BRAF-mediated NAMPT overexpression induces a melanoma cell dedifferentiation program leading to metabolic reprogramming and intrinsic resistant to BRAF inhibitors

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INTRODUCTION: In order to support continued proliferation and growth, tumor cells must metabolically adapt to balance their bioenergetic and biosynthetic needs. In metastatic melanoma (MM) oncogenic BRAFV600E signaling is a critical regulator of this process. Consistently, treatment with BRAF/MEK inhibitors (BRAFi/MEKi) leads to a block of glycolysis, promoting energy stress mediated-apoptosis. However, resistance to therapy develops rapidly, with paradoxical activation of the MAPK/ERK signaling pathway and the acquisition of novel metabolic phenotypes.

Nicotinamide phosphoribosyltransferase (NAMPT) is a key enzyme in the biosynthesis of NAD, an essential cofactor in redox reactions and a substrate of key cellular enzymes, including sirtuins. This enzyme may also be secreted in the extracellular space (eNAMPT), where it helps establish a cyto-protective microenvironment, favoring immunosuppression and resistance to therapy. Consequently, NAMPT inhibitors are being tested as cancer therapeutics.

METHODS: NAMPT expression and activity was studied using biochemical, enzymatic, immunohistochemical and ELISA assays. In silico analysis using TCGA database, RNAseq and functional/metabolic analyses were performed. Stable or inducible NAMPT overexpression was established using lentiviral vector. Xenografts of melanoma were set up using immunocompromised mice treated with NAMPT inhibitor (NAMPTi) alone or in combination with BRAFi.

RESULTS: We found that the BRAF oncogenic signature converges transcriptionally on the overexpression of NAMPT, leading to a marked increase in NAD levels, which in turn support a metabolic switch toward glycolysis or OXPHOS, both strategies exploited by BRAF-mutated melanomas to adapt to chronic exposure to BRAFi. NAMPT up-regulation in BRAFi resistant cells was confirmed in serial biopsies from melanoma patients. Furthermore, NAMPT could be dosed in patient plasma where it correlated with disease burden, response to therapy and overall survival.

Treatment of melanoma cells with NAMPTi depleted NAD, inducing mitochondrial stress, cell cycle arrest and apoptosis *in vitro*, while they were highly effective in controlling the disease in melanoma xenografts. Exogenous overexpression of NAMPT in BRAF-mutated melanoma cell lines leads to a net increase of NAD

and ATP, rendering cells intrinsically resistant to BRAFi and supporting a proliferative/invasive phenotype *in vitro* and *in vivo*. Mechanistically, NAMPT overexpressing cells switch to a dedifferetiation/epigenetic program leading to a downregulation of microphthalmia-associated transcription factor (MITF).

CONCLUSION: In conclusion, this work links oncogenic BRAF signaling to metabolic reprogramming through NAD biosynthesis and identifies NAMPT as an actionable target for melanoma patients with BRAF mutations.