Doctoral Thesis/Dissertation Digest Form

Thesis/Dissertation Title: Regulation of Interleukin-6 (*Il6*) Gene Expression by the CpG Dinucleotides in the Downstream of the Transcription Initiation Site

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Approved Digest

In this study, Interleukin 6 (*Il6*) gene was used as the research model. I used the PAMP, bacterial lipopolysaccharide (LPS), which induces the expression of the *Il6* gene via a PRR namely Toll-like receptor 4 (TLR4). The downstream region of the *Il6* gene's promoter, specifically at the Exon 2 of the *Il6* gene, has three actively methylated CpG dinucleotides (CpG +286, CpG +348, and CpG +381). Elevation of the methylation rate at the CpG dinucleotides located at the downstream area of the *Il6* gene's promoter by using CRISPR/dCas9-MQ1(WT) fusion protein, reduced the *Il6* gene expression after LPS stimulation. This pilot study indicates the DNA methylation rate in the CpG dinucleotides situated downstream of the promoter of the *Il6* gene modulates the gene expression.

To investigate the role of DNA methylation for the *Il6* gene expression, I generated *Tet1*, *Tet2* and *Tet3* knockout macrophage cells. The *Tet1* and *Tet2* knockout RAW264.7 cells showed increased expression of *Il6* in response to LPS, whereas *Tet3* knockout RAW264.7 cells showed decreased expression of *Il6* in response to LPS. Nevertheless, all the *Tet* genes knockout RAW264.7 cells did not disturb the TLR4 signaling pathway. The actively methylated CpG dinucleotides at the Exon 2 also showed significant changes. Therefore, these results suggest that the rate of DNA methylation in the actively methylated CpG dinucleotides at the Exon 2 in *Il6* gene can regulate the overall expression of *Il6* after LPS stimulation.

To further investigate the role of each CpG dinucleotide in the Exon 2 of the *Il6* gene, I used the CRISPR/dCas9 methylation (dCas9-MQ1(Q147L)) and demethylation (dCas9-TET1) plasmid system to manipulate the DNA methylation at the targeted CpG dinucleotide. This study targets only CpG +286 and CpG +348. These two CpG dinucleotides have similar DNA methylation rate to the primary cells such as bone marrow-derived macrophage, peritoneal macrophage, and alveolar macrophage.

The increase in methylation of CpG+286 in RAW264.7 cells alone suppressed the *Il6* gene

expression, while demethylation at the CpG+286 promoted the higher expression of the *Il6* gene upon gene activation by LPS. On the other hand, the high methylation rate at CpG+348 had a dominant functional role over the CpG+286. An increase in methylation at CpG+286 in both the *Tet1*, but not *Tet2* knockout RAW264.7 cells did not suppress the *Il6* gene expression after LPS stimulation. Removal of CpG+348 showed a decrease in *Il6* gene expression in the presence of LPS. Therefore, these data propose that CpG dinucleotides in the Exon 2 of the *Il6* promoter gene play essential functions in modulating gene expression through their DNA methylation rate.

To further understand how CpG+348 regulates *Il6* expression, I focused on a transcription factor CCCTC-binding factor (CTCF). From the chromatin immunoprecipitation (ChIP) assay, I found that CTCF bind to *Il6* locus at around CpG+348 in RAW264.7 cells and the CTCF binding is increased by LPS simulation. In addition, CTCF binding was lowered in CpG+348 deletion cells with or without LPS stimulation. These findings suggest that LPS stimulation recruits CTCF to *Il6* locus and the methylation of CpG+348 required for the CTCF recruitment.