Functional Studies of Methyl-CpG-binding Domain Protein 4 (MBD4)

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The DNA glycosylase methyl-CpG-binding domain protein (MBD4) is commonly found to be mutated in cancers, autoimmune disorders, and neurological disorders. MBD4, a base-excision repair enzyme that removes thymine from T:G mismatches, plays a crucial role in epigenetic DNA modifications and DNA repair pathways. Despite advances in the understanding of MBD4's catalytic mechanisms, the factors contributing to MBD4's structural integrity and stability remain elusive. My analysis of published MBD4-DNA structures led to an observation that Arg512 formed hydrogen bonds with seven amino acid residues near the catalytic pocket. This unusually high number of Arg512-mediated hydrogen bonds led to the hypothesis that Arg512's hydrogen-bond network plays a crucial role in maintaining MBD4's structural integrity and catalytic activity. To test this hypothesis, I compared the enzyme activity of wild-type MBD4 and MBD4 mutants bearing Arg512Lys or Arg512Gln mutation. DNA glycosylase activity assays with denaturing PAGE revealed that the Arg512Lys MBD4 mutant successfully cleaved T from T:G mismatches, whereas Arg512Gln MBD4 mutant did not exhibit any glycosylase activity, highlighting that the identity at the 512 position significantly affects the enzyme activity. Molecular modeling with PyMOL program predicted that Arg512Lys residue forms a similar hydrogen-bond network to that of Arg512, whereas Arg512Gln residue is unable to. Based on these results, I concluded that the Arg512-mediated hydrogen-bond network plays a fundamental role in the stabilization and catalytic activity of MBD4. This discovery would advance our knowledge of the structure-function relationship of MBD4 in maintaining genome integrity and would aid the development of more effective cancer treatments.

Awards Won: