DOI 10.18699/SBB-2022-599

G-quadruplex formed by the promoter region of the *hTERT* gene: structure-driven effects on DNA mismatch repair functions

Pavlova A.[1], Savitskaya V.[1], Dolinnaya N.[1], Monakhova M.[2], Litvinova A.[3], Zvereva M.[1], Kubareva E.[2]*

[1] *Chemistry Department, Lomonosov Moscow State University, Moscow, Russia*

[2] *Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow, Russia*

[3] *Faculty of Bioengineering and Bioinformatics, Lomonosov Moscow State University, Moscow, Russia*

* *kubarevaea@gmail.com*

Key words: DNA mismatch repair, MutS, MutL, G-quadruplexes, *hTERT* promoter

Motivation and Aim: G-quadruplexes (G4s) are a unique class of stable four-stranded noncanonical DNAs that play a key role in cellular processes. In particular, G4s have been extensively studied as promising anti-cancer targets for clinical application. Here we have focused on the G4-forming 68-nt promoter region of the human telomerase reverse transcriptase (hTERT) oncogene. hTERT is the catalytic subunit of telomerase, an enzyme primarily responsible for the immortality of cancer cells. Genetic studies have identified point mutations in the *hTERT* promoter sequence that are directly associated with increased *hTERT* expression. Also known is the ability of G4s to modulate the exposure of some DNA promoter sites to various repair systems, thus influencing their efficiency. It was shown that G4s have a diverse effect on the functioning of the base and nucleotide excision repair systems. We focused on studying the impact of *hTERT* G4s, including mutated forms of quadruplex structures, on the functioning of various DNA mismatch repair (MMR) pathways.

Methods and Algorithms: Using chemical probing and spectroscopic methods, the effect of four clinically significant nucleotide substitutions in the G4 structure formed by the single-stranded (ss) 68-nt *hTERT* promoter region on the G4 folding was studied. Mutually exclusive mutations occur most frequently at positions –124 bp (position 1295228 of chromosome 5, the mutation is designated as G228A) and –146 bp (position 1295250 – G250A) relative to the ATG start codon of the *hTERT* gene. A less common is double substitution at positions –138/–139 bp from the start codon (G242A/G243A). We elucidated how ss *hTERT* multiG4 structure and its mutated forms affect the binding affinities and functional responses of key proteins, MutS and MutL, involved in the initial stage of DNA mismatch repair (MMR) from two bacterial species, *E. coli* and *Neisserria gonorhoeae*. The latter have different repair mechanism, but some common features with the human MMR system.

Results: Characteristic features of the temperature dependence of UV absorption at 295 nm indicate G4 unfolding with a melting temperature of 58°C for the ss 96-nt WT *hTERT* promoter region (ss96-WT). The CD spectra displayed characteristics of a parallel G4 topology of ss96-WT. The DMS probing results suggest that formation of stacked three-G4 structure actually occurs in WT and mutant 96-nt DNA fragments of the *hTERT* promoter. No significant difference was observed in the binding of both MutS and MutL from *E. coli* to WT and mutant *hTERT* G4s, suggesting these MMR proteins recognize

the G4 conformation through a structure-based mechanism. We have shown that *N. gonorhoeae* MutL (ngMutL) binding to G4 has a 3-fold higher affinity than to double-stranded DNA of the same length. The introduction of mutations in the G4 *hTERT* sequence had no significant effect on complex formation with ngMutL as in the case of *E. coli* MutL. For the first time, the hydrolysis of linear ss *hTERT* substrates was carried out by ngMutL. Protein cleaves ss96-WT between G4s, but not within quadruplex motifs. Mutations in *hTERT* region did not significantly affect the endonuclease function of ngMutL.

Conclusion: It has been shown for the first time that MutS and MutL proteins from MMR system effectively interact with three tandem parallel quadruplexes of the G-rich strand of *hTERT* promoter despite single nucleotide substitutions in it. Formation of G4 in the *hTERT* promoter prevents the introduction of a nick by the ngMutL, thereby suppressing MMR.

Acknowledgements: The study is supported by Russian Science Foundation (grant 21-14-00161).