Inhibition of CpG methylation improves the barrier integrity of bronchial epithelial cells in asthma.

Paulina Wawrzyniak[1], Krzysztof Krawczyk[1], Acharya Swati[2], Ge Tan[1], Marcin Wawrzyniak[1], Emmanuel Karouzakis[3], Anita Dreher[4], Bogdan Jakiela[5], Can Altunbulakli[6], Marek Sanak[5], Liam O'Mahony[7], Kari Nadeau[2], and Cezmi Akdis[3]

[1]Swiss Institute of Allergy and Asthma Research
[2]Stanford University
[3]University of Zurich
[4]Swiss Institute of Allergy and Asthma Research (SIAF)
[5]Jagiellonian University Medical College
[6]Swiss Institute of Allergy and Asthma Researc
[7]University College Cork National University of Ireland

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To the Editor:

Asthma is a complex and heterogeneous chronic airway inflammatory disease with the involvement of environmental factors through epigenetic mechanisms.[1] Accordingly, repeated injury, repair and regeneration of the airway epithelium following exposure to environmental factors and inflammation results in histological changes and functional abnormalities in the airway mucosal epithelium, which are associated with the pathophysiology of asthma.[2]Epigenetics is defined by heritable changes in gene expression without changes in the DNA sequence.[3] Regulation of gene expression is mediated by different mechanisms such as DNA methylation, histone modifications and RNA-associated silencing by small non-coding RNAs. CpG sites are dinucleotides consisting of guanine and cytosine concentrated in clusters referred to CpG islands found at important regulatory sites, such as promoter and enhancer regions.[4] Their *de novo* methylation occurs in response to various cellular stressors and signals by DNA methyltransferases (DNMT3a and 3b), which add a methyl group to position 5 of cytosine residues at the CpG site. During DNA replication both of the separated strands of DNA carry one methylated cytosine to be used as a template for duplication. Daughter DNA duplex strands will thus be hemi-methylated, which is recognized by a different DNA methyltransferase isoform (DNMT1).[5] Because DNA methylation is a reversible process, the DNMTs are considered as a therapeutic target. Several DNMT inhibitors have been identified recently, among the non-nucleoside inhibitors, 4-aminoquoline-based inhibitors, such as SGI-1027 showed potent inhibitory activity. SGI-1027 occupies the binding site of DNMTs resulting in the prevention of access of target DNA to the substrate binding pocket.[6]

We have demonstrated in previous studies from our laboratory that human primary bronchial epithelial cells (HBEC) isolated from patients with asthma showed lower barrier integrity compared to controls.[7] To investigate the level of global methylation in HBEC, we investigated control and asthma samples for the long interspersed nuclear element-1 (LINE-1) methylation levels (Figure 1A). HBEC from asthma patients showed a tendency for higher global methylation levels, together with higher expression of 5-methylcytosine (5-mc) in immunofluorescence staining (Figure 1B). Next, we performed methylation profiling (Illumina Infinium EPIC array) to investigate genes methylated in ALI cultures of HBEC. Interestingly, in a highly methylated

group of top 100 genes, we found many genes associated with cell growth, ion transport, and cytoskeletal remodeling (Figure S1). We kept our attention on the methylated epigenetic and tight junction (TJ) genes and further focused on TJs, especially zonula occludens and claudins which showed higher methylation in contrast to occludin, which was not methylated (Figure S2). As higher methylation levels were observed in HBEC of asthmatic origin, we inhibited the DNA methyltransferase enzyme with a specific inhibitor, SGI-1027, to demonstrate the role of CpG methylation on epithelial barrier integrity. ALI cultures were treated with the DNA methyltransferase inhibitor for 72 hours. Significantly decreased expression of 5-mc was observed after 48 hours of DNA-methyltransferase inhibition, demonstrating that the methylation of 5-methylcytosine (5-mc) in bronchial epithelium was reversed (Figure 2A). This prompted us to investigate the changes triggered by the inhibitor in epithelial cells. Further experiments showed increased transepithelial electric resistance (TER) in bronchial epithelial cells, in ALI from asthmatic donors after 48 hours of DNMT inhibition (Figure 2B). The link between barrier integrity and TER results were confirmed by the significantly decreased paracellular passage of FITC-labelled 4kD dextran after inhibition of DNMTs (Figure 2C). The reconstitution of TER in asthmatic ALI was associated with decreased protein DNMT1 expression and increased ZO-1 and claudin-18 proteins (Figure 2D). We also observed increased claudin-4, but not occludin expression upon DNMT inhibition (Figure S3). Increased expression of ZO-1 with an intact and honeycomb-like structure in the immunofluorescence staining of bronchial epithelial cells confirmed the effect on protein expression of bronchial epithelial barrier in asthma donors (Figure S4).

Defective epithelial barrier has been established in asthma in addition to several chronic inflammatory diseases.[8] Direct targeting of the epithelial barrier leakiness for the treatments represents an important target, however so far there is no treatment possibility targeting epigenetic mechanisms. The present study demonstrates an increased global methylation level in HBEC from asthmatic individuals. CpG methylation of specific genes is essential for the defect of epithelial barrier integrity, which is reversed upon DNMT inhibition. The inversion of CpG methylation, restores leakiness in the epithelium in asthma by increasing TER, decreasing paracellular flux and improves the structure of bronchial epithelial cells by increasing the expression of TJ proteins. The better understanding of the importance of epigenetic memory in chronic tissue inflammatory diseases together with the availability of treatment modalities targeting epigenetic mechanisms and transition of these molecules into the clinical studies may lead to curative treatment of allergic and autoimmune inflammatory diseases.[9]

Paulina Wawrzyniak[1], PhD,

Krzysztof Krawczyk[1,3], MSc,

Swati Acharya[5], PhD,

Ge Tan[1,7], PhD,

Marcin Wawrzyniak[1], PhD,

Emmanuel Karouzakis[4], PhD,

Anita Dreher, Sci. Tech.,

Bogdan Jakiela[2], MD, PhD,

Can Altunbulakli[1], PhD,

Marek Sanak[2], MD, PhD,

Liam O'Mahony[1,6], PD, PhD,

Kari Nadeau[5], MD, PhD,

Cezmi A. Akdis[1], MD

[1]Swiss Institute of Allergy and Asthma Research (SIAF), University of Zürich, Davos, Switzerland, Christine Kühne-Center for Allergy Research and Education (CK-CARE)

[2]Department of Medicine, Jagiellonian University Medical College, Krakow, Poland

[3]Faculty of Biology and Environmental Protection, Department of Cellular Immunology, Lodz, Poland

[4]Department of Rheumatology, University Hospital of Zurich

[5]Departament of Medicine, Stanford University, United States

[6] Department of Medicine and School of Microbiology, APC Microbiome Ireland, University College Cork, Cork, Ireland.

[7] Functional Genomics Center Zurich, ETH Zurich/University of Zurich

Corresponding author:

Paulina Wawrzyniak

Swiss Institute of Allergy and Asthma Research (SIAF), University of Zürich, Davos, Switzerland

Obere Strasse 22,

7270 Davos, Switzerland

Tel: +41 81 410 08 48

Fax: +41 81 410 08 40

paulina.wawrzyniak@uzh.ch

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Abbreviations:

ALI: air-liquid interface,

BEGM: bronchial epithelial growth medium,

DNMT: DNA methyltranferase

FITC: fluorescein isothiocyanate,

HBEC: human bronchial epithelial cells,

LINE-1: long interspersed nuclear element-1

5-mc: 5-methylcytosine

TER: transepithelial electric resistance,

ZO: zonula ocludens

FIGURE LEGENDS :

Figure 1 . Increased global methylation in bronchial epithelial cells in asthma

1. Global LINE-1 methylation levels in bronchial epithelial cells from healthy control (n=4) and asthma patients (n=3) on the baseline. (B) Representative immunofluorescence staining and data showing the mean intensity of ALI cultures of differentiated bronchial epithelial cells from controls (n=5) and asthma patients (n=8) for 5-methylcytosine (5mc). Data are presented as mean±SD, (**P < 0.01), Mann Whitney U test.
2. **Figure 2. DNA methyltransferase inhibitor decreases global methylation and improve the barrier integrity of bronchial epithelial cells in asthma**

Representative immunofluorescence staining of 5-methylcytosine (5mc) and data showing the mean intensity in asthmatic ALI cultures of HBEC treated with 8 μM DNA methyltransferase inhibiitor, SGI-1027 for 48 hours, (n=8). (B) Increased TER in ALI cultures of HBEC from asthmatic individuals treated with 8 μM of DNA methyltransferase inhibitor-SGI-1027, n=5 per group. (C) The decreased paracellular flux of 4kDa FITC-dextran in response to 48 hours treatment of 8 μM DNA methyltransferase inhibitor SGI-1027, n=5 per group. (D) Western blots of DNMT1, ZO-1 and claudin-18 from ALI cultures of asthmatic HBEC treated with DNA methyltransferase inhibitor-SGI-1027 in diferent doses (2 μM, 4 μM, 8 μM, 16 μM), n=5. Data represent mean±SD, (*$P < 0.05$), (**P < 0.01), Mann Whitney U test.

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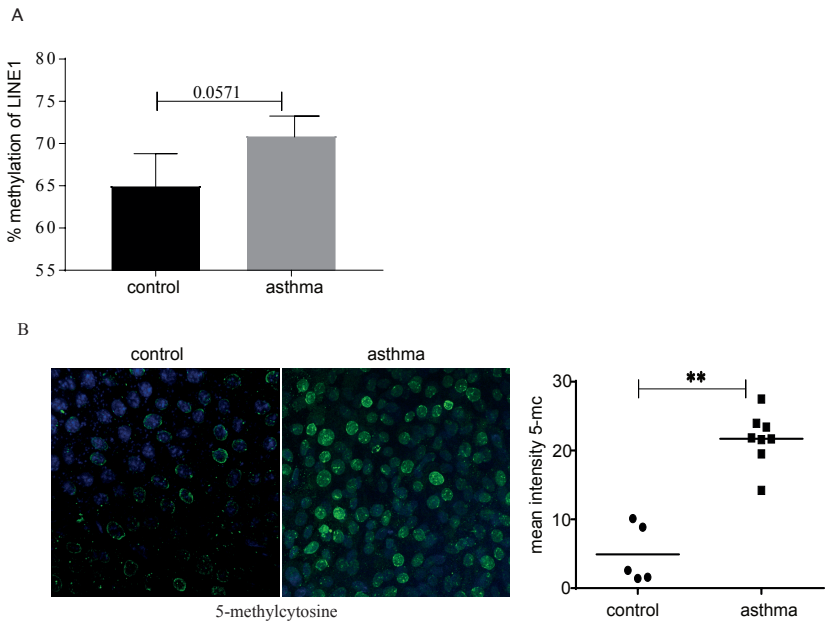

Figure 1. Wawrzyniak et al.

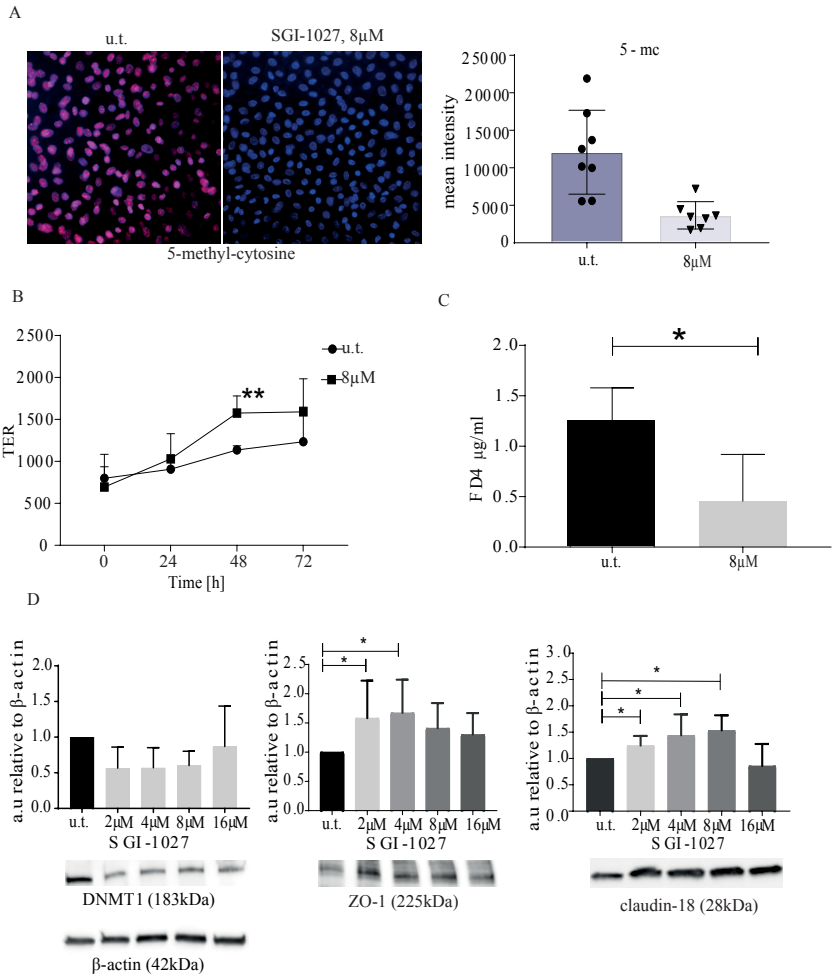

Figure. 2 Wawrzyniak et al.