Abstract

Acute myeloid leukemia (AML) is a severe hematooncologic disorder, which is difficult-to-treat and for some of its subtypes does not exist suitable treatment so far. This diploma thesis is focused on one of these novel approaches targeting an interaction between proteins p53 and Mdm2 by small molecules RITA and RG7112. Each of the selected inhibitors targets one of the interaction partners: while RG7112 binds into Mdm2-binding site of p53, RITA interacts with p53 in the binding domain of Mdm2. An effect of these inhibitors on proliferation and viability of AML cell lines was investigated and two of them, MOLM-13 and MV4-11, were selected as displaying opposite sensitivity. More detailed analysis confirmed stress reaction and changes of apoptosis-related proteins in the sensitive lines. In addition to the cell lines, primary AML cells were also examined. Second aim of this thesis was to validate a method of isolation and labeling of exosomes and to investigate the type of exosome interaction with cell membrane. Confocal microscope monitoring revealed that the type of interaction between a target cell and an exosome depends on the recipient cell type: tight contact is observed between AML cells and exosomes, while K562 and HS-5 cell lines internalize exosomes, regardless their origin. Furthermore, we detected differentially expressed exosome markers (CD9, CD63, CD81) in exosomes obtained from different AML lines or primary patients samples. Simultaneously, variability of nucleolar and apoptotic protein content in exosome samples has been found. The last part of the thesis combines the two previous approaches and tests an impact of exosome coincubation on the drugs effects. However, we didn´t observe any effect on the sensitivity or resistance to RG7112 and RITA, neither at the level of cell proliferation and viability, nor in changes in protein levels.

Key words: AML, RITA, RG7112, p53-Mdm2 interaction, exosomes, inhibitors of p53-Mdm2 interaction