DOI 10.18699/SBB-2022-574

Functional role of active site amino acid residues of human ALKBH2 during recognition of methylated DNA bases

Davletgildeeva A.T.[1], Zhao M.[2]*, Fedorova O.S.[1], Kuznetsov N.A.[1, 2]

[1] *Institute of Chemical Biology and Fundamental Medicine, SB RAS, Novosibirsk, Russia*

[2] *Department of Natural Sciences, Novosibirsk State University, Novosibirsk, Russia*

** hdzhaomingxing@163.com*

Epigenetic regulation of gene expression is an important control method over the implementation of genetic information by living organisms. Site-specific methylation of DNA is one of the crucial ways of performing this regulation. The enzymatic oxidative demethylation is aimed to control different types of DNA methylation. The main pathway of the removal of methyl groups in DNA is catalyzed by nonheme Fe(II)/α-ketoglutarate–dependent DNA dioxygenases. Commonly accepted, that the AlkB family dioxygenases are the repair enzymes that catalyze the direct removal of alkyl groups from DNA bases such as 3-methylcytosine, 1-methyladenine, 1,N6-ethenoadenine. Recently was shown, that ALKBH2, one of the human orthologues of AlkB, is able to oxidize alkyl groups not only in the damaged bases, but also methyl group in 5-methylcytosine, which is the main epigenetic mark. It was suggested that the ability of the ALKBH2 to process 5-methylcytosine strongly depends on the ability to accommodate the damaged base in the active site of enzyme. We used the active site mutational remodeling strategy to identify the functional role of Tyr122, Ile168 and Asp173 amino acid residues in the active site. A set of ALKBH2 mutant forms was constructed by site-directed mutagenesis. The amino acid residues Tyr122, Ile168 and Asp173, which form contacts with the methylated bases accordingly to the structural data, were substituted to alanine. The PAGE and pre-steady-state kinetics experiments were conducted to analyze the activity of these mutant forms towards 5-methylcytosine, 3-methylcytosine and 1-methyladenine. It allowed us to evaluate the role of the active site residues in ALKBH2 enzyme's specificity. The data obtained provide insights into the mechanism of substrate recognition by ALKBH2 in epigenetic DNA demethylation process.

Acknowledgements: This work was supported by Russian Science Foundation Grant No. 21-14-00018.