ABNORMAL METHYLATED DNA REGIONS INDICATE AN ATYPICAL RESPONSE OF THE CD4+ T CELLS IN IGA NEPHROPATHY PATIENTS

Fabio Sallustio1,2, Grazia Serino1, Sharon N. Cox1, Alessandra Dalla Gassa1, Claudia Curci2, Giuseppe De Palma3, Barbara Bannelli5, Gianluigi Zaza1, Massimo Romani1 and Francesco Paolo Schena1,2.

1 Department of Emergency and Organ Transplantation, University of Bari, Bari, Italy; 2 Consorzio CARSO and Scheda Foundation, Valenzano, Ba, Italy; 3 University of Salento, Lecce, Italy; 4 University of Verona, Department of Medicine - Nephrology, Verona, Italy; 5 IRCSS AOI San Martino, IST, Genova, Italy.

INTRODUCTION AND OBJECTIVES

Immunoglobulin A nephropathy (IgAN) is the most common form of primary glomerulonephritis worldwide among patients undergoing renal biopsy. The pathogenesis of this disease seems to have a strong genetic component but so far, no genetic variants or genes involved in its pathogenesis are well understood. In this setting, the DNA methylation could be an important factor influencing the pathology (2). Rapid advances in the field of epigenetics are now revealing a molecular basis for how information obtained from DNA sequence can influence gene function (2-4).

To assess possible changes in CpG methylation in IgA nephropathy, we analyzed the CpG methylation in a genome-wide manner on the DNA derived from CD4+ cells of 6 IgAN patients and 6 healthy controls.

METHODS

To assess possible changes in CpG methylation in IgAN, we analyzed the CpG methylation in a genome-wide manner on the DNA derived from CD4+ cells of 6 IgAN patients and 6 healthy controls (HS). DNA methylation analysis was performed by the Illumina HumanMethylation450 BeadChip that interrogates DNA methylation at more than 485,000 CpGs. All statistical analysis was performed using R and the RoBeads R package for comprehensive analysis of DNA methylation data. Identified CpGs, differentially methylated, were further validated on ten different samples of IgAN and HS. Gene expression studies by Real-time PCR were performed on isolated methylated genes/promoters to verify the correspondence between the methylation status and the gene expression.

RESULTS

Figure 1. Differential methylation analysis between 6 IgAN patients and 10 HS was conducted both on site and region level according to the beadchip protocols. We selected for our analysis only those having a threshold of a CI of 0.8 or p < 0.05. We found 281 CpGs sites differentially methylated in IgAN patients respect to HS, 130 hypermethylated and 143 hypomethylated.

Figure 2. Some most significant differentially methylated regions included genes specifically expressed in T cells and involved in the T cell receptor signaling. In particular, we found TRIM27 and DUSP5 hypermethylated in correspondence to the promoter region and the 7UTR region, respectively. They function as signal transducers of the T cell receptors. Moreover, in the chromosomes 3 we found the VTN2A-1 gene (known in promoter inactive RNAseq-786) as one of most strongly and extensively hypermethylated region in IgAN patients respect to HS. It is methylated in the promoter as well as in the first intron and it is also part of a CpG island. Blue box in the figures represent CpG islands. Black vertical lines represent methylation probes.

CONCLUSIONS

We described, for the first time, some specific chromosomal regions abnormally methylated in IgAN patients, some of which including genes involved in the T cell receptor (TCR) signaling and in the CD4+ T cell response and proliferation. These methylated regions led to the altered expression of genes of the TCR signal transduction, indicating a dysregulation response of the CD4+ T cells of IgAN patients.

REFERENCES:


Figure 3. A. Representative pyrogram of methylation in 10 IgAN patients and 10 HS for TRIM27, DUSP5 and VTN2A-1. Methylation in TRIM27, DUSP5 and VTN2A-1 was validated by sequencing on the same regions found methylated in the genome assay on 10 IgAN patients and 10 HS. We confirmed that TRIM27 and DUSP5 were hypermethylated in IgAN patients (p<0.05). The VTN2A-1 region was confirmed hypermethylated in IgAN patients with a mean difference in methylation levels of 29%.

Figure 4. To further confirm the regulatory role of DNA methylation in DUSP5, TRIM27 and VTN2A-1 expression, we treated the CD4+ HEL-T7-8 cells for 2 days with the DNA methyltransferase inhibitor 5-Aza-20-deoxycytidine which causes DNA demethylation or beta-dexamethasone. DNA demethylation can regulate gene expression by "opening" the chromatin structure. We then determined both the DNA methylation status of these three DNA regions and the gene expressions by methyltransferase specific primers (MSP analysis and Real-time PCR, respectively). Methylation levels within these regions decreased from 92% in the non-treated cells (block) to 21.5% for DUSP5, from 37.9% to 28.2% for TRIM27 and from 25.2 to 17.2% for VTN2A-1. Conversely, the gene expression levels increased of 1.5-fold for DUSP5, 1.5-fold for TRIM27, and 1.5-fold for VTN2A-1, respectively.

Figure 5. Since the mRNA levels of VTN2A-1 were found responsible for the regulation of the cell proliferation (5), we studied whether also IgAN T cells can regulate the proliferation rate. Results showed that, the transient transfection of a CD4+ T cells with 259 kb of self- coding promoter led to a decrease of the proliferation rate of 30% (p<0.01). The proliferation reduced only when cells were stimulated with CD3 and CD28 molecules. Instead, no difference was detected in unstimulated cells.