Iodine-125-labelled Triplex-forming oligonucleotides: Studies on cytotoxicity of multi-binding-site TFOs and on specific gene expression alterations caused by single-binding-site TFOs

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Introduction: Triplex-forming oligonucleotides (TFOs) are able to bind DNA in a sequence specific manner and are a promising tool to manipulate genes or gene regulatory units in a cellular environment. TFOs might have also therapeutic potential e.g. as a carrier molecule for Auger-Electron-Emitter (AEE) to target specific DNA structures of tumour cells. We established a method for the effective labelling of TFOs with the AEE iodine-125 (I-125) and studied the influence of labelled TFO with regard to cell survival and appearance of DNA Double-Strand-Breaks (DSB). Furthermore the ability of TFOs to alter gene expression of targeted genes was examined.
Methods: TFOs specific for the genes BCL*2*, GAPDH and BRCA*1* were designed employing TFO Target Sequence Search (Univ. of Texas). TFO labelling with I-125 was performed using the primer extension method. Formation of DNA triplexes was visualized with MS Imaging Plates employing a FLA-5000 Imaging System (Fujifilm) and electrophoretic mobility shift assay (EMSA). Cell survival and DNA DSB frequency in SCL-II cells after transfection with an I-125-labelled Multi-Binding-Site (MBS) TFO (~ 7000 binding sites) were analyzed with the Colony-Forming Assay (CFA) and the 53BP1-Foci Assay. SCL-II cells transfected with TFOs binding to single DNA targets in specific genes were analyzed for gene expression alterations of the targeted genes with qRT-PCR on a 7500 Real Time PCR System (Applied Biosystems).
Results: The MBS I-125-TFO transfected SCL-II cells showed a reduction of colony forming ability of ~ 45 % and the number of 53BP1-Foci was ~ 1.5-times increased when compared to sham-transfected negative control. The transfection with single binding site I-125-TFOs lead to a 1.7-times increased expression for BCL*2* and a 0.5-times reduced expression for GAPDH. No altered gene expression was detected for BRCA*1*.
Conclusions: I-125-labelled MBS TFOs have a pronounced cytotoxic effect and induce DNA DSB in SCL-II cells. Single gene targeting TFOs can alter gene expression in a gene-specific manner.

Funded by the Bundesministerium für Bildung und Forschung *(*BMBF*),* Kompetenzverbund Strahlenforschung *(*KVSF*),* Project No.: *02*NUK*00*5A