Psoriasis skin	Up (P<0.01)	4,106	5,042	952	8,196
	Not-Up	643	10,583	3,252	7,974
		P-value = 0.00E+00		*P*-value = 1.29E-232	
		Odds ratio = 13.40		Odds ratio = 0.28	
		Upper 95% CI = 14.66		Upper 95% CI = 0.31	
		Lower 95% CI = 12.25		Lower 95% CI = 0.26	

		GSE14905 Psoriasis skin			
		Down (P<0.01)	Not-Down	Up (P<0.01)	Not-Up
GSE13355 Psoriasis skin	Down (P<0.01)	3,652	3,788	916	6,524
	Not-Down	552	12,382	3,833	9,101
		P-value = 0.00E+00		*P*-value = 1.89E-174	
		Odds ratio = 21.63		Odds ratio = 0.33	
		Upper 95% CI = 23.82		Upper 95% CI = 0.36	
		Lower 95% CI = 19.63		Lower 95% CI = 0.31	

Table 22: The annotation clusters identified for up-regulated genes in psoriasis keratinocytes by DAVID Functional Classification Tool

Group number	Associated biology	Enrichment score
1	Nucleotide binding	15.87
2	Mitotic cell cycle	12.78
3	DNA replication	9.57
4	Mitochondrial membrane, cellular respiration	7.36
5	ATPase	7.03
6	Peptidase activity	6.69
7	DNA repair, DNA metabolic process	6.48
8	Chromosomal segregation	5.19
9	Cellular protein localization	5.13
10	Interphase	4.97
11	ATP metabolic process	4.76
12	RNA splicing	4.75
13	Replication, mismatch repair	4.52
14	Phosphorylation	4.48
15	Lymphocyte activation	4.14
16	Cytoskeleton organization	3.59
17	Double-strand break repair	3.54
18	Regulation of lymphocyte differentiation	3.01
19	Regulation of apoptosis	2.57

Table 23: The annotation clusters identified for down-regulated genes in psoriasis keratinocytes by DAVID Functional Classification Tool

Group number	Associated biology	Enrichment score
1	Transcriptional regulation	19.51
2	Membrane fraction	13.28
3	Regulation of gene expression	11.73
4	Response to hormone stimulus	9.39
5	Transition metal ion binding	8.70
6	Regulation of cell migration	8.34
7	Respiratory system development	8.13
8	Cell-cell adhesion	7.70
9	Transcription factor binding	6.70
10	Regulation of cell migration	6.63
11	TGF-beta signaling pathway	6.46
12	Cytoskeleton	6.23
13	Blood vessel development	5.22
14	Steroid hormone receptor activity	5.05
15	Skeletal system development	4.85
16	Zinc-finger	4.80
17	Leucine-zipper	4.58
18	Nucleotide-binding	4.57
19	Plasma membrane part	4.52

Intersections between differential expression of psoriasis and gene containing LINE-1

To evaluate the correlation between gene-containing LINE-1 and differential expression in psoriasis expression array, the CU-DREAMX program was used. Gene-containing LINE-1 was classified into four groups depending on the regulation of genetic and the presence of intragenic LINE-1. We evaluated the statuses of intragenic LINE-1 on gene expression for the first time in psoriasis. Gene expression in keratinocytes was compared with genes possessing intragenic LINE-1 and the rest. We discovered higher prevalence of down-regulated genes containing LINE-1s of psoriasis compared to normal keratinocytes (*p*-value GSE13355 = 3.84×10^{-27}; OR = 1.83 and *p*-value GSE14905 = 2.14×10^{-21}; OR = 1.79, respectively). Interestingly, genes with antisense LINE-1s were strongly indicated that higher prevalence of down-regulated genes containing LINE-1s of psoriasis keratinocytes (*p*-value GSE13355 = 7.25×10^{-15}; OR = 1.76 and *p*-value GSE14905 = 1.47×10^{-13}; OR = 1.79, respectively) (Table 24).

By bioinformatics analysis, the high prevalence of down-regulated genes containing LINE-1s involved in the biological processes of cell such as cell tumor suppressor function, proliferation, apoptosis, cell differentiation, cell survival, immune regulation and cell motility (Table 25). That may be involved in pathogenesis of psoriasis. Moreover, down-regulated genes containing LINE-1s found to be tumor suppressor genes including MIPOL1, PDS5B, UBR5, DAPK1, PTEN, STK3 and MCC (Table 25-26).

Table 24: The expression status of genes containing LINE-1s in psoriasis keratinocytes. Two psoriasis expression microarrays from GEO dataset were enrolled in this study including GSE13355 and GSE14905. The Chi-square test to evaluate intragenic LINE-1 influence psoriasis keratinocyte expression by experiment GSE14905 and GSE13355. Gene-containing LINE-1 was classified into 4 groups depending on the regulation of genetic and the presence of intragenic LINE-1. (A) The expression status of genes containing LINE-1s compared between psoriasis vs. normal skin. (B) The expression status of genes containing LINE-1s compared between uninvolved vs. normal skin. (C) The expression status of genes containing LINE-1s compared between psoriasis vs. uninvolved skin.

Table 24.1: The expression status of genes containing LINE-1s compared between psoriasis vs. normal skin.

	Genes containing L1s (1454 genes)		Genes with sense L1s (336 genes)		Genes with antisense L1s (832 genes)		Genes with both sense and antisense L1s (286 genes)	
	P-value	OR	P-value	OR	P-value	OR	P-value	OR
GSE13355 Psoriatic skin VS. Normal skin Up-regulation (*p*-value ≤ 0.01)	2.87E-13	0.65	8.09E-03	0.73	5.41E-05	0.74	2.32E-10	0.42
GSE13355 Psoriatic skin VS. Normal skin Down-regulation (*p*-value ≤ 0.01)	3.84E-27	1.83	2.45E-08	1.87	7.25E-15	1.76	6.06E-06	1.74
GSE14905 Psoriatic skin VS. Normal skin Up-regulation (*p*-value ≤ 0.01)	2.02E-07	0.68	1.89E-01	0.83	3.69E-03	0.76	3.68E-07	0.37
GSE14905 Psoriatic skin VS. Normal skin Down-regulation (*p*-value ≤ 0.01)	2.14E-21	1.79	2.14E-06	1.78	1.47E-13	1.79	1.12E-03	1.56

Table 24.2: The expression status of genes containing LINE-1s compared between uninvolved vs. normal skin.

	Genes containing L1s (1454 genes)		Genes with sense L1s (336 genes)		Genes with antisense L1s (832 genes)		Genes with both sense and antisense L1s (286 genes)	
	P-value	OR	P-value	OR	P-value	OR	P-value	OR
GSE13355 Uninvolved skin VS. Normal skin Up-regulation (*p*-value ≤ 0.01)	8.57E-08	0.64	4.63E-02	0.72	2.94E-04	0.68	4.86E-04	0.50
GSE13355 Uninvolved skin VS. Normal skin Down-regulation (*p*-value ≤ 0.01)	6.29E-08	1.47	2.88E-01	1.18	6.60E-07	1.57	1.87E-02	1.44
GSE14905 Uninvolved skin VS. Normal skin Up-regulation (*p*-value ≤ 0.01)	2.45E-02	0.81	7.16E-01	1.06	2.96E-01	0.89	3.66E-04	0.39
GSE14905 Uninvolved skin VS. Normal skin Down-regulation (*p*-value ≤ 0.01)	1.04E-04	1.40	4.92E-01	1.14	1.81E-04	1.50	1.41E-01	1.32

Table 24.3: The expression status of genes containing LINE-1s compared between psoriasis vs. uninvolved skin.

	Genes containing L1s (1454 genes)		Genes with sense L1s (336 genes)		Genes with antisense L1s (832 genes)		Genes with both sense and antisense L1s (286 genes)	
	P-value	OR	P-value	OR	P-value	OR	P-value	OR
GSE13355 Psoriatic skin VS. Uninvolved skin Up-regulation (*p*-value ≤ 0.01)	2.57E-07	0.72	1.18E-01	0.82	1.29E-02	0.82	6.18E-08	0.43
GSE13355 Psoriatic skin VS. Uninvolved skin Down-regulation (*p*-value ≤ 0.01)	4.05E-20	1.69	4.87E-06	1.68	1.83E-13	1.72	4.96E-03	1.42
GSE14905 Psoriatic skin VS. Uninvolved skin Up-regulation (*p*-value ≤ 0.01)	7.71E-04	0.81	4.32E-01	0.91	4.63E-02	0.85	1.54E-03	0.64
GSE14905 Psoriatic skin VS. Uninvolved skin Down-regulation (*p*-value ≤ 0.01)	6.85E-13	1.54	3.36E-05	1.64	2.33E-09	1.59	2.27E-01	1.18

Table 25: Psoriasis down-regulated genes with LINE-1 insertion. Official full name of these genes were showed in Appendix B (Table 4B).

Phenotype function	LINE-1 down-regulated gene in psoriasis keratinocytes
Tumor suppressor genes	MIPOL1, PDS5B, UBR5, DAPK1, PTEN, STK3, MCC
Proliferation	ACVR1C, RICTOR, PARD3B, PDGFRA, ZAK, WDR19, PTPRM, PLD1, BBX, PHIP, UACA, PTK2, PRKCA, RBMS3, NEK1, PLCE1, CUL3, KDM5A, STAG1, PTPRK, UVRAG, STK3, NEO1, CDK14, DDR2, EGF, ANK3, MMP16, NFATC1, EVI5
Apoptosis	ACVR1C, PDGFRA, WDR19, PTPRM, PHIP, UACA, PTK2, RBMS3, HIVEP1, ATG5, DAPK1, MAP3K7, MMP16, NFATC1, ANKHD1
Cell differentiation	ACVR1C, PLCE1, PTPRK, NEO1, DDR2, EGF, MMP16, NFATC1
Cell survival	RICTOR
Immune regulation	ITGB1, PDE4D, HIVEP1, IL7
Polarity and motility	DST, UACA, PTK2, ELMO1, ANK3, MMP16

Table 26: Psoriasis down-regulated genes with LINE-1 insertion act as tumor suppressor function.

Number	Official name	Full name
1	MIPOL1	Mirror-image polydactyly1
2	PDS5B	PDS5, regulator of cohesion maintenance homolog B
3	UBR5	Ubiquitin protein ligase E3 component n-recognin 5
4	DAPK1	Death-associated protein kinase 1
5	PTEN	Phosphatase and tensin homolog
6	STK3	Serine/threonine kinase 3
7	MCC	Mutated in colorectal cancers

Intersections between differential expression of psoriasis and promoter methylated genes

Previous results showed that LINE-1 is prominent hypomethylation in psoriasis keratinocytes. To investigate whether methylated genes control human gene expression in psoriasis keratinocytes, gene expression in keratinocytes were compared with promoter-methylated genes and the rest. We demonstrated of Chi-square tests of gene expression and promoter methylation in psoriasis keratinocytes by using experiment GSE14905 and GSE13355. We found higher prevalence of up-regulated genes associated with hypomethylated genes in psoriasis (*p*-value GSE13355 = 1.06×10^{-7}; OR = 1.50 and *p*-value GSE14905 = 7.55×10^{-9}; OR = 1.60, respectively). Moreover, the high prevalence of down-regulated genes were found to associate with hypermethylated genes in psoriasis (*p*-value GSE13355 = 1.90×10^{-1}; OR = 1.13 and *p*-value GSE14905 = 3.90×10^{-1}; OR = 1.10, respectively) (Table 27).

Table 27: 2x2 table of Chi-square test to evaluate hypermethylated or hypomethylated genes influence psoriasis keratinocyte expression by experiment GSE14905 and GSE13355. The down-or up-regulation of a gene (denoted by "Up" and "Down") was evaluated by paired t-test (*P*-value threshold is set at 0.01). Meanwhile, the genes with hypomethylation or without hypomethylation were denoted by hypomethylation and no hypomethylation, respectively. The genes with hypermethylation or without hypermethylation were denoted by hypermethylation and no hypermethylation, respectively. The *p*-values of 2x2 tables were obtained from Chi-square distribution.

	GSE13355 Psoriasis skin			
	Up (P<0.01)	Not-Up	Down (P<0.01)	Not-Down
Hypomethylation	389	323	241	471
No hypomethylation	8,759	10,903	7,199	12,463
	P-value = 1.06E-07		*P*-value = 1.32E-01	
	Odds ratio = 1.50		Odds ratio = 0.89	
	Upper 95% CI = 1.74		Upper 95% CI = 1.04	
	Lower 95% CI = 1.29		Lower 95% CI = 0.76	

	GSE13355 Psoriasis skin			
	Up (P<0.01)	Not-Up	Down (P<0.01)	Not-Down
Hypermethylation	204	292	195	301
No hypermethylation	8,944	10,934	7,245	12,633
	P-value = 8.73E-02		*P*-value = 1.90E-01	
	Odds ratio = 0.85		Odds ratio = 1.13	
	Upper 95% CI = 1.02		Upper 95% CI = 1.36	
	Lower 95% CI = 0.71		Lower 95% CI = 0.94	

	GSE14905 Psoriasis skin			
	Up (P<0.01)	Not-Up	Down (P<0.01)	Not-Down
Hypomethylation	230	482	149	563
No hypomethylation	4,519	15,143	4,055	15,607
	P-value = 7.55E-09		*P*-value = 8.44E-01	
	Odds ratio = 1.60		Odds ratio = 1.02	
	Upper 95% CI = 1.88		Upper 95% CI = 1.22	
	Lower 95% CI = 1.36		Lower 95% CI = 0.85	

	GSE14905 Psoriasis skin			
	Up (P<0.01)	Not-Up	Down (P<0.01)	Not-Down
Hypermethylation	75	421	110	386
No hypermethylation	7,674	15,204	4,094	15,784
	P-value = 1.26E-05		*P*-value = 3.90E-01	
	Odds ratio = 0.58		Odds ratio = 1.10	
	Upper 95% CI = 0.74		Upper 95% CI = 1.39	
	Lower 95% CI = 0.45		Lower 95% CI = 0.89	

The role of LINE-1 in keratinocytes of psoriasis

A Chi-square test of differential expressed and methylated genes in both psoriasis and methylation microarray with intragenic LINE-1 were performed to explore whether intragenic LINE-1 can control the expression of genes in psoriasis with methylation phenomena. The result showed that the higher prevalence of down-regulation of genes with hypomethylation in both psoriasis and methylation array is more prevalent in gene containing LINE-1 than genes without LINE-1 (*p*-value GSE13355 = 2.00×10^{-6}; OR = 2.54 and *p*-value GSE14905 = 1.00×10^{-5}; OR = 2.72, respectively) (Table 28). Therefore, hypomethylation intragenic LINE-1 represses transcription of genes in psoriasis by several mechanisms. Official full name of these genes were showed in Appendix B (Table 5B).

Table 28: 2x2 table of Chi-square test to evaluate intragenic LINE-1 influence methylation levels in keratinocytes of psoriasis. The down-or up-regulation genes in psoriasis expression array were denoted by up and down. The hypomethylated or hypermethylated genes in psoriasis were denoted by hypo and hyper. Meanwhile, the genes containing or without intragenic LINE-1 were denoted by LINE-1 and no LINE-1, respectively. The *p*-values of 2x2 tables were obtained from Chi-square distribution.

	GSE13355 Psoriasis skin and Methylation Array			
	Up and Hypo	Not-Up and Not-Hypo	Down and Hyper	Not-Down and Not-Hyper
LINE-1	28	835	15	655
No LINE-1	361	10,068	180	11,978
	P-value = 0.7369		*P*-value = 0.1183	
	Odds ratio = 0.94		Odds ratio = 1.52	
	Upper 95% CI = 1.40		Upper 95% CI = 2.66	
	Lower 95% CI = 0.62		Lower 95% CI = 0.86	

	GSE14905 Psoriasis skin and Methylation Array			
	Up and Hypo	Not-Up and Not-Hypo	Down and Hyper	Not-Down and Not-Hyper
LINE-1	12	1,055	12	912
No LINE-1	218	14,088	98	14,872
	P-value = 0.3001		*P*-value = 0.0219	
	Odds ratio = 0.74		Odds ratio = 2.00	
	Upper 95% CI = 1.35		Upper 95% CI = 3.75	
	Lower 95% CI = 0.39		Lower 95% CI = 1.04	

	GSE13355 Psoriasis skin and Methylation Array			
	Up and Hyper	Not-Up and Not-Hyper	Down and Hypo	Not-Down and Not-Hypo
LINE-1	3	842	29	637
No LINE-1	201	10,092	212	11,826
	P-value = 0.0009		*P*-value = 2.00E-06	
	Odds ratio = 0.18		Odds ratio = 2.54	
	Upper 95% CI = 0.58		Upper 95% CI = 3.83	
	Lower 95% CI = 0.05		Lower 95% CI = 1.67	

	GSE14905 Psoriasis skin and Methylation Array			
	Up and Hyper	Not-Up and Not-Hyper	Down and Hypo	Not-Down and Not-Hypo
LINE-1	1	1,070	21	889
No LINE-1	74	14,134	128	14,718
	P-value = 0.0536		*P*-value = 0.00001	
	Odds ratio = 0.18		Odds ratio = 2.72	
	Upper 95% CI = 1.18		Upper 95% CI = 4.42	
	Lower 95% CI = 0.01		Lower 95% CI = 1.66	

Table 29: Thirty-four gene-containing LINE-1 with down-regulation and promoter hypomethylation in psoriasis kertinocytes

L1_Candidate	Official Full Name
GRIA3	Glutamate receptor, ionotrophic, AMPA 3
OPHN1	Oligophrenin 1
AR	Androgen receptor
ACSL4	Acyl-CoA synthetase long-chain family member 4
EDA	Ectodysplasin A
HNT	Neurotrimin
NHS	Nance-Horan syndrome (congenital cataracts and dental anomalies)
ARHGEF9	Cdc42 guanine nucleotide exchange factor (GEF) 9
MAOA	Monoamine oxidase A
GPC3	Glypican 3
ATRX	Alpha thalassemia/mental retardation syndrome X-linked (RAD54 homolog, S. cerevisiae)
AFF2	AF4/FMR2 family, member 2
YIPF6	Yip1 domain family, member 6
SLC22A3	Solute carrier family 22 (extraneuronal monoamine transporter), member 3 or Organic cation transporter 3 (OCT3)
SH3BGRL	SH3 domain binding glutamic acid-rich protein like
NEBL	Nebulette
EPB41L3	Erythrocyte membrane protein band 4.1-like 3 (Protein 4.1B/Dal-1)
RGS7	Regulator of G-protein signaling 7

TMLHE	Trimethyllysine hydroxylase, epsilon
ESR1	Estrogen receptor 1
BRWD3	Bromodomain and WD repeat domain containing 3
FIP1L1	FIP1 like 1 (S. cerevisiae)
CHM	Choroideremia (Rab escort protein 1)
CNKSR2	Connector enhancer of kinase suppressor of Ras 2
PLCL1	Phospholipase C-like 1
ATP8B1	ATPase, class I, type 8B, member 1
CPD	Carboxypeptidase D
THRB	Thyroid hormone receptor, beta
NEK7	NIMA (never in mitosis gene a)-related kinase 7
DIAPH2	Diaphanous homolog 2 (Drosophila)
LNX1	Ligand of numb-protein X 1
TUSC3	Tumor suppressor candidate 3
NEO1	Neogenin homolog 1 (chicken)
SPATA18	Spermatogenesis associated 18 homolog (rat)

Table 30: The functional classification of gene-containing LINE-1 with down-regulation and promoter hypomethylation in psoriasis kertinocytes

Phenotype function	Down-regulated genes-containing LINE-1 with hypomethylation in psoriasis keratinocytes
Tumor suppressor genes	HNT, GPC3, TUSC3
Proliferation	AR, NEK7
Cell differentiation	AR, EDA
Cell adhesion	NEO1
Cell shape	EPB41L3, CNKSR2
Cell signaling	ARHGEF9, SH3BGRL, LNX1
Activation of transcription	ESR1
Homeostasis	AR
Cell migration	OPHN1, NHS

CHAPTER V

DISCUSSION AND CONCLUSION

Psoriasis is chronic skin autoimmune disease. The common clinical form is called chronic plaque-type psoriasis, which affects about 90% of psoriasis patients [53, 103]. Psoriasis is characterized by abnormal keratinocyte proliferation, differentiation, and inflammation in both epidermis and dermis. The development of psoriasis pathogenesis has been indicated to be implicated in oncogenes and tumor suppressor genes [104]. DNA methylation is reported to be involve in many biological processes and associated with various diseases such as malignancies and autoimmune disorders including psoriasis. Moreover, methylation on repetitive elements such as LINE-1 and Alu has been reported in several cancers. But, the studies on LINE-1 and Alu methylation in psoriasis are still limited.

The Alteration of methylation in LINE-1 repetitive sequences is associated with various malignancies including SCC [8, 14, 28, 29, 82, 105-109]. Moreover, the LINE-1 methylation level at different loci has been reported to be distinctive between loci [33]. Recently, DNA hypomethylation has been shown in pathogenesis of autoimmune diseases such as SLE, inflammatory, arthritis, and chronic plaque-type psoriasis. The present study is the first demonstrated alteration of methylation in non-LTR retrotransposons in psoriatic epidermis. We explored methylation level and pattern of LINE-1 and Alu using COBRA-LINE-1 technique in psoriasis compared to normal skin and SCC. LINE-1 hypomethylation was found in both psoriatic keratinocytes as same as SCC but the patterns of LINE-1 methylation are rather different. On the contrary, The Alu methylation level was not significant change in psoriasis compared to normal individuals. This finding represented both common and different in pathogenesis of both diseases. Dissimilar with SCC,

inflammation and immunological factors play important role in keratinocyte hyperproliferation and abnormal differentiation in psoriasis. Previous reports reveal global hypomethylation and Alu hypomethylation in various cancers [27-31]. In opposition, global hypermethylation of keratinocyte and PBMC of psoriasis has been reported [45]. As well as, Alu methylation was not altered in psoriatic keratinocyte by the present study. It should be note that genomic instability does not occur in psoriasis which is unlike cancers.

Our data may reveal the LINE-1 methylation changes in sex particularly in psoriatic disease. Previous data, LINE-1 methylation are directly associated with age commonly at 49 years and older [93]. It is possible that this phenomenon was found in psoriatic B-lymphocytes correlated with aging process.

Methotrexate has been used in low dose for psoriasis or inflammatory arthritis treatment [75]. It has been reported that can influence DNA methylation by induction of DNA hypomethylation in genome via reduction of methyl donors [110]. In previous result, the hypomethylation of LINE-1 in psoriasis was showed. Methotrexate is not significant alter methylation in psoriatic keratinocyte. In contrast, this data revealed that the methotrexate treatment might increase LINE-1 methylation levels specifically in $CD4^+$ lymphocyte in psoriasis correlated with responding to treatment. The hypermethyaled loci of LINE-1 was significantly higher in B-, CD4+ and CD8+ lymphocytes from methotrexate treated psoriasis than psoriasis free-treatment. This connection of psoriatic treatment and DNA methylation is not clear. But, we suggested that after treatment with methotrexate can reverse DNA methylation in white blood cells. It may be a compensate mechanisms to redeem the methylation loss in keratinocytes. However, these mechanisms need to clarify in the future.

The bioinformatics analysis of microarray data between genes containing LINE-1 and gene expression from Gene Expression Omnibus (GEO) revealed that the expression of genes containing L1s had a significantly higher probability to be suppressed in cancer and

hypomethylated normal cells [8]. The phenomena of LINE-1 hypomethylation but hypermethylation of promoter region of genes has been observed in various cancers. Moreover, promoter hypermethylation in cancer is frequently linked with transcriptional silencing of tumor suppressor genes [34, 35, 37]. Similar to cancers, our data demonstrated that DNA methylation of LINE-1 from epidermal keratinocytes revealed atypically hypomethylation compared to healthy donors and associated with higher prevalence of down-regulation of gene-containing LINE-1 in psoriasis. We explored the functional classification of down-regulated gene containing LINE-1 by bioinformatics analysis. The most down-regulated genes involved in the biological processes trigger psoriasis pathogenesis including tumor suppressor function, proliferation, apoptosis, cell differentiation or immune regulation. We revealed that gene containing LINE-1s might trigger down-regulation of tumor suppressor gene consequentially in abnormal proliferation in psoriasis skin. Previous LINE-1 data showed that the LINE-1 methylation represented the function of the cellular genes. Previous reports revealed down-regulation of tumor suppressor genes in psoriatic epidermis resulting in abnormal proliferation and differentiation of keratinocyte [40, 42, 44]. It is possible that hypomethylation of LINE-1 down-regulated tumor suppressor genes resulting in acanthosis and hyperkeratosis in psoriatic epidermis. Moreover, hypomethylation of LINE-1 may manipulate other genes involving in disease induction and maintenance including cell proliferation, apoptosis, differentiation and immune regulation.

Gene-containing LINE-1 with down-regulation and promoter hypomethylation in psoriasis kertinocytes involved in the biological processes of cell such as tumor suppressor function, cell proliferation, cell differentiation, cell adhesion and cell signaling. That may be involved in pathogenesis of psoriasis. Moreover, it found to be tumor suppressor genes. The gene-containing LINE-1 with promoter hypomethylation may trigger down-regulation of

tumor suppressor gene consequentially in abnormal proliferation in pathogenesis of psoriasis.

Previous report revealed that intragenic LINE-1 could promote gene repression in cancer depending on degree and site of LINE-1 hypomethylation. Postranscriptional mechanism by AGO2 and siRNA has been identified to be the mechanism that hypomethylation of intragenic LINE-1 represses gene in cancer [8]. However, our data demonstrated the association of hypomethylation of LINE-1 with significant down-regulation of gene-containing LINE-1 in psoriasis. It is probable that hypomethylation of intragenic-LINE-1 could repress gene in psoriasis resembling in cancer. However, the mechanism which involves in LINE-1 hypomethylation and down regulation of genes in psoriasis is unclear and need further study.

Previous data demonstrated that the % $^{u}C^{u}C$ has better power than % of overall LINE-1 methylation in various cancer detection [38, 94]. % $^{u}C^{u}C$ could differentiate between PBMCs of oral malignancies and healthy individuals as a potential biomarker for cancer diagnosis [38]. Comparable to cancer, our study established the significant association of the hypomethylated loci ($^{u}C^{u}C$) with psoriasis. We found that the % $^{u}C^{u}C$ pattern of LINE-1 from psoriatic patients was significantly higher than epidermal skin from healthy subjects. ROC curve analysis revealed that the potential of $^{u}C^{u}C$ pattern as a biomarker for psoriasis diagnosis with moderate specificity and sensitivity. Furthermore, the association between the percentages of partial methylation (% $^{u}C^{m}C$) of LINE-1 in severe cases of psoriasis when compared to mild psoriasis cases was found. We found that the % $^{u}C^{m}C$ and % partial methylation were significantly lower in severe psoriasis. LINE-1 methylation level ($^{u}C^{m}C$) was suitable for using as novel biomarker with highly specific and sensitive for detecting the severity of psoriasis than other LINE-1 methylation pattern with a cut-off value of $\leq$ 10.87% (AUC = 0.881; sensitivity = 83.33%; specificity = 100.00%).

Finally, the COBRA technique demonstrated that various LINE-1 DNA methylation patterns. Moreover, these patterns were showed the efficacy of particular DNA methylation detection on LINE-1 retrotransposable elements [94]. Furthermore, cancer and normal DNA differentiation was improved by determining with specific loci of LINE-1 [94]. Also, the measurement of LINE-1 methylation level of each locus may improve PCR-based methylation analysis to allow for a potential specificity and sensitivity detection [7, 94].

These can be concluded that LINE-1 hypomethylation play significant role in psoriasis and demonstrated significant down-regulation of gene-containing LINE-1. The distinctive pattern of LINE-1 methylation was reveal in psoriasis. Furthermore, the % $^{u}C^{u}C$ was associated with psoriatic compared to normal individuals whereas; the % $^{u}C^{m}C$ was very highly specific and sensitive for psoriatic severity. LINE-1 methylation may be use as a biomarker for therapy and monitoring of psoriasis in near future.

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APPENDICES

APPENDIX A

BUFFER AND REAGENT

1. 1 M Tris

Tris base	12.11	g
Distilled water	100	g

Adjust volume to 100 ml with distilled water. Adjust pH to 7.2. The solution was mixed and sterilizes by autoclaving at 121 ºC for 15 minutes.

2. EDTA

EDTA	37.22	g
Distilled water	200	ml

Adjust volume to 200 ml with distilled water. The solution was mixed and sterilizes by autoclaving at 121 ºC for 15 minutes. Keep refrigerated.

3. Proteinase K 10 mg/ml

Proteinase K	100	mg
Distilled water	10	ml

Mix the solution and store at -20 ºC

4. 10X Tris-boric acid buffer (TBE) 1000 ml

Tris base	108.0	g
Boric acid	55	g
0.5 m EDTA Ph 8.0	40	ml

Adjust volume to 1 liter with distilled water. The solution was mixed and sterilized by autoclaving at 121 ºC for 15 min.

APPENDIX B

Table 1B: The annotation clusters identified for hypermethylated genes in psoriasis keratinocytes by DAVID Functional Classification Tool

Group number	Associated biology	Enrichment score
1	Signal peptide, glycoprotein and extracellular region	12.99
2	Plasma membrane part	4.90
3	Regulation of glucose	3.07
4	Cytoplasmic and extracellular membrane	2.64
5	C-type lectin binding	2.57
6	Inflammatory response	2.52
7	Protease inhibitor activity, complement and coagulation	2.31
8	Regulation of protein kinase cascade	2.19
9	Embryonic development	2.12
10	Lipoprotein	2.10
11	Response to hormone stimulus	1.99
12	Chemotaxis	1.90
13	Calcium ion homeostasis	1.75
14	Response to growth hormone stimulus	1.73
15	Biological adhesion	1.72
16	Regulation of glucose	1.71
17	Cell migration and motility	1.69
18	Regulation of inflammatory response	1.41

Table 2B: The annotation clusters identified for hypomethylated genes in psoriasis keratinocytes by DAVID Functional Classification Tool

Group number	Associated biology	Enrichment score
1	Plasma membrane part	6.12
2	Lymphocyte activation	5.18
3	Lipoprotein	5.01
4	Tumor antigen	3.46
5	SH3/SH3 adaptor activity, protein binding	3.02
6	Regulation of cellular biosynthetic process Regulation of transcription	2.81
7	Regulation of cytokine biosynthetic process	2.73
8	Inflammatory response	2.79
9	Immune system development	2.16
10	Lymphocyte proliferation	2.08
11	Immune response-regulation signaling transduction	2.01
12	Cytokine activity	1.72
13	Chemotaxis	1.71
14	Interleukin-1	1.68
15	Myeloid cell differentiation	1.61
16	Induction to apoptosis	1.46

Table 3B: Psoriasis down-regulated genes with LINE-1 insertion act as tumor suppressor function

Number	Official name	Full name	Molecular and Phenotypic function
1	MIPOL1	Mirror-image polydactyly1	To be associated only with a developmental disorder, specifically suppresses in vivo tumor formation. Candidate tumor suppressor gene in nasopharyngeal carcinoma. (Cheung, A, PNAS 2009: 106; 14478-14483).
2	PDS5B	PDS5, regulator of cohesion maintenance homolog B	Plays a role in androgen-induced proliferative arrest in prostate cells. This protein is also a negative regulator of cell proliferation. APRIN/Pds5B is lost in many cancers and it is a putative tumor suppressor (Denes, V, Oncogene. 2010 Jun 10;29(23):3446-52).
3	UBR5	Ubiquitin protein ligase E3 component n-recognin 5	Tight regulation of p53 is essential for its central role in maintaining genome stability and tumor prevention. URB5 induces cell cycle arrest by increasing p53 levels (Smits VA, Cell Cycle. 2012 Feb 15;11(4):715-20).
4	DAPK1	Death-associated protein kinase 1	Apoptosis, DAPK1 mediates the G1 phase arrest in human nasopharyngeal carcinoma cells induced by grifolin, a potential antitumor (Luo, XJ, Eur J Pharmacol. 2011 Nov 30;670(2-3):427-34).
5	PTEN	Phosphatase and tensin homolog	The lipid phosphatase activity is critical for its tumor suppressor function. PTEN are tumor suppressor proteins that bind through their C-termini to the PDZ domain containing-hDlg scaffolding protein (Sotelo, NS, J Cell Biochem. 2012). PTEN Protein Phosphatase Activity Correlates with Control of Gene Expression and Invasion, a Tumor-Suppressing Phenotype (Tibarewal P, Sci Signal. 2012 Feb 28;5(213):ra18).
6	STK3	Serine/threonine kinase 3	Stress-activated, pro-apoptotic kinase which, following caspase-cleavage, enters the nucleus and induces chromatin condensation followed by inter-nucleosomal DNA fragmentation. (Growth suppressor), plays a pivotal role in organ size control and tumor suppression by restricting proliferation and promoting apoptosis. (From Gene Cards)
7	MCC	Mutated in colorectal cancers	Negatively regulate cell cycle progression (Ishikawa S, Gene. 2001 Apr 4;267(1):101-10).

Table 4B: Two hundred and thirty down-regulated genes containing LINE-1 in psoriasis keratinocytes from both psoriasis expression arrays (GSE14905 and GSE13355)

WFDC2	WAP four-disulfide core domain 2
C6orf199	cordon-bleu homolog (mouse)
COBL	Protein cordon-bleu
ACVR1C	Activin A receptor, type IC
MIPOL1	Mirror-image polydactyly 1
HERPUD2	HERPUD family member 2
SLC2A13	Solute carrier family 2 (facilitated glucose transporter), member 13
CASC4	Cancer susceptibility candidate gene 4 protein; gene associated with HER-2/neu overexpression
RICTOR	RPTOR independent companion of MTOR, complex 2
CADM2	Cell adhesion molecule 2
SCAMP1	Secretory carrier membrane protein 1
SYN2	Synapsin II
PDE5A	Phosphodiesterase 5A, cGMP-specific
RASEF	RAS and EF-hand domain containing
PARD3B	Par-3 partitioning defective 3 homolog B
DST	Dystonin
NEGR1	Neuronal growth regulator 1
PALM2	Paralemmin 2
PALM2-AKAP2	PALM2-AKAP2 readthrough
ITGB1	Integrin, beta 1
MACROD2	MACRO domain containing 2
CHRM3	Cholinergic receptor, muscarinic 3
ZNF644	Zinc finger protein 644
VPS13B	Vacuolar protein sorting 13 homolog B

CYP39A1	Cytochrome P450, family 39, subfamily A, polypeptide 1
MSRB3	Methionine sulfoxide reductase B3
KIAA1128	Family with sequence similarity 190, member B
C3orf15	Chromosome 3 open reading frame 15
ZNF638	Zinc finger protein 638
TTBK2	Tau tubulin kinase 2
HNMT	Histamine N-methyltransferase
FIP1L1	FIP1 like 1 (S. cerevisiae)
THADA	Thyroid adenoma associated
PPA2	Pyrophosphatase (inorganic) 2
TTC12	Tetratricopeptide repeat domain 12
PDS5B	PDS5, regulator of cohesion maintenance, homolog B (S. cerevisiae)
STAU2	Staufen, RNA binding protein, homolog 2 (Drosophila)
L3MBTL4	l(3)mbt-like 4 (Drosophila)
PDE4D	Phosphodiesterase 4D, cAMP-specific
RABGAP1L	RAB GTPase activating protein 1-like
RALGPS1	Ral GEF with PH domain and SH3 binding motif 1
PDGFRA	Platelet-derived growth factor receptor, alpha polypeptide
PRR16	Proline rich 16
ZNF678	Zinc finger protein 678
FUT8	Fucosyltransferase 8 (alpha (1,6) fucosyltransferase)
OTUD7B	OTU domain containing 7B
FHL5	Four and a half LIM domains 5
EVC2	Ellis van Creveld syndrome 2
ZAK	Sterile alpha motif and leucine zipper containing kinase AZK
MAGI1	Membrane associated guanylate kinase, WW and PDZ domain containing 1
WDR19	WD repeat domain 19

CAMTA1	Calmodulin binding transcription activator 1
CLASP2	Cytoplasmic linker associated protein 2
SDCCAG10	CWC27 spliceosome-associated protein homolog (S. cerevisiae)
ANTXR2	Anthrax toxin receptor 2
BBS9	Bardet-Biedl syndrome 9
CFI	Complement factor I
IMMP2L	IMP2 inner mitochondrial membrane peptidase-like (S. cerevisiae)
PTPRM	Protein tyrosine phosphatase, receptor type, M
ZNF385B	Zinc finger protein 385B
UBR5	Ubiquitin protein ligase E3 component n-recognin 5
ITFG1	Integrin alpha FG-GAP repeat containing 1
JMJD2C	Lysine (K)-specific demethylase 4C
SLC8A1	Solute carrier family 8 (sodium/calcium exchanger), member 1
SORCS1	Sortilin-related VPS10 domain containing receptor 1
LEPR	Leptin receptor
ITGBL1	Integrin, beta-like 1 (with EGF-like repeat domains)
PLD1	Phospholipase D1, phosphatidylcholine-specific
BBX	Bobby sox homolog (Drosophila)
DPY19L2	Dpy-19-like 2 (C. elegans)
PHIP	Pleckstrin homology domain interacting protein
RASAL2	RAS protein activator like 2
ATP6V1H	ATPase, H+ transporting, lysosomal 50/57kDa, V1 subunit H
ZHX2	Zinc fingers and homeoboxes 2
CHIC1	Cysteine-rich hydrophobic domain 1
FGGY	Carbohydrate kinase domain containing
USP31	Ubiquitin specific peptidase 31
DIXDC1	DIX domain containing 1
UACA	Uveal autoantigen with coiled-coil domains and ankyrin repeats

LPP	LIM domain containing preferred translocation partner in lipoma
DNM3	Dynamin 3
EDIL3	EGF-like repeats and discoidin I-like domains 3
DOCK4	Dedicator of cytokinesis 4
NRXN1	Neurexin 1
FNIP1	Folliculin interacting protein 1
C21orf34	Chromosome 21 open reading frame 34
PTK2	PTK2 protein tyrosine kinase 2
C15orf29	Chromosome 15 open reading frame 29
ADAMTSL3	ADAMTS-like 3
MCCC2	Methylcrotonoyl-CoA carboxylase 2
PRKCA	Protein kinase C, alpha
LOC389634	No
SPAG16	Sperm associated antigen 16
CCDC66	Coiled-coil domain containing 66
RBMS3	RNA binding motif, single stranded interacting protein 3
HIVEP1	Human immunodeficiency virus type I enhancer binding protein 1
DNAH3	Dynein, axonemal, heavy chain 3
ARHGEF7	Rho guanine nucleotide exchange factor (GEF) 7
LYST	Lysosomal trafficking regulator
RORA	RAR-related orphan receptor A
TBC1D8B	TBC1 domain family, member 8B
SOX6	SRY (sex determining region Y)-box 6
WDR72	WD repeat domain 72
NEK1	NIMA (never in mitosis gene a)-related kinase 1
ATP9B	ATPase, class II, type 9B
CWF19L2	CWF19-like 2, cell cycle control
PLCE1	Phospholipase C, epsilon 1

FAM13A1	Family with sequence similarity 13, member A
ZNF93	Zinc finger protein 93
NSUN6	NOP2/Sun domain family, member 6
PGM2L1	Phosphoglucomutase 2-like 1
TTC3	Tetratricopeptide repeat domain 3
SOX5	SRY (sex determining region Y)-box 5
FMO5	Flavin containing monooxygenase 5
APP	Amyloid beta (A4) precursor protein
DDX24	DEAD (Asp-Glu-Ala-Asp) box polypeptide 24
CUL3	Cullin 3
IGF2R	Insulin-like growth factor 2 receptor
PYGB	Phosphorylase, glycogen; brain
PPP1R12A	Protein phosphatase 1, regulatory subunit 12A
SEC63	SEC63 homolog (S. cerevisiae)
CPD	Carboxypeptidase D
ST6GAL1	ST6 beta-galactosamide alpha-2,6-sialyltranferase 1
JARID1A	Lysine (K)-specific demethylase 5A
STAG1	Stromal antigen 1
FNDC3A	Fibronectin type III domain containing 3A
SENP6	SUMO1/sentrin specific peptidase 6
SPOCK1	Sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican) 1
ATG5	ATG5 autophagy related 5 homolog (S. cerevisiae)
APPBP2	Amyloid beta precursor protein (cytoplasmic tail) binding protein 2
ITPR2	Inositol 1,4,5-trisphosphate receptor, type 2
PHKB	Phosphorylase kinase, beta
NAG	Neuroblastoma amplified sequence
PTPRK	Protein tyrosine phosphatase, receptor type, K

BAZ2B	Bromodomain adjacent to zinc finger domain, 2B
DAPK1	Death-associated protein kinase 1
UVRAG	UV radiation resistance associated gene
RCAN2	Regulator of calcineurin 2
PGCP	Plasma glutamate carboxypeptidase
MET	Proto-oncogene (hepatocyte growth factor receptor)
TMOD1	Tropomodulin 1
PRKAR2B	Protein kinase, cAMP-dependent, regulatory, type II, beta
SSBP2	Single-stranded DNA binding protein 2
DMD	Dystrophin
PLCB4	Phospholipase C, beta 4
HEPH	Hephaestin
NEBL	Nebulette
UTX	Lysine (K)-specific demethylase 6A
ICA1	Islet cell autoantigen 1, 69kDa
SCHIP1	Schwannomin interacting protein 1
PHACTR2	Phosphatase and actin regulator 2
PTEN	Phosphatase and tensin homolog
STK3	Serine/threonine kinase 3
TPST1	Tyrosylprotein sulfotransferase 1
GULP1	GULP, engulfment adaptor PTB domain containing 1
EDNRB	Endothelin receptor type B
KIAA0427	CBP80/20-dependent translation initiation factor
NEO1	Neogenin 1
GOLIM4	Golgi integral membrane protein 4
SKAP2	Src kinase associated phosphoprotein 2
VPRBP	Vpr (HIV-1) binding protein
MAOA	Monoamine oxidase A

ELMO1	Engulfment and cell motility 1
CHL1	Cell adhesion molecule with homology to L1CAM (close homolog of L1)
PFTK1	Cyclin-dependent kinase 14
DKFZP564O0823	Prostate androgen-regulated mucin-like protein 1
GMDS	GDP-mannose 4,6-dehydratase
AUH	AU RNA binding protein/enoyl-CoA hydratase
ARHGAP26	Rho GTPase activating protein 26
LAMA2	Laminin, alpha 2
UST	Uronyl-2-sulfotransferase
DDR2	Discoidin domain receptor tyrosine kinase 2
GALK2	Galactokinase 2
ESR1	Estrogen receptor 1
NR3C2	Nuclear receptor subfamily 3
GLRB	Glycine receptor, beta
DBT	Dihydrolipoamide branched chain transacylase E2
ZBTB20	Zinc finger and BTB domain containing 20
DCLK1	Doublecortin-like kinase 1
GHR	Growth hormone receptor
DIAPH2	Diaphanous homolog 2 (Drosophila)
PRRG1	Proline rich Gla (G-carboxyglutamic acid) 1
DTNA	Dystrobrevin, alpha
CYP3A5	Cytochrome P450, family 3, subfamily A, polypeptide 5
NRXN3	Neurexin 3
PDE4DIP	Phosphodiesterase 4D interacting protein
PRKD1	Protein kinase D1
RELN	Reelin
CREB5	cAMP responsive element binding protein 5

SETBP1	SET binding protein 1
EPHA3	EPH receptor A3
MCC	Mutated in colorectal cancers
MEOX2	Mesenchyme homeobox 2
EDA	Ectodysplasin A
DLG2	Discs, large homolog 2 (Drosophila)
EGF	Epidermal growth factor
RYR3	Ryanodine receptor 3
OPHN1	Oligophrenin 1
PRLR	Prolactin receptor
ANK3	Ankyrin 3, node of Ranvier (ankyrin G)
LDB2	LIM domain binding 2
APBA1	Amyloid beta (A4) precursor protein-binding, family A, member 1
IL7	Interleukin 7
PGDS	Prostaglandin D2 synthase 21kDa (brain)
MAP3K7	Mitogen-activated protein kinase kinase kinase 7
SEMA3E	Sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3E
MMP16	Matrix metallopeptidase 16 (membrane-inserted)
ALDH1A2	Aldehyde dehydrogenase 1 family, member A2
SLC16A7	Solute carrier family 16, member 7 (monocarboxylic acid transporter 2)
PRKG1	Protein kinase, cGMP-dependent, type I
CDH12	Cadherin 12, type 2 (N-cadherin 2)
DZIP3	DAZ interacting protein 3, zinc finger
ZNF343	Zinc finger protein 343
RAB28	The Rab subfamily of Ras-related small GTPases
P4HA1	Prolyl 4-hydroxylase, alpha polypeptide I
MAGI2	Membrane associated guanylate kinase, WW and PDZ domain2

DAZ1	Deleted in azoospermia 1
DAZ2	Deleted in azoospermia 2
DAZ3	Deleted in azoospermia 3
DAZ4	Deleted in azoospermia 4
MEF2C	Myocyte enhancer factor 2C
C18orf1	Chromosome 18 open reading frame 1
COL21A1	Collagen, type XXI, alpha 1
NFATC1	Nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1
KCNAB1	Potassium voltage-gated channel, shaker-related subfamily, beta member 1
EVI5	Ecotropic viral integration site 5
PGR	Progesterone receptor
AKAP13	A kinase (PRKA) anchor protein 13
SNTB1	Syntrophin, beta 1 (dystrophin-associated protein A1, 59kDa, basic component 1)
ANKHD1	Ankyrin repeat and KH domain containing 1

Table 5B: Thirty-eight down-regulated genes containing LINE-1 with hypomethylation in psoriasis keratinocytes from both psoriasis expression arrays (GSE14905 and GSE13355)

GRIA3	Glutamate receptor, ionotrophic, AMPA 3
OPHN1	Oligophrenin 1
AR	Androgen receptor
ACSL4	Acyl-CoA synthetase long-chain family member 4
EDA	Ectodysplasin A
HNT	Neurotrimin
NHS	Nance-Horan syndrome (congenital cataracts and dental anomalies)
ARHGEF9	Cdc42 guanine nucleotide exchange factor (GEF) 9
MAOA	Monoamine oxidase A
GPC3	Glypican 3
ATRX	Alpha thalassemia/mental retardation syndrome X-linked (RAD54 homolog, S. cerevisiae)
AFF2	AF4/FMR2 family, member 2
YIPF6	Yip1 domain family, member 6
SLC22A3	Solute carrier family 22 (extraneuronal monoamine transporter), member 3 or Organic cation transporter 3 (OCT3)
SH3BGRL	SH3 domain binding glutamic acid-rich protein like
NEBL	Nebulette
EPB41L3	Erythrocyte membrane protein band 4.1-like 3 (Protein 4.1B/Dal-1)
RGS7	Regulator of G-protein signaling 7

TMLHE	Trimethyllysine hydroxylase, epsilon
ESR1	Estrogen receptor 1
BRWD3	Bromodomain and WD repeat domain containing 3
FIP1L1	FIP1 like 1 (S. cerevisiae)
CHM	Choroideremia (Rab escort protein 1)
CNKSR2	Connector enhancer of kinase suppressor of Ras 2
PLCL1	Phospholipase C-like 1
ATP8B1	ATPase, class I, type 8B, member 1
CPD	Carboxypeptidase D
THRB	Thyroid hormone receptor, beta
NEK7	NIMA (never in mitosis gene a)-related kinase 7
DIAPH2	Diaphanous homolog 2 (Drosophila)
LNX1	Ligand of numb-protein X 1
TUSC3	Tumor suppressor candidate 3
NEO1	Neogenin homolog 1 (chicken)
SPATA18	Spermatogenesis associated 18 homolog (rat)

Table 6B: Four Up-regulated genes containing LINE-1 with hypermethylation in psoriasis keratinocytes from both psoriasis expression arrays (GSE14905 and GSE13355)

EDNRB	Endothelin receptor type B
XYLB	Xylulokinase homolog (H. influenzae)
LY96	Lymphocyte antigen 96
TRPC4	Transient receptor potential cation channel, subfamily C, member 4

BIOGRAPHY

Mr. Surasak Yooyongsatit was born on March 20th, 1982 in Kanchanaburi, Thailand. He graduated with the Bachelor degree of Science in Biology from Silpakorn University in 2005 and Master degree of Science in Medical Microbiology from Chulalongkorn University in 2007. He got Development and Promotion of Science and Technology Talents Project (DPST) Scholarship and participated in Medical Microbiology program, Graduate School, Chulalongkorn University for philosophy degree in 2012.