

**TRIPLEX-FORMING OLIGONUCLEOTIDES AS A CARRIER FOR AUGER ELECTRON  
EMITTER: STUDIES ON CYTOTOXICITY AND ON SPECIFIC GENE EXPRESSION  
ALTERATIONS CAUSED BY 125-I-LABELLED 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 possess also a therapeutic potential e.g. as a carrier molecule for Alpha- or Auger-Electron-Emitter (AEE) to target specific DNA sequences in 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 BCL2, GAPDH and BRCA1 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 I-125-labelled Multi-Binding-Site (MBS) TFO (~ 7000 binding sites) were analyzed with the Colony-Forming Assay 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-labelled-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-labelled-TFOs lead to a 1.7-times increased expression for BCL2 and a 0.5-times reduced expression for GAPDH. No altered gene expression was detected for BRCA1.

*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.

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