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Creation of a panel of mammalian cell lines with CRISPR/Cas9 knockout of DNA repair genes

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DNA repair is an important mechanism for maintaining genome integrity. The main methods of treating cancer, chemo- and radiotherapy, are based, among other things, on DNA damage. To date, many studies are aimed at finding inhibitors of DNA repair enzymes. Using the CRISPR method, we obtained cell clones of the HEK293 and A549 lines with deletions in the genes of enzymes participating in the DNA repair system, including knockout of *TDP1* (tyrosyl-DNA phosphodiesterase 1) and *PARP1* (poly-ADP-ribose polymerase 1). We use the obtained cell clones with DNA repair genes knockout as a cell model to study the role of these enzymes in DNA repair process and in the response to chemotherapy, as well as the mechanisms of action of anti-cancer therapy using inhibitors of these enzymes.

PARP1 is an enzyme that controls many cellular processes via PARylation, including repair of the topoisomerase 1 complex with DNA (TOP1-DNA) by TDP1. TDP1 removes covalent adducts from the 3’ ends of DNA formed under the action of antitumor drugs that inhibit TOP1. The antitumor activity of the drugs topotecan and irinotecan is based on the ability to inhibit the TOP1 enzyme, stabilizing TOP1-DNA complexes that leads to the accumulation of DNA breaks and the death of tumor cells. The ability of TDP1 to hydrolyze TOP1-DNA complexes leads to a decrease in the therapeutic effect of TOP1 inhibitors, so the study of potential TDP1 inhibitors is a promising strategy for searching for new anticancer drugs.

We performed transcriptomic analysis of human embryonic kidney cell lines HEK293A for wild type and *TDP1* or *PARP1* knockout, treated with topotecan and the effective TDP1 inhibitor that we found earlier. Maximal changes in gene expression levels were found with knockout of the *PARP1* gene compared to *TDP1*. The largest number of differentially expressed genes (DEGs) in *PARP1* knockout are associated with DNA repair, ribosome biogenesis, transcription and cancer development, proteasome, apoptosis, cell cycle control and protein processing. Several hundred DEGs in signaling pathways associated with protein processing, apoptosis, cell cycle control, and proliferation were found in *TDP1* knockout or drug-treated cell clones. A number of DEGs were found among metastasis-related genes common to *TDP1* knockout and TDP1 inhibitor treatment.

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