Study on DNA sequence specific binding of I-125 labeled Triplex-forming oligonucleotides in the cellular environment with focus on target specificity, DNA damage induction and cell survival

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Introduction: Triplex-forming oligonucleotides (TFOs) are known for their ability to bind DNA in a sequence specific manner and are therefore a promising tool to manipulate genes or gene regulatory units in a cellular environment. Auger-Electron-Emitter-labeled TFOs can induce complex but localized damage to the DNA. Using radionuclide-labeled TFO the specificity of the target binding and the subsequent damage in the cellular environment were analysed by comparing the effects in two cell lines of which only one possessed the proper TFO target sequence.
Methods: The human carcinoma cell line SCL-II wildtype (WT) was stably transfected with a vector system (p2RT) producing a transgenic strain, SCL-II p2RT, containing the TFO-p2RT target sequence. TFO labeling with I-125 or P-32 was performed using the primer extension method. Efficient delivery of labeled TFO into SCL-II p2RT as well as SCL-II WT cells was ensured by electroporation with the Nucleofector I system (Lonza GmbH, Basel). Subsequently, specific target binding was visualized by restriction digest of isolated genomic DNA and autoradiographic analysis. DNA damage and survival rate were determined with the 53BP1-foci and the colony forming assay (CFA).
Results: Autoradiographic analysis revealed a specific triplex formation at the p2RT vector target sequence, confirming the specific binding. However, the 53BP1-foci assay and the CFA detected a similar increased level of DNA damage and a pronounced reduction in cell survival in both cell strains per cumulated decays. Thus, the different target status in the two cell strains was not reflected on damage level and in survival rate.
Conclusions: Even though a high degree of specific TFO-p2RT binding to its target site could be verified, the 53BP1 and CFA data indicate that also a considerable amount of alternative DNA damaging is occurring.