Targeting specific DNA sequences with I-125-labelled Triplex-forming-oligonucleotides

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Purpose: 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 have also therapeutic potential e.g. as a carrier for Auger-Electron-Emitter (AEE) to target DNA of tumour cells. We established a method for the effective labelling of TFOs with I-125 and studied the DNA binding capabilities of labelled TFOs in vitro. Furthermore we examined the intracellular biokinetic of TFOs with the focus on the transfer from the cytoplasm into the cell nucleus.

Methods: TFOs specific for the genes cdkn2a, bcl2, brca1, chk2, cdk4 were designed using TFO Target Sequence Search (Univ. of Texas). TFO labelling with I-125 was performed with the primer extension method. Formation of DNA triplexes was visualized with MS Imaging Plates on a FLA-5000 Imaging System (Fujifilm, Düsseldorf) and electrophoretic-mobility-shift-assay (EMSA). For biokinetic studies SCL-II cells were transfected by electroporation with Alexa488-labelled TFOs. Transfected cells were subsequently cultured for 1, 6, 12, 18, 24, 30, 48 and 72 h and TFO signal intensity was determined in single cells and in isolated cell nuclei by flow cytometry (FACS-Canto II, BD).

Results: The desired Triplex-DNA-formation could be confirmed for 53 % of all tested TFOs by EMSA. Triplex-formation of I-125-labelled TFOs was confirmed for 10 % by autoradiographic analysis. The biokinetic studies showed that TFO-Alexa488-positive cells were detectable as soon as 1 h after transfection and the signal intensity remained constant for at least 30 h. 72 h after transfection the signal was less intense but still detectable. A substantial loss of TFO-Alexa488-labelled positive cell nuclei was observed within the first 6 h post-transfection followed by a significant increase up to 18 h post-transfection.

Conclusions: Labelling of TFOs with I-125 has a strong influence on their binding capacities. TFOs initially located in the cytoplasm are re-located to the cell nucleus within 12 h after delivery of the TFOs probably during cell division.