The promoter methylation of S-adenosylmethionine decarboxylase-1 gene in psoriasis

การเกิด ดี เอ็น เอ เมทิลเลชั่น ที่บริเวณโปรโมเตอร์ของยีน S-adenosylmethionine decarboxylase-1 ในโรคสะเก็ดเงิน

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ABSTRACT

S-adenosylmethionine decarboxylase-1 (AMD1) is an initial enzyme for polyamine biosynthetic pathway. Recent published microarray data shown that, the expression of *AMD1* is up-regulated in epidermis from psoriatic skins. We analyzed *AMD1* promoter methylation in microdissected epithelium of skin and total leukocytes from patients with psoriasis and normal control. The methylation both epidermis and total leukocytes were not significantly different between psoriasis and normal control. Our results suggest that, *AMD1* mRNA expression in psoriasis is not controlled by *AMD1* promoter methylation.

บทคัดย่อ

S-adenosylmethionine decarboxylase**-1** (*AMD1*) เป็นเอนไซม์สำคัญในกระบวนการสังเคราะห์สารในกลุ่ม polyamine ซึ่งผลิตภัณฑ์ของกระบวนการนี้เกี่ยวข้องกับการเติบโตของเซลล์ การแบ่งตัวและเพิ่มจำนวน รวมทั้งกระบวนเหนี่ยวนำให้เกิดมะเร็ง มีรายงานการวิจัยแสดงออกของยีนในระดับเอ็ม อาร์ เอ็น เอ โดยใช้เทคนิคไมโครอาเรย์พบว่าการแสดงออกของยีน AMD1 เพิ่มขึ้นในผิวหนังชั้นหนังกำพร้าของผู้ป่วยโรคสะเก็ดเงิน เราได้ทำการทดสอบการเกิด ดี เอ็น เอ เมทิลเลชั่นที่บริเวณโปรโมเตอร์ของยีน *AMD1* ในผิวหนังชั้นหนังกำพร้าที่ได้จากการทำไมโครไดเส็ค และใน total leukocyte ของผู้ป่วยโรคสะเก็ดเงินและคนปกติ พบว่าระดับการเกิด ดี เอ็น เอ เมทิลเลชั่นดังกล่าวไม่มีความแตกต่างกัน จากผลการทดลองอาจสรุปได้ว่า การเพิ่มขึ้นในระดับ เอ็ม อาร์ เอ็น เอ ของยีน *AMD1* ไม่ได้มาจากกลไกการควบคุมด้วย การเกิด ดี เอ็น เอ ดี เมทิลเลชั่น ที่บริเวณโปรโมเตอร์ของยีน *AMD1*

Key Words : Psoriasis, methylation, S-adenosylmethionine decarboxylase**-1**

คำสำคัญ : โรคสะเก็ดเงิน ดี เอ็น เอ เมทิลเลชั่น S-adenosylmethionine decarboxylase**-1**

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Introduction

Psoriasis is a chronic, inflammatory skin disease that affects about 1-3% in many populations. Thick, red, sharply plaques covered with silvery scale are the hallmark of psoriasis. Histologically, keratinocytes show abnormal differentiation and hyperproliferation in dermis, the inflammatory cells infiltrated in both the dermis and the epidermis, and neo blood vessel (neoangiogenesis) (Ortonne,1999; Lowes, 2007). Plaque-type psoriasis is the most common clinical type of the disease, occurring more than 80% of cases (Biondi, 1989). The cause of psoriasis is unknown. Genetic and environmental factors are believed to be contributing factor in development of disease (Bowcock and Krueger, 2005). S-adenosylmethionine decarboxylase (AdometDC, AMD1) is transcribed by *AMD1* gene on chromosome 6. *S*-Adenosylmethionine Decarboxylase (AdoMetDC) is an initial enzyme in the polyamine synthetic pathway. AdoMetDC catalyzes the removal of the carboxylate group from *S*-adenosylmethionine (AdoMet) to *S*-adenosyl-5′-3-methylthiopropylamine. This acts as the *n*-propylamine donor to synthesize spermidine and spermine from putrescine. Thus the expression and activity of AdometDC are related to concentration of spermidine and spermine. These products are low molecular weight aliphatic amines essential for cellular proliferation, differentiation and tumor promotion (Bale, 2009). Recently, Bowcock et al, and Zhou et al, study the expression profiles from uninvolved and lesional psoriatic skin by microarray show that AMD1 mRNA is higher upregulation in lesional psoriatic skin (Zhou, 2003; Reischl, 2007).

DNA methylation is the part of Epigenetic. DNA methylation is a fundamental mechanism in controlling biological process especially in the regulation of genes and the stability of the genome without changing the DNA sequence. DNA methylation mechanism occurs by add the methyl group to the 5- carbon of CpG dinucleotide by DNA methyltransferase. DNA methylation usually occurs in the CpG islands. Methylation in promoters of DNA usually down regulate of mRNA transcription either by directly interfering with the binding of transcription factors or by allowing chromatin condensation resulting in loss of gene function (Attwood, 2002; Luczak, 2006; Rodenhiser, 2006).

To date, they have only been four studies in DNA methylation in psoriasis. Ruchusatsawat et al., observed a significant degree of demethylation in SHP-1 promoter2 in psoriasis with reverse correlation of SHP-1 isoform2 mRNA level in psoriatic skin (Ruchusatsawat, 2006). Other groups reported demethylation of p16 gene in PBMCs from psoriasis patients and hypermethylation of psoriatic epidermis (Zhang, 2007; Chen, 2008). Finally, Zhang K. et al, found that p15 and p21 promoter hypomethylation related to increased transcription levels in psoriasis comparison to normal volunteers (Zhang, 2009).

We hypothesized that the up-regulation of *AMD1* expression is mediated by the demethylation of *AMD1* promoter.

Materials and methods

Cell lines, patient samples and DNA extraction

Twelve epithelial cell lines, HCT16, HSC2, OM1, OM2, TSU, Hela, Caski, RKO, SW480, HEp2, HepG2 and HaCat, and hematopoietic cell lines, Daudi, Jurkat, Molt4, HL-60, K562, UACC903 were used.

The paraffin-embedded tissues from normal skin (n=6) and lesional psoriasic skin (n=6) were dissected using laser-capture microdissection. Normal skins were collected during plastic surgery and all patients taking biopsy were diagnosed clinically and pathologically by an experienced dermatologist.

Total leukocytes were separated from fresh blood sample carry in heparinized venous blood by centrifugation.

Genomic DNA was extracted from all of the samples with QIAamp DNA mini kit™ (QIAGEN).

Methylation Analysis

After extraction, all genomic DNA samples were treated with sodium bisulfite (Zymo Research, Orange, CA, USA), according to the manufacturer's specifications. The methylation status of *AMD1* promoter was determined by methylation specific PCR (MSP) which MSP primers specifically recognized the methylated or unmethylated DNA after bisulfite conversion. Methylation-specific primer sequences were 5' -GAG-TCG-GTT-AGA-GTT-CGA-GTC- 3' and 5'- CCA-AAA-AAT-AAC-GCA-TCG-T- 3' , and unmethylation-specific primer sequences were 5' -TAG-GAG-TTG-GTT-AGA-GTT-TGA-GTT-3' and 5' -CCA-AAA-AAT-AAC-ACA-TCA-TCC-C- 3'. Both of amplification products, 149 bp were visualized by UV illumination on 2% agarose gel that contained ethidium bromide.

Cloning and Sequencing

To observe the accuracy of MSP, TSU and HaCat DNA which already processed by Bisulfite conversion were amplified *AMD1* promoter by polymerase chain reaction using the following primers: AMD1seqF 5'-ATG-TTT-TTT-GTT-AAT-TAT-TTT-TTT-TTT-3' and AMD1seqR 5'-ATA-AAA-ACA-AAT-ACA-ATT-CAA-TCT-CT-3'. Condition for PCR were follows : Activation hot start for 5 min at 95°C followed by 35 cycles of denaturing for 60 sec at 95°C, annealing for 60 sec at 60°C and extension for 60 sec at 72°C. The PCR products were cloned into the pGEM-T easy cloning vector (Promega). DNA sequencing was performed on an Applied Biosystems DNA sequencer using M13primers.

Results and discussion

The previous study showed AMD1 mRNA was up-regulated in lesional psoriatic skin. We hypothesize that AMD1 expression is controlled by promoter methylation. Then, we investigated the methylation level of AMD1 promoter in several cell lines by MSP technique. Almost of cell lines (HCT16, HSC2, OM2, TSU, Hela, Caski, RKO, SW480, HEp2, HepG2, Daudi, Jurkat, Molt4, HL-60, K562 and UACC903) revealed only unmethylatated band. The OM1 and HaCat cell lines revealed both methylated and unmethylated bands as shown in Figure 1a. To verify MSP technique, bisulfite cloning and sequencing of AMD1 promoter methylation of TSU and Hacat was done as shown in Figure 1b.

To explore methylation status of AMD1 promoter in psoriatic skins, lesional skin from 5 psoriatic patients and normal skin from 5 healthy subjects was compared using MSP. The methylation status of AMD1 promoter was not significantly difference between psoriasis and normal controls (Fig 2a). We also compared AMD1 promoter methylation in total leukocytes from psoriatic patients and healthy subjects. The AMD1 promoter methylation was not different in psoriasis compared to normal controls. Four of 5 psoriatic patients and normal controls showed only unmethylated bands. One of 5 psoriatic patients and normal controls showed both methylated and unmethylated bands (Fig 2b).

Conclusions

The present study demonstrated promoter methylation of AMD1 was not different between psoriasis patients compared to normal healthy subjects. Previous study shows up-regulation of *AMD1* mRNA in psoriatic epidermis. Thus, the mechanism controlled up-regulation of *AMD1* mRNA in psoriatic epidermis, should not be DNA methylation. Further studies need to clarify pathogenic mechanism in up-regulation of *AMD1* mRNA expression in psoriasis.

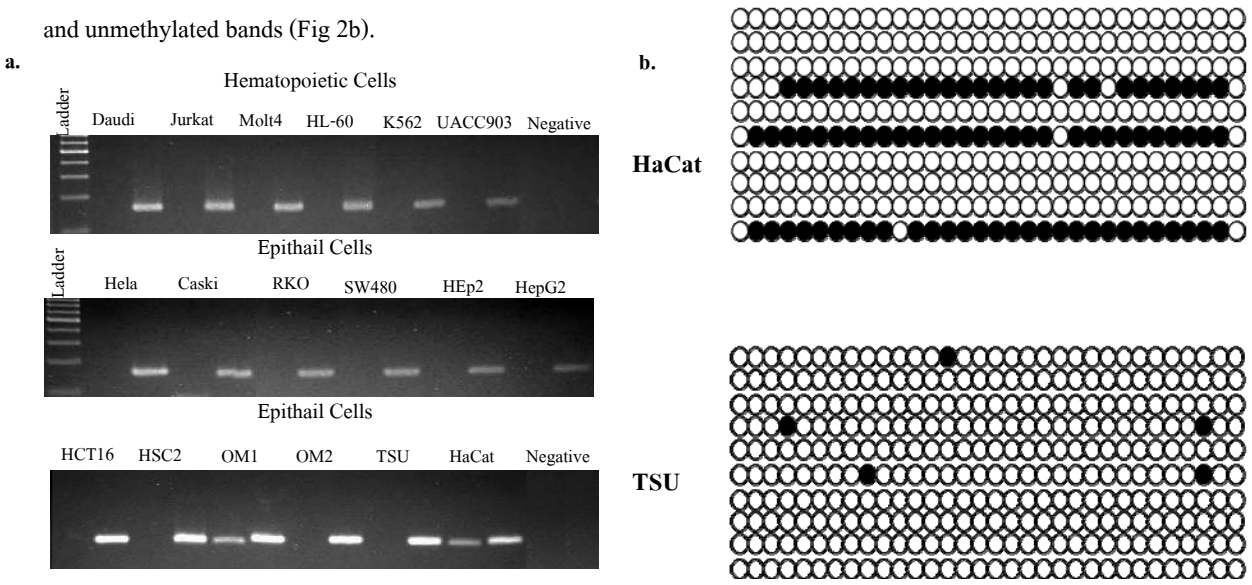

Figure 1 *AMD1* promoter methylation in various cell lines.

a) Promoter methylation is detectable by MSP. U is unmethylated amplicons, and M is methylated amplicons. Both of amplification products are 149 bp. Cell sources of genomic DNA are listed above each lane.

b) Methylation status of CpG nucleotides at promoter of TSU and HaCat by bisulfite sequencing. The numbers of circle indicate CpG nucleotides. Each circle represents the methylation status of each selected clone. Black and white circles are methylated and nonmethylated CpG dinucleotides, respectively.

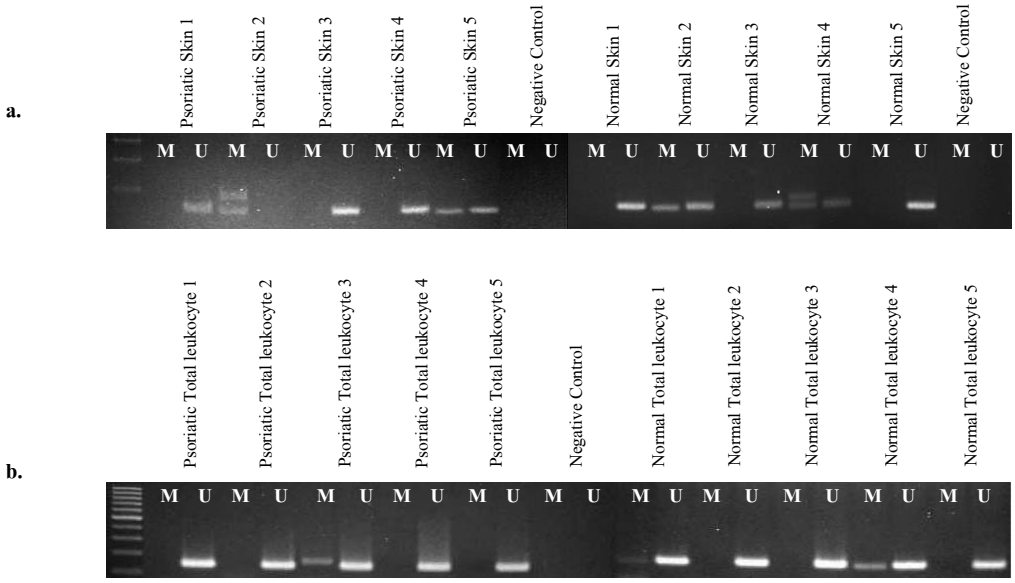

Figure *AMD1* **2** promoter methylation of psoriatic skins and normal skins (a.) and total leukocytes (b.) are detectable by MSP. U is unmethylated amplicons, and M is methylated amplicons.

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