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Interaction of triazinylamidophosphate-containing oligonucleotides with cells and DNA-binding proteins

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Motivation and Aim: A distinctive feature of the currently most widely applied anticancer therapy is the use of cytotoxic methods, namely radiation therapy and chemotherapy, which are accompanied by side effects that harm the patient's body. Increasing the effectiveness of the impact on tumor cells, which is not accompanied by nonspecific toxic effects on the body, as well as expanding the range of combination therapy, is an important and urgent task.

To develop strategies for synthesis of triazinylamidophosphate derivatives of oligonucleotides with different modifiers that may improve stability, cell uptake and interaction with key proteins of DNA repair.

Methods and Algorithms: A scheme for the introduction of a modified unit on a solid-phase support during the oxidation step with highly reactive 2-azido-4,6-dichloro[1, 3, 5]triazine according to the Staudinger reaction was proposed and implemented, followed by the insertion of various aliphatic residues upon treatment with the corresponding amines. Modifying reagents (azidotriazines) differed in the number of previously introduced functional groups into the triazine backbone. The most effective was the modifier with two acceptor substituents in the form of acid chloride groups [1].

To study the regularities of triazinylamidophosphate oligodeoxyribonucleotides (TAP-ODN) interaction with cells and target proteins within them, a panel of human cell lines including oncotransformed cells of different origins: HeLa, MCF-7, BJAB, T98G and immortalized non-cancer HEK293T cells were selected.

Affinity modification with chemically reactive DNAs containing TAP-ODN and either photoactivatable nucleotide at the 3'-end or 5'-deoxyribose phosphate residue (5'-dRP) or AP site was applied for search of target proteins in whole-cell extracts (WCE).

The degree of damage to genomic DNA as a result of exposure to genotoxic compounds was assessed by the comet method. TAP-ODN conjugates with fluorophores (Flu-TAP-ODN) were used to track the intracellular accumulation of these DNAs.

Results: It has been shown that Ku antigen (double strand break sensor) and PARP1 (single and double strand break sensor) are the main targets of DNA probes, with WCE being significantly different in the content of Ku and PARP1. This difference was confirmed by Western blot analysis. HEK293T and T98G, cell lines with high and low Ku antigen and PARP1 content, and WCE prepared from them were used in further experiments.

For Ku and PARP1 (both for purified proteins and in the extracts) the fundamental possibility of the formation of complexes with TAP-ODNs has been shown; moreover, the efficiency of protein binding to ODNs with the largest in volume substituent was significantly higher than for the native counterpart. It was found that, in comparison with

unmodified oligonucleotides, their triazinylamidophosphate derivatives exhibit increased resistance to the action of cellular nucleases, moreover this stability can be significantly increased by introduction of additional phosphorylguanidine (PG) groups.
It was shown that TAP-ODNs and duplexes containing them act as activators of the synthesis of poly (ADP-ribose) catalyzed by PARP1, only moderately yielding to unmodified ODN counterparts.
It was found that Ku antigen and PARP1 play an important role in the protection of DNAs containing TAP-ODNs from hydrolysis by intracellular nucleases, which was confirmed by experiments with the addition of these proteins to the extract with a low concentration of its own Ku and PARP1.
By flow cytometry experiments, it was shown that the intracellular accumulation of Flu-TAP-ODNs is comparable to the transfection efficiency of the control oligonucleotides using the standard Lipofectamine 2000 transfectant for HEK293T and two times lower for T98G. For a set of oligonucleotides with different numbers of PG groups, it was shown that under standard transfection conditions (4 hours, without serum), the greatest penetration efficiency is observed for ODN with two PG groups, and with an increase in their number under the same transfection conditions, the penetration efficiency decreases.
When studying the localization of Flu-TAP-ODNs in the cells and the effect of PG groups on this parameter, it was shown that both oligonucleotides with and without these groups actually penetrate the cell membrane and are localized in the cytoplasm both within endosomal structures and diffusely.
During a series of kinetic experiments, it was shown that with an increased transfection time (8 and 24 hours), the efficiency of the ODN accumulation in the cells increases. The factors influencing the efficiency of Flu-TAP-ODNs penetration were determined, and a conclusion about the possibility of using these constructs for the delivery of ODN into cells was made.
The impact of TAP-ODNs on the cytotoxic effect of several DNA-damaging agents was evaluated on the example of HEK293T and T98G cells (the cell lines with high and low content of both Ku antigen and PARP1 respectively). It was shown that TAP-ODNs practically does not affect the cytotoxicity of temozolomide on HEK293T cells, while for T98G, a moderate increase in the cytotoxicity in presence of ODNs is observed. The slightly enhanced cytotoxicity of bleomycin and etoposide against T98G cells, but not HEK293T was also observed.
Conclusion: Thus, representatives of the class of TAP-ODNs derivatives were obtained for the first time. These TAP-ODNs are able to penetrate into cells without transfectant and interfere with cytotoxicity of temozolomide, bleomycin and etoposide with the influence being dependent of the amount of endogenous Ku antigen and PARP1 in the cells.
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References

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