Title of the project: Testing of new anticancer therapeutics based on low-molecular-weight inhibitors of DNA-PK

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Summary of 2017 results

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DNA-dependent protein kinase (DNA-PK) is a member of phosphatidylinositol 3-kinase (PI3K) related protein kinase family (PIKK). It is the major regulator of the cellular answers to DNA double strand breaks (DSB), that can be caused by ionizing radiation or chemotherapeutic drugs. DNA-PK helps the cells dealing with DSBs through activation of non-homologous end joining (NHEJ).

In the last year, the screening of 18 derivates in combination with doxorubicin was tested. The compound A17 was chosen for detailed testing on the HT-29 cell line of human colorectal carcinoma. Western blot analysis was used for detection of specific proteins that regulate cell cycle and response to cellular stress, namely p53 protein and its phosphorylation on serine 15, checkpoint kinases Chk1 and its phosphorylation on serine 345, Chk2 and its phosphorylation on threonine 68. Chk1 was phosphorylated at serine 345, Chk2 threonine 68 and p53 at serine 15 after application of doxorubicin and combination with both inhibitors. From this result, we may hypothesize that inhibitor A17 does not inhibit ATM/ATR signaling pathway, as the proteins are phosphorylated. The induction of apoptosis was confirmed after administration of the combination therapy using the Caspase-Glo assay kit. Significant increases in the activity of the caspase 8 and 9 and effector caspases 3/7 were noted after 48 hours of activity. Distribution of HT-29 cells within the cell cycle was monitored by flow cytometry. It was found that the combination of doxorubicin with the A17 or NU7026 inhibitor caused accumulation in the G2 phase, but there was no statistically significant proportional difference between combination therapy and doxorubicin alone. Due to promising results, the compound will be applied in mouse models.

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