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Abstract

Medulloblastomas (MB) are devastating brain tumors originating in the cerebellum most commonly in children. There are four distinct subgroups of medulloblastoma: WNT (wingless), SHH (sonic hedgehog), group 3, and group 4. The most malignant tumors possess an aggressive phenotype characterized by c-Myc amplification and deletions to chromosome 17p; they belong to group 3. Prior investigations into the significance of genes on 17p revealed that miR-1253, which is found on locus 17p13.3, is significantly downregulated in medulloblastoma and has important tumor suppressive properties. Amongst its oncogenic targets is B7-H3 (CD276), a highly deregulated oncoprotein that attenuates the immune response to MB tumors. We chose to elucidate the oncogenic properties of B7-H3 in group 3 MB using synthetic inhibitors. After screening 100,000 different compounds for: 1) docking ability, 2) oral bioavailability, 3) potential CNS activity, and 4) number of metabolic side reactions, we selected two N-terminal inhibitors: B7-H3-Ni1 and B7-H3-Ni3. In HDMB03 cells (with c-Myc amplification and i17q), we found an IC_{50} of 3.7 μM for B7-H3-Ni1 and no discernible effect of B7-H3-Ni3. We confirmed CD276 expression inhibition using B7-H3-Ni1 via Western blotting and concurrently noted elevations in cleaved PARP (apoptosis) and reduction in p-Akt (proliferation marker), providing us preliminary insights into the mechanism of inhibition. Notably, a remarkable decline in migration and wound healing and abrogation of colony formation were observed with B7-H3-Ni1. Collectively, our findings substantiate the inhibitory properties of B7-H3-Ni1 *in vitro*, potentially serving as a therapeutic agent for *in vivo* group 3 MB tumors.

Background

- Group 3 medulloblastomas are one of the most aggressive malignant pediatric tumors of the central nervous system. They express frequent c-Myc amplification and i17q.[1,2]
- Our prior studies have revealed that miR-1253, found on the terminal end of chromosome 17, is epigenetically silenced and has important tumor suppressive properties.[1]
- Two identified oncogenic targets of miR-1253 include CDK6 and CD276 (B7-H3).[1]
  - B7-H3 is deregulated in group 3 tumors of MB patients.
- B7-H3 is a transmembrane immune checkpoint protein that is overexpressed in medulloblastoma. It inhibits tumor infiltration by T cells and promotes metastasis.[3]
- Previous clinical investigations have demonstrated promising results in targeting B7-H3 with conjugated monoclonal antibodies (mAbs).[4]
- Other methods of targeting B7-H3, including the use of small-molecule inhibitors and chimeric antigen receptor T cells, are currently being investigated.[4]
  - An inhibitor binding to B7-H3 blocks the interaction between the receptor on the immune cell and the ligand on the tumor cell, recovering immune cell function.[5]
- Expression patterns of B7-H3 across different malignancies, especially in pediatric brain tumors, makes it an important target for cancer therapy.[5]

Methodology

Establish that B7-H3 is highly upregulated in Group 3 medulloblastoma. (Figure 1) → **Select top inhibitors based on established criteria. (Figure 2)** → **Determine IC_{50} of selected inhibitors with MTT assay. (Figure 3)** → **Evaluate B7-H3 expression, apoptotic/ proliferation markers. (Figure 4)** → **Analyze colony formation, migration, and wound healing. (Figure 5)**

Hypothesis

Synthetic inhibitors of B7-H3 will demonstrate an anti-neoplastic response in Group 3 medulloblastoma.

Results

B7-H3 is deregulated in Group 3 medulloblastoma.

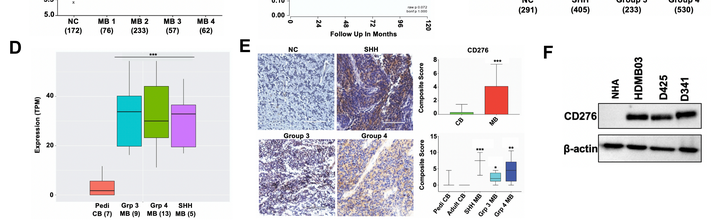
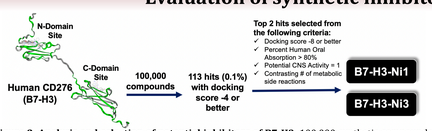


Figure 1. Differential expression of B7-H3 in medulloblastoma. (A) *In silico* analysis showing the expression of B7-H3 across MB datasets (R2 platform; NC=normal cerebellum; MB=medulloblastoma). **(B)** Kaplan Meier curve showing MB patient survival (Cavali *et al.*). Subgroup specific expression of B7-H3 in patient samples **(C)** GSE124814 and **(D)** GSE148390. **(E)** Immunohistochemical analysis of B7-H3 in MB patient samples (pedi CB n = 10, adult CB n = 5, SHH n = 3, Grp3 n = 6, Grp4 n = 10). **(F)** B7-H3 expression across three MB cell lines compared to normal human astrocytes. Statistical analysis performed via Mann-Whitney U test (D), ANOVA (A and C), and Student's *t*-test (E). **p* <0.05, ***p* <0.01, ****p* <0.001. β-Actin served as an internal loading control. Scale bar:100μm.

Evaluation of synthetic inhibitors of B7-H3.

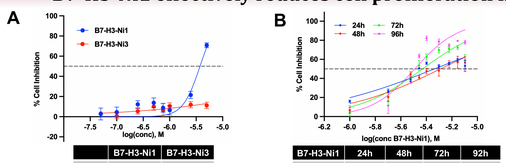


N-terminal site blockers				
Inhibitor	Docking score	#metab	%OralAbs	CNS score
B7-H3-Ni1	-9.381	6	81.038	1
B7-H3-Ni2	-9.094	3	100.000	-1
B7-H3-Ni3	-8.694	3	100.000	1

C-terminal site blockers				
Inhibitor	Docking score	#metab	%OralAbs	CNS score
B7-H3-Ci1	-6.158	7	100.00	1
B7-H3-Ci2	-5.538	3	74.302	-2

Figure 2. Analysis and selection of potential inhibitors of B7-H3. 100,000 synthetic compounds were analyzed using the crystal structure of human B7-H3, which was modelled through the YASARA structure program. The docking ability of these molecules on either the N- or C- terminus of B7-H3 was evaluated. From a total of top 113 hits, two inhibitors were selected for further investigation based on established criteria.

B7-H3-Ni1 effectively reduces cell proliferation in HDMB03 cells.

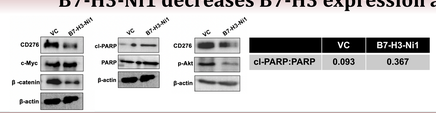


	B7-H3-Ni1	B7-H3-Ni3
IC_{50}	3.7 μM	0.002 M

B7-H3-Ni1	24h	48h	72h	92h
IC_{50}	4.6 μM	5.1 μM	3.7 μM	3.2 μM

Figure 3. Cell inhibition in response to drug treatment for HDMB03 cell line measured by the MTT assay. (A) IC_{50} values were determined following 48h treatment with 0.05, 0.1, 0.25, 0.5, 0.75, 1, 2.5, and 5 uM concentration of B7-H3-Ni1 and B7-H3-Ni3. **(B)** IC_{50} values following treatment with 1, 2, 3, 3.5, 4, 5, 6, 7, and 8 uM concentration of inhibitor B7-H3-Ni1 with 24, 48, 72, and 96h incubation periods.

B7-H3-Ni1 decreases B7-H3 expression and favors apoptosis.



	VC	B7-H3-Ni1
cl-PARP:PARP	0.093	0.367

Figure 4. Analysis of expression of B7-H3 and apoptotic/tumor cell proliferation markers. A significant decrease in expression of B7-H3 was observed at the translational level with B7-H3-Ni1 treatment. Elevations in an apoptotic marker (cleaved-PARP) and reduction in a tumor proliferation marker (p-AKT) were also noted. The ratio of cleaved PARP to total PARP is tabulated. β-Actin served as an internal loading control.

Results

B7-H3-Ni1 treatment demonstrates anti-neoplastic properties.

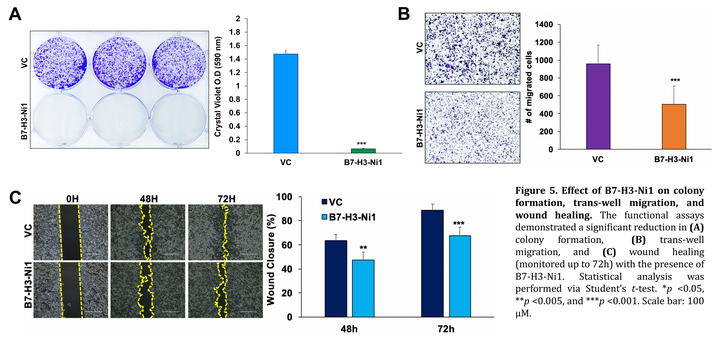


Figure 5. Effect of B7-H3-Ni1 on colony formation, trans-well migration, and wound healing. The functional assays demonstrated a significant reduction in **(A)** colony formation, **(B)** trans-well migration, and **(C)** wound healing (monitored up to 72h) with the presence of B7-H3-Ni1. Statistical analysis was performed via Student's *t*-test. **p* <0.05, ***p* <0.005, and ****p* <0.001. Scale bar: 100 μM.

Conclusions and Future Directions

Conclusions

- B7-H3-Ni1 effectively attenuated CD276 expression and favored apoptosis.
- B7-H3-Ni1 exhibited anti-tumorigenic properties by reducing colony formation, migration, and wound healing.

Future Directions

- Re-capitulate inhibitory effects in additional medulloblastoma cell lines, such as D341 and D425.
- Assess impact on expression of downstream effectors of B7-H3, such as NF-κB, PI3K, HIF-1α, and STAT3.[6]
- Further explore colonogenic inhibition by inhibitor via medullosphere assay and assess stem cell markers.
- Conduct co-culturing experiments of medulloblastoma cell lines with immune cells (T-cells) in the presence of B7-H3-Ni1.
- Perform *in vivo* orthotopic experiments to assess the therapeutic potential of B7-H3-Ni1 on tumor growth and survival.

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