Summary (ENG)

Title of the dissertation: Proteomic analysis of gamma-irradiated human leukemic cells

In the presented doctoral thesis, we aimed to elucidate molecular mechanisms underlying radiosensitization of MOLT-4 cell line (T-ALL) by specific inhibition of kinases from the phosphatidylinositol-3 kinase-related kinases (PIKKs) family. We tested two highly potent inhibitors of ATR and ATM, VE-821 and KU55933, respectively, for their effects on proliferation, viability, and cell cycle of sham-irradiated and irradiated MOLT-4 cells. Both inhibitors proved to radiosensitize MOLT-4 cells and furthermore, 10 μM VE-821 was shown to act as a strong antiproliferative agent in sham-irradiated MOLT-4 cells.

To further describe cellular mechanisms underlying the VE-821-mediated radiosensitization of MOLT-4 cells, we employed high-resolution mass spectrometry to identify and quantify changes in proteome and phosphoproteome of irradiated VE-821-treated cells. As the detection and quantification of phosphorylated peptides in complex biological samples is challenging due to their low stoichiometry, we first compiled and optimized protocol for their enrichment. The protocol was then applied to study changes in radiosensitized MOLT-4 cells. In concordance with our expectations, VE-821 did not cause any significant changes on the proteome level one hour after irradiation. However, we detected 623 differentially regulated phosphorylation sites; most of them (431) were upregulated in response to VE-821 treatment. Using bioinformatic tools, we revealed changes in DDR related pathways and kinases, but also pathways and kinases involved in maintaining cellular metabolism. Notably, we found downregulation of mTOR, the main regulator of cellular metabolism, which was most likely caused by an off-target effect of the inhibitor, and we proposed that mTOR inhibition could be one of the factors contributing to the phenotype observed after treating MOLT-4 cells with 10 μM VE-821.

To investigate the potential modulation of cellular metabolism, we performed a targeted metabolomic analysis of irradiated MOLT-4 cells pre-treated by 10 μM VE-821. In this analysis, 206 intermediary metabolites were quantified. Subsequent data analysis showed that VE-821 potentiated metabolic disruption induced by IR and affected response to IR-induced oxidative stress. Our data indicated that upon IR, recovery of damaged deoxynucleotide triphosphates might be affected by VE-821, hampering DNA repair by their insufficiency.

Thus, in this thesis we described a complex scenario of cellular events that might be dependent on ATR or triggered by ATR inhibition by VE-821 in irradiated MOLT-4 cells. Importantly, data presented in this work might serve as a resource for follow-up studies and provide a platform for future work with other kinase inhibitors.