DESCRIPTION FOR THE GENERAL PUBLIC

Inhibitors of anti-apoptotic proteins from the BCL-2 family as agents enhancing $BRAF^{V600E}$ inhibition in melanoma cells.

Melanoma is characterized by poor response to available therapeutics. Inhibition of the activity of $BRAF^{V600E}$, a kinase mutated in 50-70% of melanoma cases, by selective targeted drugs has much improved melanoma therapy. Although $BRAF^{V600E}$ inhibitors rapidly inhibit cell proliferation, they exert poor potency to induce programmed cell death, apoptosis, which can be related to high basal expression levels of multiple anti-apoptotic proteins in melanoma cells. In addition, resistance mechanisms exist or occur shortly after the initiation of treatment with targeted inhibitors leading to the tumor relapse. The aim of the project is to define the cellular and molecular effects of encorafenib, a potent inhibitor of $BRAF^{V600E}$ kinase activity, and to clarify the efficacy of simultaneous inhibition of $BRAF^{V600E}$ and specific anti-apoptotic proteins from the BCL-2 family. As an experimental model, melanoma cell populations derived from surgical specimens and grown *in vitro* in culture medium containing growth factors EGF and bFGF will be used. This pre-clinical model of melanoma takes advantage of the possibility to maintain characteristics of the original tumor, and enables to sustain the phenotypic diversity of melanoma cell populations observed at the level of morphology and activity of signaling pathways crucial for melanoma development and maintenance. To elucidate encorafenib activity, cell growth inhibition, induction of apoptosis and autophagy will be verified at the cellular and molecular levels, by employing live-cell imaging, microscopy, flow cytometry, acid phosphatase activity assay, Western blotting and qRT-PCR. Moreover, recently described procedure 'Dynamic BH3 Profiling' will be used to assess whether encorafenib sensitizes melanoma cells to apoptosis by inducing partial inhibition of anti-apoptotic BCL-2 protein activity. 'Dynamic BH3 Profiling' will also indicate specific pro-survival proteins from the BCL-2 family that prevent from apoptosis initiation in phenotypically diverse populations of melanoma cells treated with encorafenib. Further, it will be validated whether targeting of particular anti-apoptotic proteins by using selective small-molecule BH3 mimetics can enhance induction of apoptosis in melanoma cell populations treated with encorafenib. To broaden the mechanism of this potential cooperation, apoptosis will be monitored at the cellular and molecular levels, including preparation of mitochondrial fraction and assessment of changes in expression levels of 84 genes associated with apoptosis, including those encoding for DEATH domain-containing proteins, caspases, BCL-2 genes and transcription factors involved in apoptosis regulation. Therefore, this project is expected to identify potential therapeutic targets among anti-apoptotic BCL-2 proteins whose inhibition could improve the outcome of encorafenib activity in melanoma cells.