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Activity of natural polymorphic variants G118V and R149I of DNA polymerase β

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DNA polymerase β (Polβ) is one of the main DNA polymerases involved in the base excision repair pathway, capable of filling single nucleotide gaps in DNA. It is known that functionally deficient Polβ mutants may have a reduced efficiency of DNA repair, which can lead to an increased occurrence of mutations in the genome. At the moment, significant amount of studies showed the association of single nucleotide polymorphisms with various cancers.

In this study we analyzed the natural mutations located in DNA-binding domain of Polβ. The analysis was carried out using software tools (SIFT, PolyPhen, CADD, REVEL, MetaLR, Provean) predicting whether an amino acid substitution will have an impact on the biological function of a protein. During the analysis, two natural polymorphic variants Gly118Val and Arg149Ile were selected with a high probability of affecting the protein functioning. Then, mutant forms of Polβ were obtained by site-directed mutagenesis and purified to experimentally analyze activity of these polymorphic variants.

The EMSA analysis revealed that both mutant forms have reduced ability to bind single nucleotide gapped DNA compare to WT Polβ. Using stopped flow analysis, it was shown that Gly118Val mutant form binds dNTP several time less effectively then WT Polβ and Arg149Ile mutant form. PAGE analysis of enzyme activity revealed that both mutant forms have a reduced ability to fill the single nucleotide gapped DNA compared to the WT Polβ.

Obtained data allow to conclude that single amino acid mutations Gly118Val and Arg149Ile mutations located in DNA-binding domain of Polβ significantly influence on the enzyme catalytic activity and interaction with DNA.

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