Microarray based analysis of regulatory networks of methylation sensitive genes in T cells

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Introduction

Eukaryotic gene expression requires not only transcription factor activation but also regional modification of chromatin structure into a transcriptionally permissive configuration through epigenetic mechanisms, including DNA methylation and histone modifications. The methylation of dC bases in CpG islands promotes a repressive chromatin structure inaccessible to transcription factors, suppressing gene expression. Hypomethylation of regulatory sequences correlates with active transcription (Figure 1).

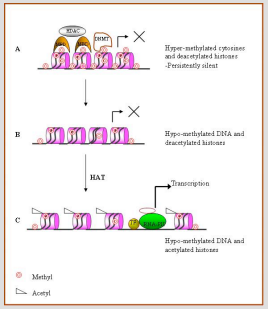

Figure 1. Activation of transcription by DNA demethylation

DNA methyltransferase inhibitors such as the cytosine analogs 5-azacytidine and 5-aza-2'-deoxycytidine can induce DNA hypomethylation.

Recent evidence indicates that environmentally-induced epigenetic changes, and in particular altered patterns of DNA methylation, contribute to the environment–host interaction in some forms of autoimmunity. T cell DNA hypomethylation has been implicated in the pathogenesis of idiopathic and drug-induced human lupus. However, the genes and the regulatory pathways affected by DNA hypomethylation are largely unknown.

Here we report an elucidation of the regulatory networks orchestrating the transcriptional changes observed by microarray experiments on methylation sensitive genes as a meaningful approach in identification of the effect of epigenetic changes on disease mechanism.

Materials and Methods

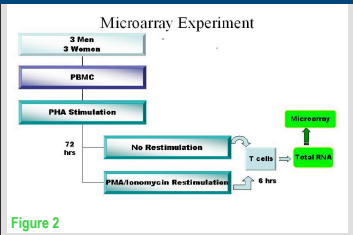

Figure 2

RNA samples were analyzed on Affymetrix (Santa Clara, CA) GeneChip Human Genome Plus 2.0 (HG-133 Plus 2.0) microarrays by the University of Michigan Comprehensive Cancer Center (UMCCC) Affymetrix and Microarray Core Facility. Microarray data analysis was performed using Genomatix program (http://www.genomatix.de/) with a FDR below 4%.

Results

We identified 165 significantly regulated genes for unrestimulated and 215 genes for PMA+ ionomycin restimulated T cells.

Gene Ontology (GO) representation

Term	ID	Total	Observed	Expected	ZScore
immune system process	GO:0002376	767	39	10.57	9.03
immune response	GO:0006955	596	32	8.21	8.52
regulation of myeloid leukocyte differentiation	GO:0002761	15	4	0.21	8.41
response to stimulus	GO:0050896	1932	66	26.62	8.21
response to wounding	GO:0009611	367	21	5.06	7.23
defense response	GO:0006952	491	25	6.76	7.17
apoptosis	GO:0006915	657	30	9.05	7.16
programmed cell death	GO:0012501	663	30	9.13	7.1
death	GO:0016265	705	31	9.71	7.04
cell death	GO:0008219	705	31	9.71	7.04
negative regulation of apoptosis	GO:0043066	198	14	2.73	6.92
regulation of apoptosis	GO:0042981	452	23	6.23	6.87
negative regulation of programmed cell death	GO:0043069	201	14	2.77	6.84
myeloid leukocyte differentiation	GO:0002573	33	5	0.45	6.8
regulation of programmed cell death	GO:0043067	458	23	6.31	6.79
response to external stimulus	GO:0009605	531	25	7.32	6.7
cell development	GO:0048468	1026	37	14.13	6.34
cellular developmental process	GO:0048869	1490	47	20.53	6.19
cell differentiation	GO:0030154	1490	47	20.53	6.19
cell communication	GO:0007154	3129	79	43.11	6.16
signal transduction	GO:0007165	2826	73	38.93	6.08
leukocyte differentiation	GO:0002521	91	8	1.25	6.08
anti-apoptosis	GO:0006916	159	11	2.19	6.02
inflammatory response	GO:0006954	269	15	3.71	5.96
developmental process	GO:0032502	2728	70	37.58	5.86
cell-cell signaling	GO:0007267	575	24	7.92	5.86
cytokine production	GO:0001816	96	8	1.32	5.86
acute-phase response	GO:0006953	28	4	0.39	5.86
myeloid cell differentiation	GO:0030099	79	7	1.09	5.72
regulation of protein metabolic process	GO:0051246	255	14	3.51	5.68
B cell activation	GO:0042113	61	6	0.84	5.68
B cell differentiation	GO:0030183	30	4	0.41	5.62
response to stress	GO:0006950	843	30	11.61	5.59
positive regulation of protein metabolic process	GO:0051247	83	7	1.14	5.53
regulation of phosphorylation	GO:0042325	46	5	0.63	5.53
regulation of myeloid cell differentiation	GO:0045637	31	4	0.43	5.51
immune system development	GO:0002520	183	11	2.52	5.41
regulation of cell motility	GO:0051270	50	5	0.69	5.24
regulation of phosphorus metabolic process	GO:0051174	50	5	0.69	5.24
regulation of phosphate metabolic process	GO:0019220	50	5	0.69	5.24
leukocyte activation	GO:0045321	168	10	2.31	5.11
regulation of locomotion	GO:0040012	52	5	0.72	5.1
locomotion	GO:0040011	53	5	0.73	5.04
regulation of G-protein coupled receptor protein signaling pathway	GO:0008277	36	4	0.5	5.02

Table 1. Table 1. Gene Ontology (GO) categories of differentially expressed methylation sensitive genes for PMA+Ionomycin stimulated T cells. GO-rankings with Z score >5 with minimum number of observed genes 4 are shown. Immune system genes formed the highest scoring group.

Methylation sensitive Immune genes

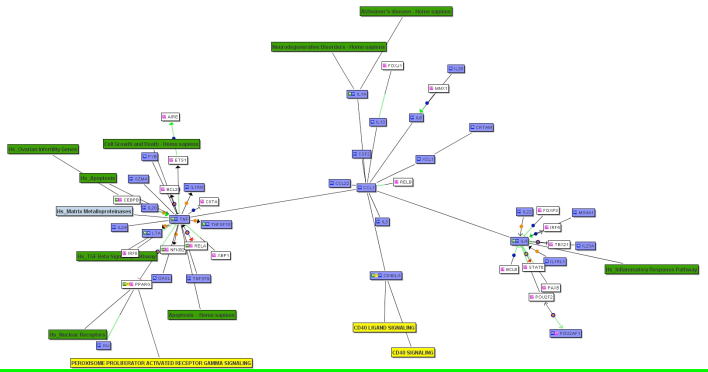

Figure 3. Regulatory network of differentially expressed immune genes upon 5-Aza treatment

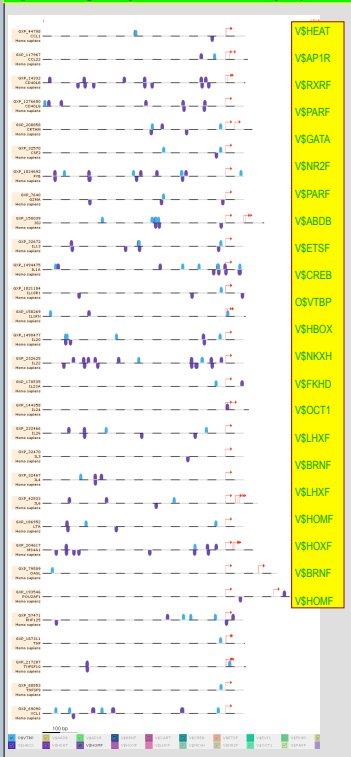

Figure 4. Promoter FrameWorker model of immune response genes

5Aza responsive Transcription Factors

Gene Symbol	Gene ID	TF Name
ATF5	22809	activating transcription factor 5
CREG1	8804	cellular repressor of E1A-stimulated genes 1
CREM	1390	cAMP responsive element modulator
CTCFL	1E+05	CCCTC-binding factor (zinc finger protein)-like
ETS2	2077	ETS2 repressor factor
HMX2	3167	H6 family homeobox 2
HOXB6	3216	homeobox B6
INSM1	3642	insulinoma-associated 1
KLF5	688	Kruppel-like factor 5
MYB	10043	human MYB binding protein
NR4A2	4929	nuclear receptor subfamily 4
POLR2K	5440	human polymerase (RNA) II (DNA directed) polypeptide K, 7.0kDa
POU2AF1	5450	POU class 2 associating factor 1

Table 2. Transcription factors upregulated by 5-Aza treatment

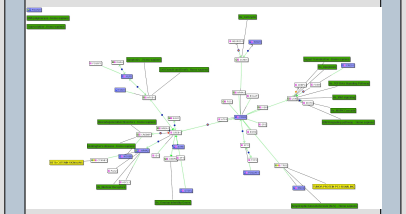

Figure 5. Regulatory network of the methylation sensitive transcription factors.

Summary

❖ DNA hypomethylation perturbs the functions of several cellular processes.

❖ GO analysis of the 5-Aza responsive genes reveal that immune response genes are preferentially affected by demethylation in T cells.

❖Some methylation sensitive immune genes appear to be co-regulated by the Transcription Factors common to the promoters of these genes.

Conclusions

Immune response genes are preferentially expressed and at higher levels in hypomethylated T cells. These data suggest that epigenetic changes associated with environmental factors may lead to stronger autoimmune T cell responses under conditions of repeated stimulation. Underlying regulatory networks enables us to gain new insights into molecular basis in autoimmune diseases.

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