

PhD Dissertation Defense: Mr. Bongyong Lee

Title: “Epigenetic Control Mechanisms in Somatic Cells Mediated by DNA methyltransferase 1”

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ABSTRACT

DNA methylation regulates gene expression through a complex network of protein/protein and protein/DNA interactions in chromatin. The maintenance methylase, DNA methyltransferase 1 (DNMT1), is a prominent enzyme in the process that is linked to DNA replication and drives the heritable nature of epigenetic modifications in somatic cells. The mechanistic details that explain how DNMT1 catalytic action is directed in a chromatin setting are not well understood. The focus of this thesis is to establish plausible mechanistic pathways that regulate global and local DNA methylation events in somatic cells that are relevant in carcinogenesis.

First, we show that DNMT1 is post-translationally modified by sumoylation and we have mapped these sumoylation sites by defined mutations. Sumoylated DNMT1 is catalytically active on genomic DNA *in vivo* and substantially increases the enzymatic activity of DNMT1 both *in vitro* and in chromatin. These data establish that sumoylation modulates the endogenous activity of a prominent epigenetic maintenance pathway in cells.

Second, we investigated novel mechanisms whereby somatic cells can erase then reset DNA methylation events in somatic cells. In this study, the relationship between DNA damage and gene silencing was explored. To this end, we generated a HeLa cell line containing a specialized GFP reporter cassette (DRGFP) containing two mutated GFP genes and a unique I-SceI restriction endonuclease site. These cells do not express GFP. A unique double strand break is then delivered by transfecting in the gene for I-SceI. About 4% of the cells produced a functional GFP by gene conversion and homologous recombination (HR); however roughly half of the GFP recombinants expressed the gene poorly and this was attributed to gene silencing. Silencing of the GFP expressing cell clones was due to DNA methylation and could be reversed using a drug that inhibits global methylation (5-aza-2'-deoxycytidine). Approximately half of the repaired genes were heavily methylated, and half were hypomethylated. That is, a key intermediate methylation state after HR repair is hemimethylated DNA, defined as methylation limited to one strand. Evidence is given that DNMT1 is acting as a *de novo* methylase at the HR repair patches in cells. Moreover, the DNA damage inducible protein, GADD45 α , interacts specifically with the catalytic domain of DNMT1 and GADD45 α binds with extremely high affinity to hemimethylated DNA sites. Thus, GADD45 α is a key regulatory element in silencing of HR repaired DNA segments and appears to inhibit the activity of DNMT1. Consistent with these results, we found that GADD45 α increased the expression of recombinant GFP following HR repair, further suggesting its role in orchestrating strand specific DNA methylation by DNMT1. Since these experiments were performed in live cells, there is strong physiological relevance.

We propose that DS DNA damage and the resulting HR process involves precise, strand selected DNA methylation mediated by the prominent methylase enzyme, DNMT1. Moreover, DS DNA break repair through HR and gene conversion, may potentially erase and reset DNA methylation patterns and therefore alter the expression of repaired genes. The overall process is tightly regulated by the DNA damage inducible protein GADD45 α , which may coordinate strand specific methylation by recruiting DNMT1 to HR repair templates. The ability of GADD45 α to modulate DNMT1 catalytic activity may explain its role as a passive mediator of demethylation that has been reported by other groups. The overall process of silencing post DNA repair is a strong evolutionary force that may predispose cells to malignant transformation.

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