Cephalothin Analogs Inhibit GD3 Synthase and Target GD2+ Breast Cancer Stem-Like Cells in Triple Negative Breast Cancer

Zoe Arvanitis[1,2], Appalaraju Jaggupilli Ph.D.[2], Vivek Anand Ph.D.[2], V.L. Battula Ph.D.[2]

Augustana College[1], Rock Island, IL

Leukemia Department, The University of Texas MD Anderson Cancer Center[2], Houston, TX

Background

- Triple Negative Breast Cancer (TNBC) is the most aggressive subtype of breast cancer, with a high rate of metastasis and poor clinical outcome.
- Breast Cancer Stem-like Cells (BCSC) comprise a small portion of the primary tumor that contribute to the aggressive phenotype of TNBC[1,2].
- BCSC have characteristics that allow them to proliferate, metastasize, and resist conventional cancer treatments.
- There are currently no drugs that target BCSCs.
- Expression of ganglioside GD2 identifies BCSCs and the enzyme GD3 Synthase (GD3S) is upregulated in GD2+ BCSCs[1].
- GD3S is a key enzyme involved in the biosynthesis of b- and c- series gangliosides including GD2.
- Inhibition of GD3S expression in TNBC cells significantly inhibits their stem-cell function and inhibits tumor growth *in vivo*.

Hypothesis

- Targeting GD3S enzyme activity using small-molecule inhibitors inhibits tumor growth and metastasis in TNBC.

Methods

- Using a structural homology modeling approach, we identified cephalothin (an FDA-approved antimicrobial agent) as a potential GD3S inhibitor[3].
- TNBC cell lines including SUM159 and MDA-MB-231 were treated with cephalothin and its analogs at different concentrations for 72 hrs. and GD2 expression was analyzed using flow cytometry.
- TNBC cell lines treated with cephalothin, and its analogs were subjected to BCSC functional assay including mammosphere formation assay.

Results

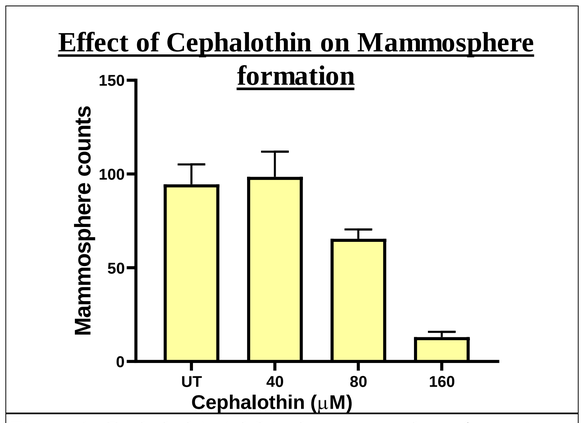

Figure 1: Cephalothin inhibited mammosphere formation by 10-fold in SUM159 cells in a dose dependent manor.

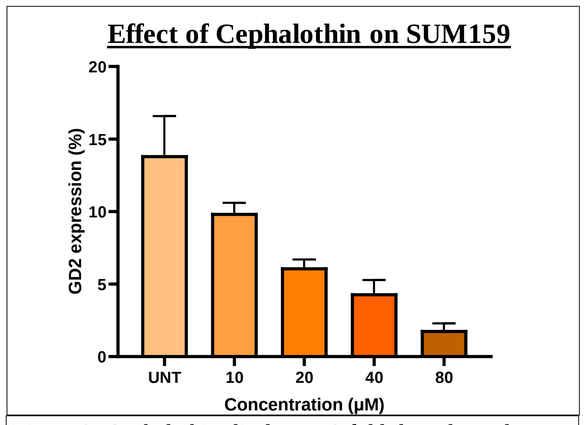

Figure 2: Cephalothin displays a 6-fold dose dependent decrease in GD2 expression in SUM159.

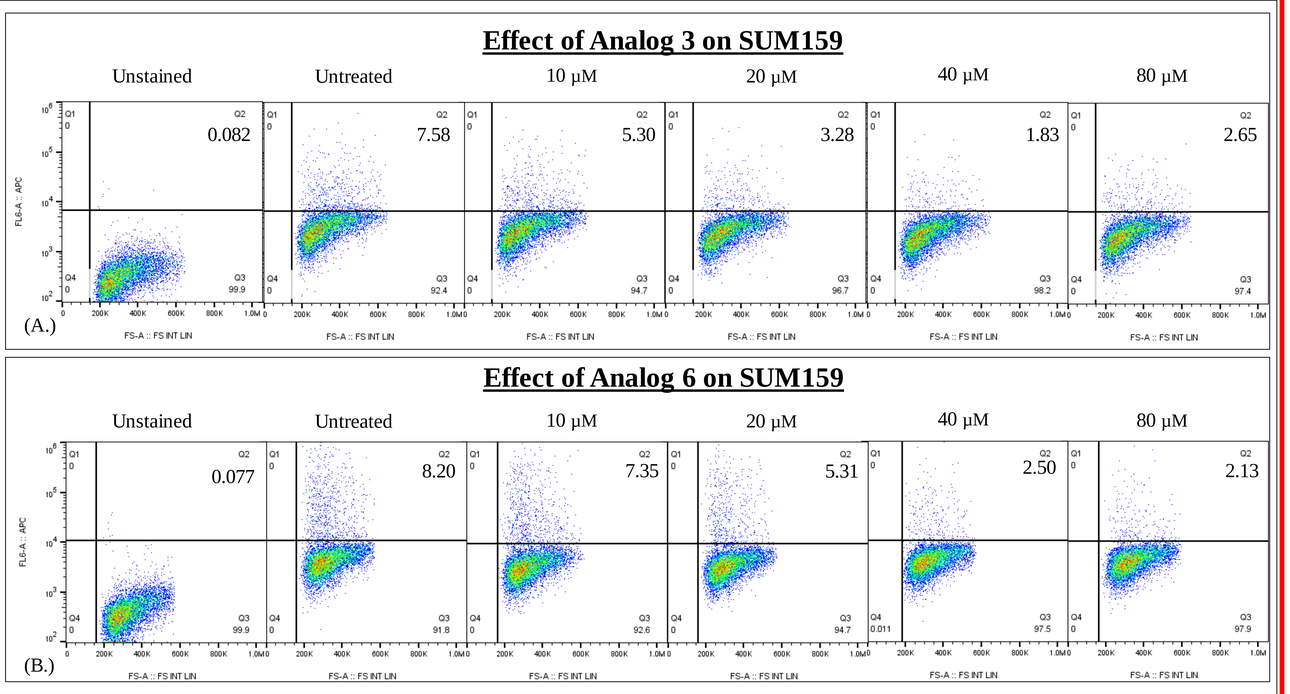

Figure 3: Flow cytometry dot plots showing the effect of (A.) cephalothin analog 3 and (B.) cephalothin analog 6 on SUM159. A dose dependent decrease is observed for both analogs. Analogs 3 and 6 decreased GD2 expression by 3-fold.

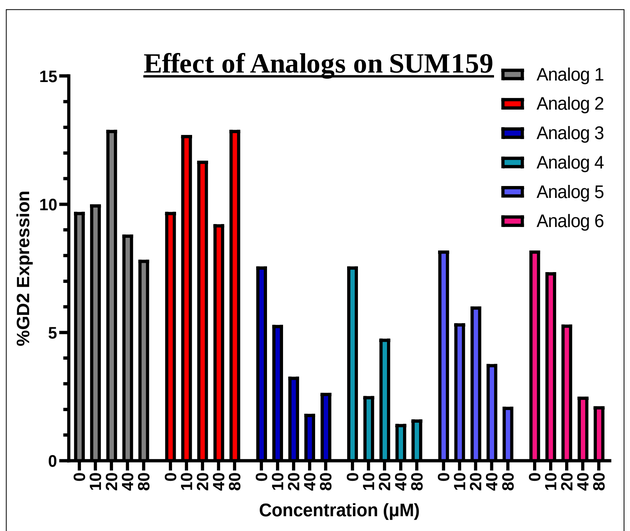

Figure 4: Comparison of all six cephalothin analogs on SUM159 cells. This graph depicts flow cytometry analysis of % GD2 expression. Analogs 3-6 shows a dose dependent decrease on SUM159 cells similar to the decrease shown by cephalothin.

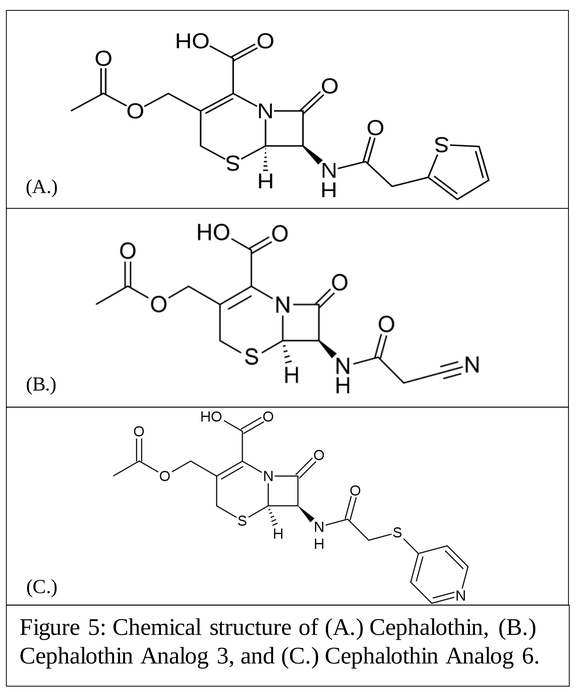

Figure 5: Chemical structure of (A.) Cephalothin, (B.) Cephalothin Analog 3, and (C.) Cephalothin Analog 6.

Conclusion

- Cephalothin inhibited mammosphere formation in a dose dependent manor, limiting the BCSC function of SUM159.
- Cephalothin has shown the most significant decrease on GD2 expression out of the analogs that were tested.
- Cephalothin analogs were more effective at reducing the GD2 expression in TNBC cell lines with higher GD2 expression such as SUM159 compared to MDA-MB-231 with lower GD2 expression.
- Cephalothin analogs 3 and 4 were the most effective on SUM159 cells, decreasing GD2 expression by 3- and 4- fold, respectively.
- Cephalothin's ability to decrease GD2 expression makes it a potential leading compound for inhibiting GD3S enzyme activity.
- Functional characteristics of cephalothin analogs is currently on going.

References

1. Battula, Venkata Lokesh, et al. "Ganglioside GD2 Identifies Breast Cancer Stem Cells and Promotes Tumorigenesis." *Journal of Clinical Investigation*, vol. 122, no. 6, 2012, pp. 2066–2078., doi:10.1172/jci59735.

2. Luo M. et al. (2015) Breast Cancer Stem Cells: Current Advances and Clinical Implications. In: Vivanco M. (eds) Mammary Stem Cells. Methods in Molecular Biology, vol 1293. Humana Press, New York, NY. https://doi.org/10.1007/978-1-4939-2519-3_1

3. Battula, V. L. "Targeting GD3 Synthase (ST8SIA1) in GD2+ Breast Cancer Stem-Like Cells to Prevent Tumor Growth and Metastases in Triple-Negative Breast Cancer". University of Texas MD Anderson Cancer Center, Aug. 2020.

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