A novel therapeutic compound VNPP433-3β inhibits medulloblastoma cells

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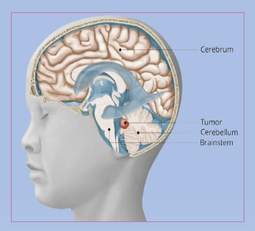
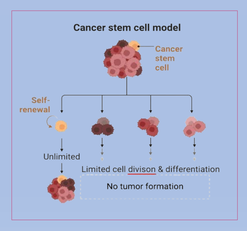
ABSTRACT

Medulloblastoma, originating in the cerebellum, is the most common malignant brain tumor in children accounting for 15-25% of pediatric brain cancers. While drug therapies are available, none are consistently effective at overcoming resistance and killing cancer stem cells (CSCs). As a result, there is an urgent need for a new therapeutic approach. This study investigates the efficacy of a newly developed compound, VNPP433-3β, and its molecular mechanism on medulloblastoma cell lines and medullospheroids derived from medulloblastoma cell lines. DAOY and D341 metastatic cell lines were used as *in vitro* models, and medullospheroids were used as an *ex vivo* model. The cells were cultured and treated with specific media containing growth factor, and their viability was assessed using the MTT assay to determine the IC50 value at 24 h, 48 h, and 72 h. Morphological assessment was conducted using an inverted microscope, and inhibitory properties were determined in a clonogenic assay. The MTT assays revealed a significant reduction in cell viability in a time and dose-dependent manner. Furthermore, treatment of cells with 1 µM of VNPP433-3β resulted in significantly fewer colonies and medullospheroids than untreated cells. The mechanism of cell death was examined using DAPI staining, which showed nuclear fragmentation and chromatin condensation, indicating cell death characteristic of apoptosis. Western blot analysis revealed that VNPP433-3β downregulated the oncoprotein FoxM1. Additionally, phospho-β-catenin expression, associated with spheroid formation, was downregulated compared with untreated cells. These findings suggest that VNPP433-3β has the potential to be a therapeutic candidate for the treatment of medulloblastomas.

BACKGROUND

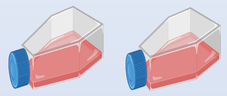




- Medulloblastomas are the most common malignant brain tumor in children and occur in the cerebellum.
- Typically, fast-growing
- It primarily affects children and teens under the age of 16 and more rarely affects adults.
- Occurs between ages 3-8.
- 5-year survival rate of 70-90%
- Urgent need to develop a drug that can kill stem cells responsible for drug resistance, disease recurrence, and metastasis.

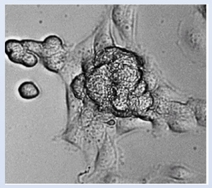
METHODOLOGY

In vitro



D341 **DAOY**

Ex vivo



Cell Culture

Cell lines were cultured with DMEM complete media containing 10% for DAOY and 20% fetal bovine serum (FBS) for D341 and 1X penicillin and streptavidin, then incubated at 37C with 5% CO2.

Medullospheroid Culture

Medullospheroids were cultured in DMEM/F-12 media containing 2% B27, 1% N2 supplement, 2 mg/mL heparin, 20 ng/mL EGF, 10ng/mL 1x AA. At day 7 full medullospheriods were formed.

MTT Assay

MTT assay for detection of IC50 value using different concentrations of VNPP433- 3β (0, 0.5, 1, 2, 4 µM for 24h, 48h).

Morphology Assessment

A morphological assessment was performed using an inverted microscope

Clonogenic Assay

The presence of colonies was tested using Crystal violet dye.

Western Blot Analysis

An equal amount of proteins (30 µg) from treated or untreated DAOY cell lysates as indicated.

DAPI Staining

Apoptotic measurement using DAPI staining.

RESULTS

Structure of VNP433-3β

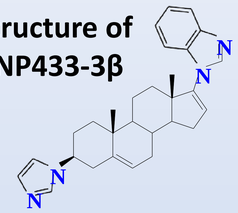


Figure 1. Cell Viability Assay

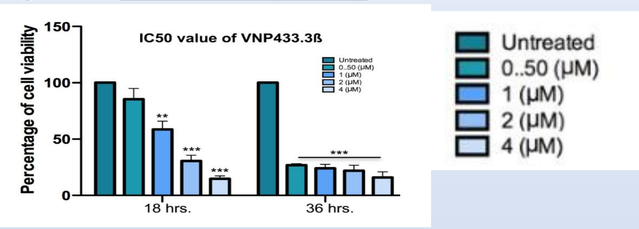
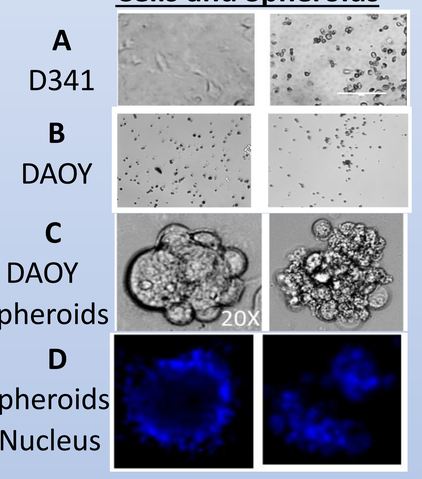


Figure 2. Morphological Impact of VNP4333β on Medulloblastoma Cells and Spheroids



DAOY and D341 cells (4x10⁴) were seeded on 96-plate for overnight. Cells were treated with 1 µM VNP433.3ß for 24h. Images were captured using and microscope (A) DAOY (B) D341. (C) Fragmented medullospheroid after treatment with 2 µM drug. (D) DAPI staining reveals nuclear fragmentation with or without drug treatment.

Figure 4. VNP433.3β suppresses Oncoprotein FoxM1 and β-catenin expression

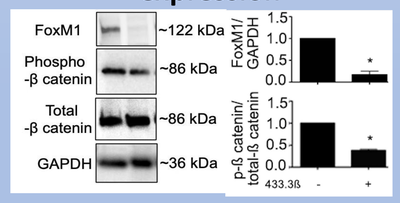
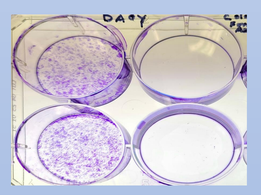
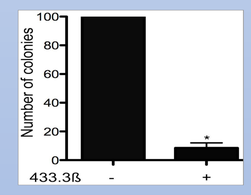


Figure 3. VNP433-3β inhibits Clonogenicity





Growth was measured by direct counting of clonal clusters (DAOY cells) stained with crystal violet in six well plate at 24h.

CONCLUSION

- VNPP433-3β has the potential to be a therapeutic candidate for Medullablastoma cells and spheroids.
- The molecular mechanism is downregulation of phospho β-catenin and oncoprotein FoxM1 protein expression.
- **FUTURE PLAN**

Drug Efficacy on xenograft model.

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