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Mutational Analysis of the Putative Dimer Interface of DNA Methyltransferase 3b

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ABSTRACT

DNA methylation is an epigenetic process involved in gene regulation that is key for cell differentiation and viability. DNA methyltransferase 3a and 3b (Dnmt3a andDnmt3b) are two enzymes that establish this epigenetic modification during early cell development. These two proteins have been linked to many cancers such as worsening the prognosis of patients with acute myeloid leukemia (AML), due to mutation of Dnmt3a. Over-expression of Dnmt3b has been shown to be involved in Immunodeficiency Centromere instability and Facial abnormalities syndrome (ICF). The crystal structure of Dnmt3a catalytic domain shows that it forms a tetramer and it was shown to methylate multiple sites on DNA by a cooperative catalytic mechanism. In absence of structural details of Dnmt3b, very little is known about the catalytic properties of this enzyme. Based on previous studies showing Dnmt3b to be non-cooperative, we hypothesize that Dnmt3a and Dnmt3b may differ in their oligomer state. We hypothesize that unlike Dnmt3a, Dnmt3b does not oligomerize and to test this hypothesis, we performed mutational analysis of the conserved residues in Dnmt3b that are critical for Dnmt3a tetrameric structure. These variant proteins were overexpressed in bacterial overexpression system and purified by affinity chromatography. Next, the catalytic activity of the variant enzymes will be compared to the wild-type enzyme. Determining the catalytic mechanism of Dnmt3b will help design specific inhibitors that can be potentially used as anti-cancer agents.

KEYWORDS

Epigenetics, Cancer, DNA Methylation