Abstract

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Title of diploma thesis: Interactions of selected anticancer drugs of the MAPK/ERK signaling pathway inhibitors group with the ABC drug transporters

ABCB1 (Pgp, P-glycoprotein) and ABCG2 (BCRP, breast cancer resistance protein) are members of a transmembrane efflux ATP dependent transporter family, so called ATP-binding cassettes (ABC). Physiologicaly they are expressed in the cellular membrane and protect body tissues against potentially toxic xenobiotics including drugs. They represent also one of the tumor defense mechanisms when being able to efflux a wide variety of cytotoxic drugs out of the cancer cells leading to treatment failure.

BRAF protein plays an important regulatory and signal role in MAPK/ERK pathway affecting cell division, differentiation and secretion. Mutations of BRAF lead to overactivity in MAPK/ERK pathway in many cancer cells and can be therefore targeted by anticancer therapy. Cobimetinib and dabrafenib are relatively new anticancer therapeutics inhibiting the signal pathway mentioned above and they are used in treatment of melanoma carrying the BRAF mutation.

The aims of this project were to investigate whether the kinase inhibitors cobimetinib and dabrafenib could inhibit the efflux transporters ABCB1 and ABCG2 and reverse drug resistance to ABCB1 and ABCG2 substrates in vitro. Using the Hoechst accumulation assay we studied the inhibitory effect of these drugs to MDCKII cell lines overexpressing ABCB1 and ABCG2 transporters. The XTT assay was further

used to study the antiproliferative effect of cobimetinib and dabrafenib and their impact on cytotoxicity of daunorubicin and mitoxantron, the model anticancer substrates of ABCB1 and ABCG2, respectively.

We found that cobimetinib and dabrafenib are able to significantly inhibit ABCB1 and ABCG2 efflux transporters in MDCKII-ABCB1 and MDCKII-ABCG2 cell lines with cobimetinib showing higher inhibitory effect on ABCB1, compared to ABCG2. Contrary to cobimetinib, dabrafenib revealed preferential inhibition of ABCG2.

Both drugs cobimetinib and dabrafenib can significantly reverse daunorubicin resistance in MDCKII-ABCB1 cells. Moreover, dabrafenib is able to reverse resistance of mitoxantrone in MDCKII-ABCG2 cells. We also showed that the presence of neither ABCB1, nor ABCG2 affected resistance of cobimetinib in MDCKII-ABCB1 and MDCKII-ABCG2 cells, indicating that these transporters do not play a role in the cellular resistance to these drugs. The sensitivity to dabrafenib (in tested concentrations to 50 μM) was minimal in all the cell lines we used regardless of presence of ABCB1 or ABCG2.

We demonstrate both anticancer agents, cobimetinib and dabrafenib, as inhibitors of ABCB1 and ABCG2 able to reverse ABC transporter-mediated drug resistance to daunorubicin and mitoxantron. These results may be taken into account when optimizing the cobimetinib- and dabrafenib- containing therapeutic regimens for the treatment of multidrug resistance melanoma patients.