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PhD DISSERTATION

Antisense oligonucleotides and their nanostructures conjugated with boron clusters; physicochemical and biological characteristics

SUMMARY

According to the available epidemiological data, the number of cancer patients progressively increases worldwide. The types of cancer most commonly diagnosed in humans are lung, skin, breast, ovarian, liver, colon, and brain cancers. Interestingly, all of them are characterized by high concentration of an oncogene encoding the epidermal growth factor receptor (EGFR). In a human body, stimulated EGFR transmits the cellular response to mediate various cellular activities, including cell proliferation, cell survival, growth and development. Abnormal functioning of the *EGFR* oncogene and its augmented expression are of key importance in development of malignant tumours and their metastasis to other organs in the body. Therefore, the development of gene therapies aiming to downregulate the genes responsible for the development of disease, is an urgent need to cure the cancer patients and to improve the quality of their life.

My PhD dissertation is focused on antisense therapeutic nucleic acids decorated with boron clusters (B-ASO), both in their linear form and embedded in nanostructures, and directed towards mRNA encoding the EGFR protein. These compounds are designed to be dual-mode therapeutics. On the one hand, they are expected to downregulate the expression of the EGFR receptor, and in this way to inhibit the growth and development of neoplastic cells. On the other hand, they are carriers of boron atoms needed in the BCNT radiotherapy aiming to kill cancer cells.

The first goal of my research was to develop a reliable dual fluorescence assay (DFA) based on the expression of a fusion plasmid coding the gene of green fluorescent protein with EGFR gene and a plasmid coding red fluorescent protein (GFP-EGFR/RFP). Such the assay allowed

me to screen the silencing activity of a library of linear B-ASO models decorated with three different boron clusters, and to demonstrate that such the B-ASOs are extremely active as inhibitors of the EGFR expression in the HeLa cells. Interestingly, the B-ASO oligonucleotides repeatedly modified with metallacarborane residues (FESAN) effectively reduced the level of EGFR, despite of highly cluster-rich decoration. Moreover, free boron cages and those built into the ASO chain have a strong affinity for proteins and nucleic acids.

Next, especially designed B-ASO dipod (synthesized by our team), in which the 1,2-dicarba-*closo*-dodecarborane cage served as a platform for conjugation with two ASO strands targeting the EGFR gen, were synthesized. I found that such the B-ASO anti-EGFR dipod is able to form higher order structures with its complementary counterpart (B-anti-ASO). AFM and cryo-TEM microscopy analyses provided valuable information about the structure, shape and size of these higher order structures. They occurred to be ring-like nano-objects composed of DNA fragments (ASO/anti-ASO) of a total 44 to 176 base pairs. These nanostructures exhibited increased nucleolytic stability and high inhibitory activity towards the EGFR both in the exogenous DFA model, tested in 3 tumour cell lines (A431, HeLa, MCF-7), and in A431 cells (an endogenous EGFR level determined by the Western Blot test).

Interestingly, the nanostructures did not induce inflammation in macrophages delivered from healthy donors, which is a beneficial property of boron carrier compounds. The ICP MS analysis confirmed that the nanostructures are able to deliver sufficient amount of boron atoms to A431 cells, fulfilling the requirements of BNCT therapy.