Targeting AXL with a Highly Stable Modified Aptamer in Medulloblastoma Cell Lines

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Abstract

Medulloblastomas are the most common types of pediatric brain tumors, that start in the cerebellum. They represent approximately 20% of pediatric brain tumors, with an average age of diagnosis between 5 and 9 years old¹. Surgery followed by radiation, and chemotherapy is the standard treatment; however, it can be ineffective due to the location of the tumor and the age of the patient. Thus, patients would benefit from non-toxic, targeted therapeutics.

AXL, a receptor tyrosine kinase, is overexpressed in many types of cancers and associated with poor patient survival. AXL is activated by its ligand, Gas6. AXL/Gas6 signaling pathway has been shown to enhance proliferation, invasion, metastasis, and drug resistance².

Aptamers are synthetic, single-stranded RNA or DNA oligonucleotides that selectively bind their target proteins with high affinity. Aptamers are considered chemical antibodies with the advantage to be non-immunogenic and easy tumor penetration due to their small size.

The lower survival in patients expressing high levels of AXL makes it a potentially viable therapeutic target in the treatment of brain cancer. Additionally, the enhanced stability of the GLB-A04 aptamer and strong binding to AXL have been shown to inhibit AXL in vivo. Here, although we find no difference in cell viability or apoptotic protein expression, experiments need to be repeated with higher concentrations of aptamer and longer treatment times.

Here, we demonstrate that overexpression of AXL in GBM patients is associated with poor survival. We then tested the ability of a highly modified DNA aptamer, targeting AXL to inhibit phospho-AXL and produce effects apoptosis by Western Blot and Annexin V/PI flow cytometry analysis. Thus, aptamer-induced inhibition of phospho-AXL may be a viable therapeutic target. Results obtained so far by us provide the strongest evidence and lay the foundation for the next stage of development to advance towards the translation of the aptamer candidates and companion biomarkers to the clinic.

GLB-A04 Preliminary Data

Figure 1. GLB-A04 Structure, Binding, and Stability. (A) Predicted 3D structure of GLB-A04 according to MFold Software. (B) Binding curve of GLB-A04 (blue) compared to scramble aptamer. (C) Interaction of GLB-A04 aptamer and scramble aptamer (D) with Axl. (E) Stability of GLB-A04 in 95% human serum.

Introduction

• AXL has been identified as an important driver of mesenchymal and stem-like phenotype in diffuse pontine glioma (DIPG).

• Our group has generated a new molecule DNA aptamer targeting phospho-AXL with better features, more stable and higher activity, and bioavailability.

• Here, we examined the effect of the modified DNA aptamer GLB-A04 on two medulloblastoma cell lines expressing high levels of AXL. DAOY and UW228 cells were treated with the DNA aptamer GLB-A04 and the effects on viability and apoptosis were assessed.

Hypothesis

Targeting AXL leads to anti-tumor activity in medulloblastoma cell lines.

Objectives

• To determine the expression of AXL in medulloblastoma cell lines and examine the effect on survivability.

• To determine the ability of GLB-A04 to inhibit phospho-AXL in medulloblastoma cell lines.

• To examine the effects of GLB-A04 to induce apoptosis.

Results

AXL expression is correlated with poor survival, is highly expressed in brain tumor and medulloblastoma cell lines, and Axl activation is inhibited by GLB-A04

Figure 2. (A) High expression of AXL is associated with lower survival in brain tumor patients (Center for Cancer Research, BioDiscovery Portal). (B) Axl is overexpressed in brain tumors compared to normal tissue⁵. CCLE data⁶ (C) and western blot analysis (D) indicate high expression of Axl in DAOY and uw426 cell lines. (E) Gas6-stimulated (200ng/mL) medulloblastoma cell line was treated with 400nM GLB-A04 and scramble aptamers to determine p-Axl expression.

Figure 3. Cells treated with concentrations of 500nM, 800nM, and 1.6µM GLB-A04 and 1.6µM scramble aptamer showed no difference in expression of pro-death apoptotic proteins

Treatment with GLB-A04 does not impact expression of apoptotic proteins in DAOY and uw426 cell lines

Figure 4. Cells were incubated with varying concentration of GLB-A04 or scramble aptamer for 72 hours and then collected, labeled with Annexin V and PI and visualized via flow cytometry. No difference in viability or apoptotic cell count was found between any of the concentrations of GLB-A04 and the scramble control.

Conclusion

• AXL is highly expressed in medulloblastoma cell lines and associated with poor patient survival.

• The GLB-A04 modified DNA aptamer downmodulated phospho-AXL in vitro.

• GLB-A04 modified DNA aptamer does not induce in vitro apoptosis at the concentrations and times tested in the medulloblastoma cell lines.

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Methods

• Cell lines: DAOY and uw426 cell lines were maintained in high-glucose DMEM supplemented with 10% FBS and 1% penicillin. Cell lines were maintained at 37°C with 5% CO2 and 95% air.

• Western Blotting: Cells were lysed in RIPA buffer with protease and phosphatase inhibitors. Protein was quantified using BCA analysis and samples were run on 4-15% polyacrylamide SDS gel. Protein was transferred to PVDF membrane and blocked for 30min in 6% milk. Antibodies were prepared in 5% milk and incubated for 48hr (p-Axl) or overnight. Secondary antibodies were prepared in 5% milk and incubated overnight (p-AXL) or for 90min. Bound antibodies were visualized using ECL lighting reagents.

• Flow Cytometry: Apoptosis assay was carried out using FITC Annexin V Apoptosis detection kit (BD Pharmingen). Cells were treated with GLB-A04 at varying concentrations for 96 or 120 hours. Cells were then collected, washed with PBS, and treated with 4µL of Annexin V and propidium iodide. After 10min incubation in the dark, samples analyzed by flow cytometry.

References

1. St. Jude Children’s Research Hospital