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Synthesis and characterization of a PNA probe targeting the Bcl-2 gene promoter: a promising tool in anticancer treatment

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Among several anti-gene strategies, the use of DNA analogues such as Peptide Nucleic Acids (PNA) represents a promising approach for the modulation of gene transcription. PNAs are mimics of DNA in which the sugar-phosphate backbone is replaced by the N-(2-aminoethyl)glycine moiety. The absence of charges on the PNA backbone allows the formation of PNA/DNA complexes provided with a higher stability than that of the corresponding natural DNA/DNA counterparts [1]. We have recently demonstrated that the PNA complementary to the 7-mer longest loop of the G-quadruplex formed by the 23-mer bcl2midG4 sequence, located 52–30 bp upstream of the P1 promoter of Bcl-2 gene, is able to selectively bind the loop of the structure. The results have shown the ability of the PNA-coated OAd5 oncolytic vectors to load and transfect their PNA cargo with a high efficiency and also the synergistic cytotoxic effect against human A549 and MDA-MB-436 cancer cell lines [2]. We have also demonstrated that the synthesized PNA does not interact with the corresponding duplex. With the aim of improving the target specificity we have investigated whether the length of the chosen PNA sequence could affect the type of interaction with the complementary DNA sequence. We extended the length of the pyrimidine-rich PNA from seven to ten bases complementary to the $N_{10–19}$ tract of the bcl2midG4 sequence target. Additionally, we synthesized the 10-mer PNA-FITC labelled analogue. PAGE, CD and CD melting experiments were performed to investigate the interaction of the PNA and its analogue with the DNA target, in both quadruplex and duplex complexes. Moreover, molecular dynamics simulations were used to investigate the stability and the structural features of the target heterotriplexes. The drugability of the new PNAs was attested by fluorescence microscopy which showed that the FITC-labelled PNA specifically enters the cell nuclei, with no significant fraction being co-localized in the mitochondria or endoplasmic reticulum organelles. Finally, preliminary cytotoxicity assays confirmed the biological activity of the new anti Bcl-2 PNA. Overall, the studies here reported extend our knowledge about the structural properties of DNA_2-PNA heterotriplexes and provide the basis for the development of new PNA-based anticancer agents for the treatment of human cancers expressing high levels of the Bcl-2 protein.

References:

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