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Resistance to BET Inhibitors Involves the Wnt/β-catenin Pathway in Uveal Melanoma

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ABSTRACT

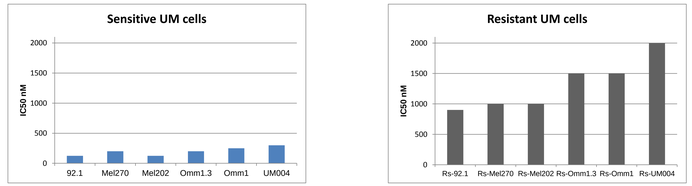
Uveal melanoma (UM) is an aggressive intraocular malignancy with high tendency to metastasize to the liver. Currently available drugs have shown limited clinical activity in patients with UM and there is an urgent need for new effective therapies. Recent findings from our laboratory demonstrated that UM cells are sensitive to BET inhibitors (BETi) through the induction of cell cycle arrest and apoptosis (Ambrosini G, Oncotarget 2015). However, despite the initial inhibitory effects, UM cells acquire resistance to this class of drugs following chronic drug exposure. In order to understand the mechanistic basis for BETi resistance in UM, we assessed genome-wide CpG methylation patterns in UM cell lines that had been rendered resistant to the clinical BET inhibitor PLX51107 (Plexxikon, Berkeley, CA). The comparison between BET inhibitor-resistant versus sensitive cell lines revealed differential methylation of 3,700 genes, including several involved in Wnt/β-catenin signaling, as well as other signaling pathways. Immunoblotting analysis confirmed that β-catenin protein was induced and activated in the resistant cells, while depletion of β-catenin by siRNA re-sensitized the resistant cells to the BET inhibitor. We then explored several combinations of PLX51107 with drugs that block β-catenin transcriptional activity through inhibition of its phosphorylation by PAK4 or through the inhibition of binding partners like Cdk8 and CBP. All these combinations increased the activity of the BET inhibitor in both sensitive and resistant cells, and these effects were synergistic, with combination indexes (CI) <1. These observations support the evidence that resistance to BET inhibitors is mediated, at least in part, by activation of the Wnt/β-catenin pathway, and inhibitors of this pathway may provide a means to overcome acquired BET inhibitor resistance in UM patients treated with this class of drugs.

CONCLUSIONS

- **Despite the initial cytotoxic activity of BET inhibitors (AACR 2016, Abstract #4462), UM cells develop resistance to this class of drugs over time.**
- **Analysis of DNA methylation of resistant versus sensitive cells revealed differential methylation of ~3,718 genes, including genes involved in the Wnt/β-catenin pathway.**
- **The combination of PLX51107 with several inhibitors of β-catenin regulators or binding partners increases sensitivity to the BET inhibitor in a synergistic manner, in both parental and resistant cell lines.**
- **Collectively, our findings suggest that the combination of PLX51107 with inhibitors of the β-catenin pathway will be beneficial to patients with uveal melanoma.**

Figure 1. UM cells are sensitive to BET inhibition but acquire resistance after long-term exposure

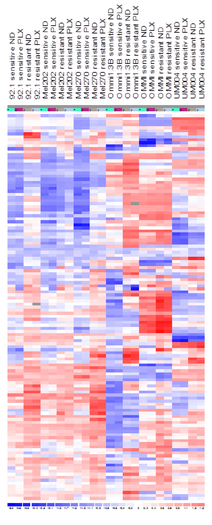
A



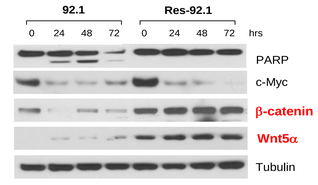
Methylation analysis of BETi resistant versus sensitive cells reveals activation of the β-catenin pathway

B. Differential DNA methylation in resistant versus sensitive cell lines

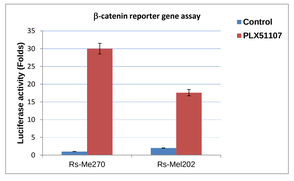
CpG Methylation Heatmap



C. The expression of Wnt5 and β-catenin is induced in the resistant cells



D. The BET inhibitor induces β-catenin transcriptional activity



E. β-catenin depletion sensitizes cells to the BET inhibitor

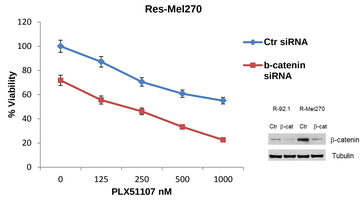
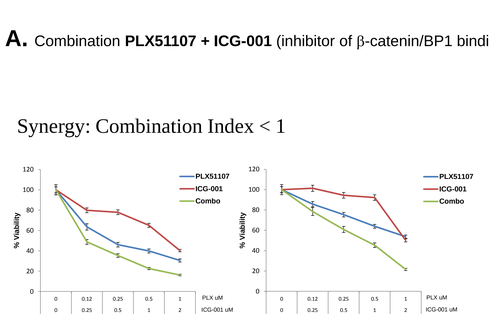


Figure 2. The combination of PLX51107 with drugs targeting the β-catenin pathway is synergistic in both sensitive and resistant UM cells

Sensitive **Resistant**

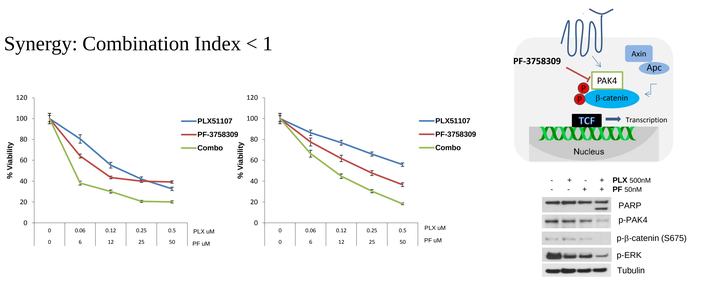
A. Combination **PLX51107 + ICG-001** (inhibitor of β-catenin/BP1 binding)

Synergy: Combination Index < 1



B. Combination **PLX51107 + PF-3758309** (inhibitor of PAK4, which phosphorylates β-catenin)

Synergy: Combination Index < 1



C. Combination **PLX51107 + PLX5552** (CDK8 inhibitor, which inhibits β-catenin transcriptional activity)

Synergy: Combination Index < 1