Effects of B4GALNT1 expression on metastatic phenotype and response to treatment in osteosarcoma cell lines

Fatemeh Zareihajiabadi, Jessie Ellis, Jared Barrott

Department of Biomedical and Pharmaceutical Sciences, Idaho State University

Introduction

GD2 is a glycosphingolipid and a member of the ganglioside family that is overexpressed in several tumor tissues such as osteosarcoma and neuroblastoma while having limited expression in most normal tissues.

GD2 is synthesized by an enzyme called GD2 synthase that is coded by the B4GALNT1 gene. Our preliminary data shows that GD2 synthase has high expression in both metastatic and non-metastatic osteosarcoma..

Recent studies in lung adenocarcinoma have shown that the expression of B4GALNT1 can be related to more malignant phenotype.

The purpose of this study is to understand the effects of B4GALNT1 expression on cancer cells' behaviour and investigating whether the expression of this gene can cause more metastatic phenotype in osteosarcoma cell lines.

Methods

All steps were performed in two different OS cell lines -HOS and SAOS- and we used B4GALNT1 plasmid for overexpression of the gene. The sensitivity and resistance of the cell lines were tested using Cisplatin, and Doxorubicin as part of the standard osteosarcoma chemotherapy protocol.

The proliferation rates were compared over a 2 week period by counting the cells every 3 days.

The migration capability of over-expressed and wild type cell lines was compared using 24 hour scratch assay and to evaluate the metastatic phenotype we compared the expression of the mesenchymal marker (N-cadherin) and transcription factors for epithelial to mesenchymal transition (Twist, Snail, and Slug).

Figure 1: Chemoresistance and chemosensitivity Analysis

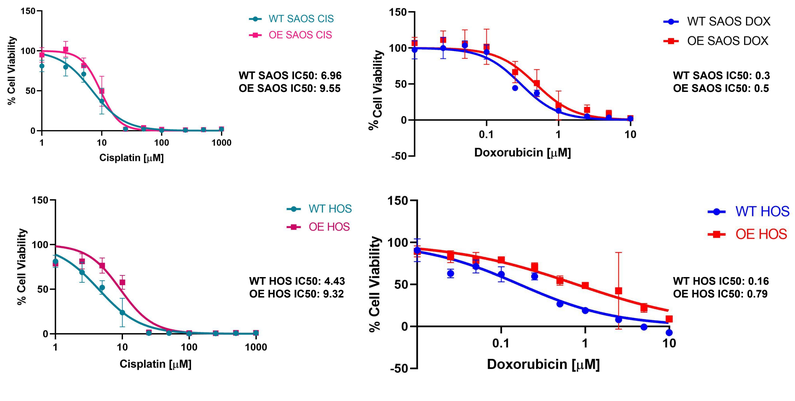

Figure 2: Proliferation

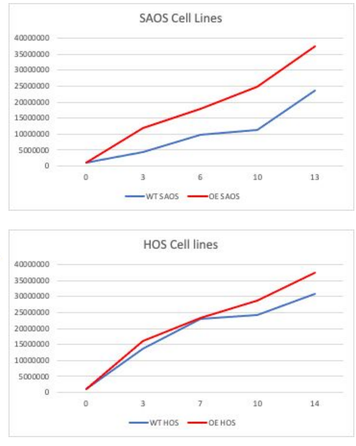

Figure 3: Scratch Assay

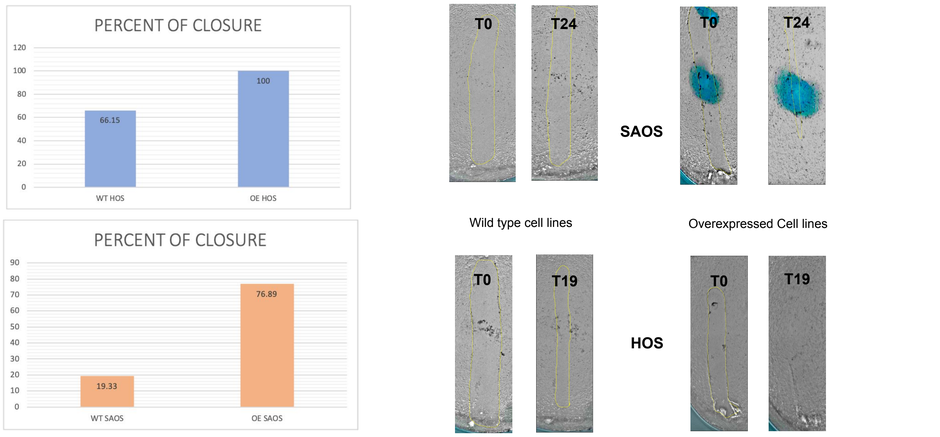

Figure 4 : RT-qPCR Gene Expression

Results

Overexpressing B4GALNT1 increased the IC50 of Cisplatin and Doxorubicin in both Cell lines, and made them more resistant to standard chemotherapeutic agents used in Osteosarcoma treatment (Figure 1). Also both of these cell lines had a higher proliferation rate when having higher expression of B4GALNT1 (Figure 2) . Looking at the scratch assay results, overexpressed cell lines had a higher percentage of closure of the scratch comparing to the wild type cell lines over the same period of time (Figure 3).Over-expression of B4GALNT1 increased expression of N-cadherin and TWIST in both cell lines, but the changes in SNAIL and SLUG were not consistent in them and none of these changes in expression of EMT and transcription factors were statistically significant. (P value > 0.05) (Figure 4).

Conclusion

This study shows that B4GALNT1 contributes to characteristics of a metastatic phenotype and chemoresistance in genetically altered osteosarcoma cell lines, despite no significant changes in expression of mesenchymal markers and EMT transcription factors. Regardless, B4GALNT1 can be a potential gene to target in the treatment of metastatic osteosarcoma.

References

Tian Jiang, Hao Wu, Miao Lin, Jun Yin, Lijie Tan, Yuanyuan Ruan, Mingxiang Feng, B4GALNT1 promotes progression and metastasis in lung adenocarcinoma through JNK/c-Jun/Slug pathway, *Carcinogenesis*, Volume 42, Issue 4, April 2021, Pages 621–630, https://doi.org/10.1093/carcin/bgaa141