BMP Signaling Pathway Mediates Quiescence and Chemoresistance in GBM Stem Cells

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Introduction

Cancer stem cells (CSCs) represent a distinct cell population that has been implicated in tumor recurrence following chemotherapy. Accumulating evidence indicates that BMP signaling plays an important role in the regulation of CSCs, and may be involved in the resistance of CSCs to cytotoxic drugs. The role of BMP pathway regulation of the CSC phenotype responsible for treatment resistance in glioblastoma (GBM) has not been explored.

Methods

Paraffin-embedded samples from GBM patients were used to evaluate the expression of markers for BMP signaling (pSMAD1/5/8), proliferation (PCNA), and stemness (SOX2) by immunostaining. We tested the self-renewal capacity of primary patient-derived glioma stem cell (GSC) lines using a sphere formation assay. Expression of stemness markers SOX2 and Bmi-1 in BMP4 treated and untreated GSCs, was analyzed by Western blotting. BrdU cell proliferation assay was used in detection and quantification of GSC proliferation post BMP4 treatment. We also performed xenograft transplantation, whereby GSCs were injected intracranially into immunedeficient mice. When tumor formation was confirmed post-injection, mice were injected with EdU and sacrificed at successive time points thereafter. The frequency of all EdU positive cells as well as ID-1positive/ EdU positive cells was quantified from the primary tumor sections throughout the labeling and chase. To study chemoresistance in glioblastoma, GSC lines were treated with graded concentrations of temozolomide (TMZ).

Conclusions

This study establishes that the BMP signaling pathway is involved in the maintenance of GSC quiescence and may play a role in glioblastoma chemoresistance.

Results

We found that pSMAD1/5/8 positive cells in primary patient tumors were largely PCNA-negative. BMP4 treatment inhibited GSC proliferation and self-renewal (sphere-formation), but did not abolish expression of markers of stemness, such as Sox2 and Bmi-1, or tumorigenicity. BMP4 treated GSCs showed a decrease in cell proliferation as evidenced by the BrdU proliferation assay. In our in vivo model, we found Id-1 positive cells retain EdU following a long-term chase. BMP4 treatment protects GSCs against TMZ-induced growth inhibition. Furthermore, pSMAD1/5/8-expressing cells do not possess the DNA damage marker gamma-H2AX following TMZ treatment.

Learning Objectives

Targeting the BMP signaling pathway may increase the sensitivity of the tumour cells to TMZ treatment, providing a potential novel therapy for patients with glioblastoma.

References

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