

DNA-Triplex-forming-oligonucleotides as a tool to target specific DNA sequences

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Purpose: Triplex-forming-oligonucleotides (TFOs) are able to bind complementary DNA sequences in a sequence specific manner and are therefore 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 for Auger-Electron-Emitter (AEE) to target DNA of tumour cells. A main obstacle is the access of the TFOs to their targets in the cell nucleus. Thus we studied the intracellular biokinetics of TFOs with the focus on the intracellular transfer from the cytoplasm into the nucleus.

Method: TFOs specific for the genes p16ink4a and survivin were designed using (TFO Target Sequence Search, Univ. of Texas). DNA-Triplex-formation was confirmed by electrophoretic-mobility-shift-assay (EMSA) in vitro. For biokinetic studies SCL-II cells were transfected by electroporation with Alexa488-labeled TFOs. Transfected cells were subsequently cultured for 1 h, 6 h, 12 h, 18 h, 24 h and 30 h and TFO signal intensity were determined in single cells and in isolated cell nuclei by flowcytometry (FACS-Canto II, BD) at each time point.

Results: Sequence design of TFOs by (TFO Target Sequence Search, Univ. of Texas) for the desired genes is generally not suitable to predict DNA-Triplex-formation in vitro as could be demonstrated by EMSA. The desired Triplex-DNA-formation could be confirmed for only two TFOs by EMSA. The biogenetic 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. TFO-positive cell nuclei were not detectable for up to 6 h. After 12 h a significant increase of TFO-Alexa488-positive cell nuclei was observed.

Conclusion: Stable Triplex-DNA-formation in vitro can not be predicted by the sequence of TFOs only. 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.