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

Zoe Arvanitis1,2, Appalaraju Jaggupilli Ph.D. 2, Vivek Anand Ph.D. 2, V.L. Battula Ph.D.2

Augustana College1, Rock Island, IL
Leukemia Department, The University of Texas MD Anderson Cancer Center2, 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 TNBC1,2.
• 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+ BCSCs1.
• 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 inhibitor3.
• 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

Effect of Cephalothin on Mammosphere formation

![Graph showing the effect of Cephalothin on mammosphere formation](Image)

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

Effect of Cephalothin on SUM159

![Graph showing the effect of Cephalothin on SUM159](Image)

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

Effect of Analogs on SUM159

![Flow cytometry dot plots showing the effect of cephalothin analogs on SUM159](Image)

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.

Effect of Analogs on SUM159

![Graph showing the effect of cephalothin analogs on SUM159](Image)

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.

Effect of Analogs on SUM159

![Chemical structure of cephalothin analogs](Image)

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 manner, 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


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