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Tyrphostin (AG-879) Decreases AKT Activation Through NGFR Inhibition in Human Glioblastoma Cell Lines

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Background

Glioblastoma (GBM), a grade IV astrocytoma, is an aggressive malignant brain cancer with a dismal 5-year survival rate of 6.9%[1-3]. Current treatments including surgical resection, radiation and chemotherapy, are not curative, thus better targeted therapy is urgently needed. During GBM development, Nerve Growth Factor Receptor (NGFR), a crucial signaling receptor for the maintenance and growth of gliomas, is increased [2].

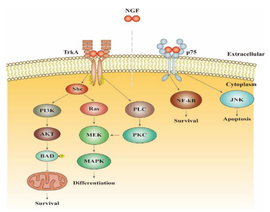
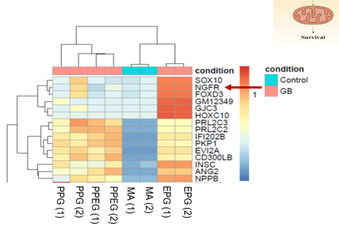
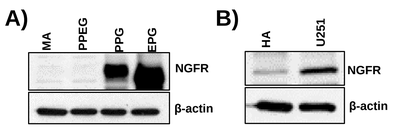


Fig. 1. NGFR signaling. Image taken from Journal of Biological Engineering Zhou et. al 2023 17:75.





MA – mouse astrocytes; PPG – PTEN $^{-/-}$; p53 $^{R172H-/-}$ & GFAP Cre; PPEG - PTEN$^{+/-}$; P53 $^{R172H\ +/-}$; EGFRvIII & GFAP Cre; EPG – EGFRvIII, p16 $^{-/-}$ & GFAP Cre

Fig. 2. NGFR expression increased in EPG mouse syngeneic cell lines, which express EGFRVIII, on RNA-seq.

Fig. 3. NGFR expression in GBM cell lines (A) NGFR expression was greater in PPG and EPG models. β-actin serves as the loading control. **(B)** NGFR expression was greater in GBM.

- Tyrphostin (AG-879) inhibits TrkA in the NGFR pathway.[1] In unpublished preliminary studies, AG-879 inhibited cell growth and survival in U118 GBM cells.

NGFR expression in GBM cells

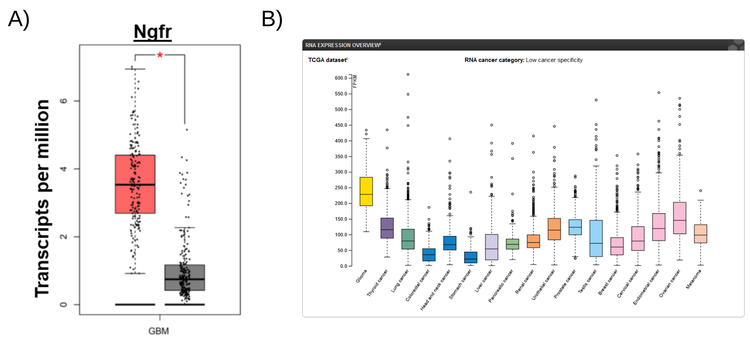
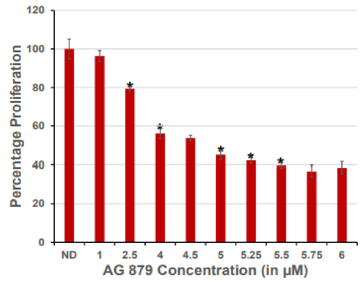


Fig.4. NGFR expression increases in GBM (A) Gene Expression Profiling Interactive Analysis (GEPIA) of NGFR expression in GBM **(B)** TCGA data of NGFR RNA expression in various types of cancer.

AG-879 causes dose dependent inhibition

Fig. 5. AG-879 inhibited growth in dose dependent fashion. MTT analysis to obtain IC_{50} values for increasing concentrations of AG-879 in U118 GBM cells. *p< 0.05
(Consistent findings with IncuCyte, data not shown).



Hypothesis

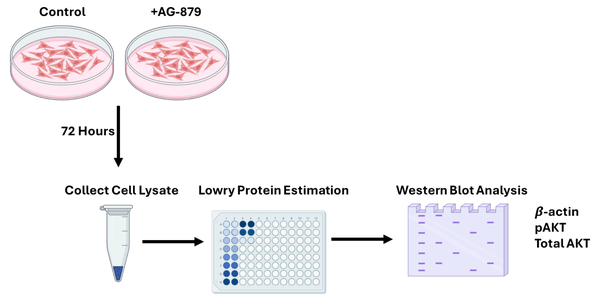
NGFR contributes to GBM progression by promoting proliferation and its pharmacological targeting will inhibit GBM growth.

Objectives

1) To determine the impact of AG-879 on proliferation
2) Characterize the underlying mechanism of anti-GBM effects of AG-879

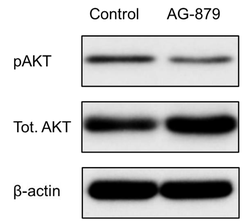
Methods

- U118 cells cultured in DMEM high glucose media
- Split and seeded into petri dishes
- Treated with 10μM concentration of AG-879 for 72 hours in 37.5°C incubator
- Cells scraped and collected
- Lowry protein estimation was performed to quantify the amount of protein in the sample
- Lysates were used in Western Blot analysis
- Membranes were probed for β-actin (loading control), pAKT (proliferation marker) and total AKT.



NGFR inhibition decreases U118 cell proliferation by inhibiting AKT activation

Fig. 6. AG-879 decreases AKT activation. U118 cells were treated with IC_{50}10μM of AG-879 for 72 hours and lysates were probed with Total AKT and Phospho-AKT (Ser473) antibodies. β-actin serves as the loading control.



Discussion

- NGFR expression is enhanced in GBM EGFRvIII mutant/ p16$^{-/-}$ tumor cells from a syngeneic mouse
- NGFR expression is elevated in mouse glioblastoma and human glioblastoma U251 cells
- AG-879 causes dose-dependent inhibition of cell growth and survivability in both U251 and U118 cell lines
- In this study, AG-879 inhibited AKT activation (pAKT) at 10μM

Future Directions

- Evaluate the effect of AG-879 on apoptotic markers (cleaved Caspase 3 and cleaved PARP) as well as on cell cycle arrest (Cyclins and CDKs)

References

1. Kumar V, Gupta AK, Shukla RK, Tripathi VK, Jahan S, Pandey A, Srivastava A, Agrawal M, Yadav S, Khanna VK, Pant AB. Molecular Mechanism of Switching of TrkA/p75(NTR) Signaling in Monocrotophos Induced Neurotoxicity. Sci Rep. 2015 Sep 15;5:14038.
2. Morrison C, Weterings E, Gravbrot N, Hammer M, Weinand M, Sanan A, Pandey R, Mahadevan D, Stea B. Gene Expression Patterns Associated with Survival in Glioblastoma. International Journal of Molecular Sciences. 2024; 25(7):3668.
3. Ostrom, Q. T., Price, M., Neff, C., Cioffi, G., Waite, K. A., Kruchko, C., & Barnholtz-Sloan, J. S. (2022). CBTRUS Statistical Report: Primary Brain and Other Central Nervous System Tumors Diagnosed in the United States in 2015-2019. Neuro-oncology, 24(Suppl 5), v1–v95

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