Cytotoxic effects and specific gene expression alterations induced by I-125-labeled triplex-forming oligonucleotides

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Abstract

Purpose : Triplex-forming oligonucleotides (TFO) bind to the DNA double helix in a sequence-specifi c manner. Therefore, TFO seem to be a suitable carrier for Auger electron emitters to damage exclusively targeted DNA sequences, e.g., in tumor cells. We studied the infl uence of I-125 labeled TFO with regard to cell survival and induction of DNA double-strand breaks (DSB) using TFO with diff erent genomic targets and target numbers. Furthermore, the ability of TFO to alter the gene expression of targeted genes was examined.

Materials and methods : TFO were labeled with I-125 using the primer extension method. DNA triplex formation and sequencespecifi c DSB were demonstrated in vitro. Cell survival was analyzed by colony-forming assay and DNA damage was assessed by microscopic quantifi cation of protein 53 binding protein 1 (53BP1) foci in the human squamous carcinoma cell line II (SCLII). Quantitative real-time polymerase-chain-reaction (qRT-PCR) was performed to analyze gene expression alterations.

Results: The sequence-specifi c induction of a single DSB in a 1695 bp long DNA double stranded fragment was demonstrated in vitro. I-125-labeled TFO binding to single and multiple targets were shown to induce a pronounced decrease in cell survival and an increase of DSB. TFO targeting multiple sites diff ering in the total target number showed a signifi cant diff erent cell killing per decay that is also in good accordance with the observed induction of DSB. Single gene targeting I-125-labeled TFO signifi cantly decreased cell survival and altered gene expression in the targeted gene.

Conclusions : I-125-labeled TFO enable specifi c targeting of DNA in vitro as well as in a cellular environment and thus induce sequence-specifi c complex DNA lesions. Therefore I-125-labeled TFO might be a very useful tool for basic DNA repair research.