Processing of Complex DNA Lesions in Mammalian Cells Induced by I-125 labeled Triplex-Forming Oligonucleotides

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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. TFOs labeled with the Auger-Electron-Emitter Iodine-125 can induce complex but localized damage to the DNA. Using radionuclide-labeled TFO the subsequent cellular damage was analyzed regarding mutation frequency, mutation type and mutation localization.

Methods: The human squameous cell carcinoma cell line SCL-II was used as the wildtype strain SCL-II WT and the transgenic strain SCL-II p2RT. In the conducted experiments the SCL-II WT strain was transiently transfected with an *in vitro* pre-formed DNA-triplex of the p2RT vector containing the target sequence and its specific TFO, the I-125-TFO p2RT. The transgenic SCL-II p2RT strain carry the stably integrated p2RT vector system harboring the specific triplex target sequence for TFO-p2RT and was, therefore, transfected with the I-125-TFO p2RT only. Efficient delivery of vector + TFO or TFO only was ensured by electroporation with the Nucleofector I system (Lonza GmbH, Basel). After storage at -150°C for decay accumulation the samples were analyzed for mutation frequency, type and localization using blue/white screening and sequencing.

Results: In the SCL-II WT cell line an almost four fold increased mutation frequency at the target region on the p2RT vector was found when compared to the negative controls. In contrast, the SCL-II p2RT transgenic strain did not show a significant increase of the mutation frequency in comparison to the negative controls. Sequencing revealed that most mutants displayed large deletions of more than 100 bp located at the TFO p2RT target site.

Conclusions: The local complex DNA damage induced by the decay of the TFO delivered Iodine-125 is likely to be accountable for the increased mutation rate in the SCL-II WT cells. Since this increase could not be detected in the SCL-II p2RT transgenic strain it can be hypothesized that the site-specific triplex formation between I-125-TFO p2RT and its target sequence is inhibited to some extent in the cellular environment.