

Dual Targeting of Oncogenic Microtubules and Mitochondria in PDAC

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Presentation Objective:

To demonstrate the dual inhibitory effects of SB-216 on oncogenic β -tubulin subtypes and mitochondrial function in pancreatic ductal adenocarcinoma cell lines.

Introduction:

Pancreatic Ductal Adenocarcinoma (PDAC) is among the deadliest cancers with a five-year survival rate of only 12%. Development of PDAC metastases is dependent on increased bioenergetics and oncogenic microtubule isotypes. We have previously demonstrated that BRD4, a transcription factor that is part of the bromo- and extra-terminal domain (BET) protein family, regulates mitochondrial complex gene expression, increases bioenergetics, and is targetable. We hypothesize that novel dual-function inhibitors (Veru-111 and a more potent derivative, SB-216) blocking mitochondrial and β -tubulin polymerization would decrease PDAC cell progression.

Methods:

Cell-free DNA (cfDNA) from PDAC patient plasma was analyzed using DNA sequencing and gene set enrichment analysis (GSEA), comparing high vs. low tumor burden samples. Commercial and patient-derived PDAC cell lines (PDCLs) were treated with Veru-111 and SB-216. Cell proliferation was assessed via IncuCyte live-cell imaging. Protein and mRNA expression were evaluated by Western blot and qRT-PCR. Mitochondrial function was assessed using Seahorse XF assays.

Results:

GSEA identified enrichment of mitochondrial electron transport chain genes in high tumor burden samples (NES 2.1, $P < 0.0001$). SB-216 and Veru-111 significantly reduced BRD4

expression and inhibited proliferation across PDCLs ($P < 0.0001$). Both agents reduced ATP production and respiration ($P < 0.05$), indicating impaired mitochondrial function. Expression of TUBB3 (β III) and TUBB4 (β IVb) was significantly decreased at both mRNA and protein levels ($P < 0.05$). Treated cells showed increased autophagy marker expression.

Conclusion:

SB-216 inhibits cell growth, expression of oncogenic β -tubulin subtypes, and mitochondrial function in multiple PDAC models. Further studies will elucidate the effect of these drugs on murine and organoid models, as well as characterize the effect of these drugs on mitochondrial function in PDAC cells with mitochondrial function assays.

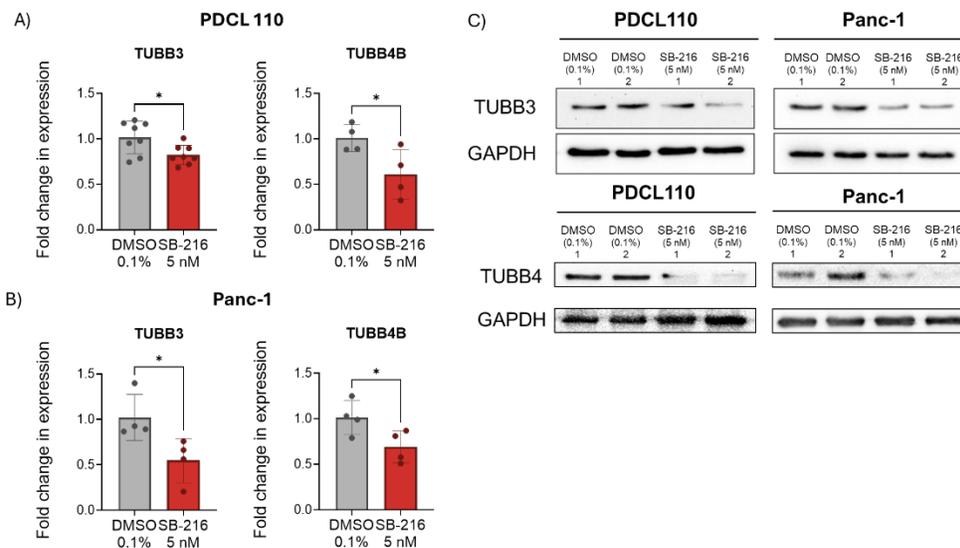


Figure : SB-216 significantly reduces mRNA expression of oncogenic tubulin subtypes β III and β IVB and protein expression of β III

A) Effect of SB-216 on mRNA expression of TUBB3 and TUBB4B in (A) PDCL110 and (B) Panc-1 cells. Cells were treated for 48 hours with indicated concentrations. RNAs were isolated and transcribed for cDNA preparation. qPCR was performed to determine mRNA expression. Actin was used as an internal control. Graphs represent relative fold expression of each tubulin mRNA expression. (Values mean \pm SD, $n=8,4$). C) Effect of SB-216 on protein expression of TUBB3 and TUBB4 was validated in Panc-1 cells after treatment with SB-216 (5nM) or DMSO (control) for 48 hours. Protein expression was evaluated with western blot. GAPDH was used as the loading control. Asterisks represent a significant difference, * $p < .05$.