

Effect of mutations in protein arginine methyltransferase 5 on tumorigenesis

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Over- and under-activation of enzymes responsible for maintaining the cell cycle and gene expression is a major cause of tumor development, and can be caused by mutations and abnormal post-translational modifications. The protein arginine methyltransferase 5 (PRMT5) catalyzes the symmetric arginine dimethylation of histone proteins, resulting in the inhibition of gene expression. It is regulated by RhoA-activated kinase (ROK) and myosin phosphatase (MP) via the Thr80 phosphorylation site. Mutations in the Thr80 regulatory side chain of PRMT5 have been described in several tumor types using the COSMIC database.

The aim of our studies is to biochemically investigate the mutations in PRMT5 Thr80. We transfected and purified tsA201 cells with a plasmid encoding wild-type and mutant PRMT5 protein. By mass spectrometry analysis, a new phosphorylation site, PRMT5 Ser74, was described for PRMT5 Thr80 mutant proteins under ROK treatment compared to wild-type PRMT5 enzyme. To investigate the effect of PRMT5 Thr80 mutations on activity, overexpressed proteins were bound to affinity gels. The bound PRMT5 protein was incubated with ROK and then examined for phosphorylation by Western blotting. While WT PRMT5 showed no increase in Ser side chains upon ROK treatment, an increase was detected in Thr80 mutants. The mutant PRMT5 showed clear PRMT5 Ser phosphorylation by in vitro ROK enzyme activity assay, which was reduced by the addition of MP. We analyzed the amount of histone substrates bound by WT and mutant PRMT5 proteins and found that ROK induced mutant PRMT5 to bind significantly more histone substrates, which was not altered by further phosphorylation. We validated our results with a liver carcinoma tissue array assay and other tumor cell lines using an antiphospho-Ser74 antibody and observed a grade- and status-dependent correlation in the phosphorylation of tissue samples.

Our studies suggest that mutations in tumors may regulate the increased activity of PRMT5 by the amount of substrate bound by the enzyme and that this may result from spatial structural changes and exposure to new activating phosphorylation sites caused by the mutations.

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