Cellular Radiobiology

Induction of the chromosomal translocation t(14;18) by targeting the BCL-2 locus with specific binding I-125-labeled Triplex-Forming oligonucleotides

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Triplex-Forming oligonucleotides (TFO) 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. TFO possess a therapeutic potential e.g. as a carrier molecule for Alpha- or Auger-Electron-Emitter (AEE) to target specific DNA sequences in tumor cells. We established a method for the effective labeling of TFO with the AEE Iodine-125 (I-125) and analyzed the influence of I-125-labeled TFO in SCL-II cells on gene expression and translocation frequency of the human *BCL2* gene.

The TFO-BCL2 employed in the present study binds to the *BCL2* gene at approximately 5400 bp upstream of the 3´-end. TFO labeling with I-125 was performed using the primer extension method. SCL-II cells were transfected with TFO via electroporation and subsequently stored at -150°C for decay accumulation up to a range from 100 to 330 decays/cell. SCL-II cells either transfected with I-125-labeled multi-binding TFO (>300,000 targets per genome) or transfected with non-labeled TFO-BCL2 served as controls. Monitoring of *BCL2* translocations was done with the Fluorescence-In-Situ-Hybridization (FISH) method. The utilized FISH probes were designed to detect a translocation of the *BCL2* gene from chromosome 18 to chromosome 14, a common translocation found in follicular lymphomas leading to an overexpression of *BCL-2* protein. Analysis of *BCL2* gene expression levels was done via quantitative Real-Time PCR on the Real Time PCR System 7500 (Applied Biosystems).

The relative gene expression of *BCl-2* in I-125-TFO-BCL2 transfected cells showed a significant up-regulation when compared to the controls. Control SCL-II cells were either transfected with I-125-labeled multi-binding TFO (>300,000 targets per genome) or non-labeled TFO-BCL2. Analysis of the *BCL2* t(14;18) translocation frequency revealed a significant 1.8- to 2-fold increase when compared to the control cells.

We conclude that I-125 decays within the *BCL2* gene facilitates the occurrence of the t(14;18) chromosomal translocation in SCL-II cells and that the increased translocation frequency of t(14;18) contributes to the observed overall enhanced *BCL-2* expression. However, it seems unlikely that these rare translocation events fully explain the observed enhanced gene expression of *BCL-2*.

Funded by Bundesministerium für Bildung und Forschung (BMBF), Grant 02NUK005A