

Development of an approach for analyzing the telomere interacting proteins

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Loss of telomeric DNA by incomplete replication in the cells might be compensated by telomerase, a reverse transcriptase, which adds single-stranded telomeric repeats to the 3' DNA strands. In most of cancer cells, where overexpression of telomerase is observed, telomeric DNA has become the target for anti-cancer therapy. Driving force in the drug discovery research might be strategy based on folding of telomeric DNA repeats (TTAGGG) into G-quadruplex conformation by small organic molecules (ligands). As a consequence, telomerase cannot effectively elongate telomeric DNA, which may lead to telomere shortening and death of tumor cells.

The aim of this project is to develop an approach for the quantification of the proteins that bind directly or indirectly to telomeres, as well as for the control of targeted proteins abundance in the presence of potential anti-cancer drugs. Nuclear and cytosolic fractions, from two cells lines transfected with the following proteins: hPOT1 and TRF2, have been investigated by mass spectrometry.

In this report we present the results obtained for POT1 transfected cell line. Cells were grown in DMEM medium supplemented with Glutamax, fetal bovine serum and penicillin/streptomycin until 95% confluence, extraction protocol was based on nuclear extraction kit (Chemicon) and finally analysis with Q-Exactive was done. According to the preliminary results, a lot of proteins of interest were found using 2D nanoLC –MSMS analysis; the latest will be integrated into a targeted quantitative method to evaluate G-quadruplex ligand treated cell models response.