**Genome-wide DNA methylation patterns in CD4+ T cells from patients with rheumatoid arthritis**

Shicheng Guo1,Dongyi He8\*

1Department of Rheumatology, Guanghua Integrative Medicine Hospital, Changning District, Shanghai 200052, China

5Guangdong Provincial Key Laboratory of Medical Molecular Diagnostics, Dongguan Scientific Research Center, Guangdong Medical University, Dongguan 523808, China

Corresponding authors: Dongyi He, Department of Rheumatology, Guanghua Integrative Medicine Hospital, Changning District, Shanghai 200052, China, Phone: +86-21-55664885, Fax: +86-21-55664885, E-mail: [dongyihe@medmail.com.cn](mailto:dongyihe@medmail.com.cn)

**Acknowledgements**

These studies were supported by research grants from the International S&T Cooperation Program of China (2013DFA30870), National Science Foundation of China (81273979), Key projects of Shanghai Municipal Health Bureau (20114027), Ministry of Science and Technology (2011BAI09B00), and the 111 Project (B13016). The computations involved in this study were supported by Fudan University High-End Computing Center.

**Abstract**

[Background] Rheumatoid arthritis is a chronic-relapsing autoimmune disease of incompletely understood etiology. Recent evidence strongly supports an epigenetic contribution to the pathogenesis of rheumatoid arthritis. To understand the extent and nature of dysregulated DNA methylation in rheumatoid arthritis T cells, we performed a genome-wide DNA methylation study in CD4+ T cells in 12 rheumatoid arthritis patients compared to 12 matched normal healthy controls. [Methods and Result] Cytosine methylation status was quantified with Illumina methylation 450K microarray (HM450K, 485512 CpG sites). We identified 810 hypomethylated and 392 hypermethylated CG sites in lupus CD4+ T cells compared to normal controls, representing 383 and 785 genes hypermethylated and hypomethylated in RA patients (P<3.4\*10-7). Cluster analysis based on significantly differential methylated loci showed distinct separation between RA and normal controls. Gene ontology analysis showed alternative splicing (P=1.2\*10-7, FDR) and phosphoprotein (1.7\*10-2, FDR) were significantly aberrant in RA patients, indicating the abnormal of transcript alternative splicing and protein modification mediated by DNA methylation might play important role in the pathogenesis of rheumatoid arthritis. What’s more, the result showed human leukocyte antigen (HLA) region were frequently hypomethylated in RA patients, including HLA-DRB6, HLA-DQA1 and HLA-E, however, HLA-DQB1 showed different methylation profiles with significant hypermethylation in CpG island region and hypomethylation in CpG shelf region. Outsite of the MHC region, The most hypermethylated genes in RA included HDAC4, NXN, TBCD and TMEM61 while the most significant hypomethylated genes included ITIH3, TCN2, PRDM16, SLC1A5 and GALNT9. [Conclusion] Genome-wide DNA methylation patterns revealed significant DNA methylation change in CD4+ T cells from patients with rheumatoid arthritis.

**Keywords:**

[DNA Methylation](http://www.tandfonline.com/action/doSearch?Keyword=DNA%20Methylation)，rheumatoid arthritis，CD4+ T cells， Genome-wide，Illumina methylation 450k microarray

Guangjie Chen,

Department of Immunology and Microbiology, Shanghai JiaoTong University School of Medicine,Shanghai 200025, China. Phone: +86-21-63846590,Email: guangjie\_chen@163.com

**Isolation of PBMCs and CD4**+ **T cells**

Mononuclear cells were prepared from blood specimens (PBMCs) by Ficoll-Hypaque centrifugation (Amersham Biosciences) using the standard protocol (ref1) and immediately processed for cell culture. CD4+ T cells were prepared from freshly isolated PBMCs by depleting cells expressing CD8, CD14, CD16, CD19, CD36, CD56, CD123,γ/δ T cell receptors, and glycophorin A using No-Touch T cell isolation kits (Miltenyi Biotec). The purity of the CD4+ T cells was 95–98%, as determined by flow cytometry using specific antibodies.

Ref1. Chen GJ, Zhang X, Li RS, Niu XY, zheng YX, He DY, Xu R, Zhang JW.. Role of Osteopontin in Synovial Th17 Differentiation in Rheumatoid Arthritis. . ***Arthritis & Rheumatism***,2010;62(10):2900–2908