Programme Point: Molecular Radiobiology

Cytotoxic effects and specific gene expression alterations induced by I-125-labelled Triplex-forming oligonucleotides

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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 posses also a potential for radiotherapy e.g. as a carrier 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.

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. Cell survival and DNA DSB frequency in SCL-II cells after transfection with a I-125-labelled Multi-Binding-Site (MBS) TFO (~ 7000 binding sites) were analyzed with the Colony-Forming Assay respectively with 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.

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 *BCL2* and a 0.5-times reduced expression for *GAPDH*. No altered gene expression was detected for *BRCA1*.

I-125-labelled MBS TFOs induce a pronounced cytotoxic effect in SCL-II cells. Single gene targeting TFOs alter gene expression in a gene-specific manner.

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