

Analysis of growth-inhibitory mechanisms of flubendazole in malignant melanoma cells

Malignant skin melanoma is at its advanced stage highly aggressive and chemoresistant to almost all currently available therapies. Modern efforts are thus aimed to identify new targets in melanoma cells or to take advantage of novel indications of already approved drugs – a process called drug repurposing. This work was focused on evaluation of cell growth inhibitory properties of one such drug – flubendazole (FLU) – a widely used anthelmintic compound belonging to benzimidazole family. This drug specifically interacts with β -tubulin, which results in disruption of microtubule structure and function in the exposed cells. Several members of the benzimidazole family (including FLU) have already shown the growth inhibitory potential in tumor cells derived from breast, colon, blood and nervous system malignancies. Still, the specific activity of FLU in malignant melanoma has not been tested to the date.

Cytotoxicity of FLU was tested in three malignant melanoma cell lines representing diverse melanoma molecular types (A-375, BOWES and RPMI-7951) during up to 72 hours using WST-1 and x-CELLigence assays. Based on achieved IC_{50} from individual cell lines, the 1 μ M FLU concentration was selected for further testing. While relatively tolerated in normal human skin melanocytes, FLU at this concentration disrupted microtubular cytoskeleton, induced G2/M cell cycle arrest and changed morphology of exposed melanoma cells. The presence of other specific changes (i.e. cell enlargement and multinucleation, overexpression of p21 and activation of caspase-2) indicated mitotic catastrophe which was followed by apoptotic cell death. Further analyses revealed that both processes were not linked by activated TP53-BAX axis and their further elucidation should be addressed in future.

FLU activity was also evaluated in melanoma cells derived from melanoma explant cultures obtained from human subjects undergoing melanoma excision in Faculty teaching hospital in Hradec Králové. In a pilot study using cells from one patient FLU showed cytotoxicity and growth inhibitory properties, however, with lesser potency as judged from higher IC_{50} value. 1 μ M FLU concentration then had cytostatic effect in exposed cells and induced a series of very unusual morphological alterations entirely dissimilar of mitotic catastrophe and classical apoptosis.