Bone Morphogenetic Protein in the Treatment of Glial Tumors

Maryam Rahman MD, Shanshan Wang PhD, Amy A. Smith MD,
Dennis Steindler PhD, Brent Reynolds PhD
University of Florida, Gainesville, FL, USA

Introduction

The origin, maintenance, and resistance of solid tissue malignancies, including human glioma, is attributed to transformed precursors that have the cardinal properties of stem cells.(5, 17) These transformed cells with stem cell-like properties are hypothesized to be resistant to conventional therapy based on the notion that conventional therapy targets the heterogeneous body of cancer cells in a relatively non-specific fashion and spares the tumor stem cells due to their unique properties.(5, 13) A body of evidence now exists suggesting that brain tumors contain this relatively rare subpopulation of cells that exhibit stem cell characteristics(3, 6, 15, 17), that this population may be responsible for treatment resistance(1) and targeting this population may be an important therapeutic strategy in treating patients with brain tumors.(17) (14)

Traditionally, cancer has been treated with cytotoxic therapy. An alternative approach to targeting the proliferating (and relatively quiescent stem) cells with cytotoxic therapy is to induce stem and progenitor cell differentiation, causing them to lose their stem and proliferative qualities. This regimen would make these tumors less aggressive and more sensitive to cytotoxic treatment.(2) This approach has been confirmed using retinoic acid in hematologic malignancies for differentiation of leukemic stem cells.(9, 10) Use of all-trans retinoic acid (ATRA) with chemotherapy raised the complete remission rate of acute promyelocytic leukemia (APL) from 75% to 90% by forcing leukemic stem cells to differentiate.(18) These studies, together with our recently published work, gives promise to using differentiation therapy in the treatment of brain tumors.(12)

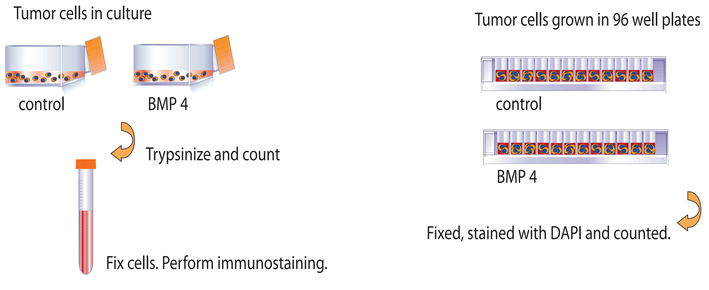
Bone morphogenetic proteins (BMPs) have broad roles in regulating stem cell biology. In vitro cultured NSC exposed to BMPs show age dependent disposition in terminal fate choice that mimics the in vivo developmental differentiation process.(11, 12, 16) BMPs inhibit neurogenesis and promote exit from the cell cycle which may be a result of differentiation.(4, 8) BMPs also cause differentiation of neural progenitors from the subventricular zone (SVZ) and olfactory bulb where NSC are concentrated.(7) These functions of BMPs have led to interest in using them for decreasing the population of tNSC in human central nervous system (CNS) tumors by forcing them to differentiate.

Having previoulsy shown that BMP 4 decreases proliferation by inducing differentiation in human glioblastoma multiforme (GBM), we tested the effects of BMP 4 on the proliferation of less aggressive human glial tumors.

Methods

GBM, anaplatic pleomorphic xanthoastrocytoma (PXA), ependymoma, pilocmyxoid astrocytoma, and juvenile pilocytic astrocytoma (JPA) cell lines established from primary human tumors were used for the experiments. Cells were grown in serum free media with growth factors. Cells were treated with 200ng/mL of BMP 4 in culture for 5-14 days, depending on growth rate. After treatment, cells were trypsinized, counted and fixed with 90% methanol at -20C for 15minutes. Immunohistochemistry was performed with antibodies against BMPR1(R&D©), BMPR1B (Invitrogen©), BMPRII (R&D©), Smad 1/5/8 (Cell Signal©), Ki67 (Invitrogen©), MCM2 (Santa Cruz©), Nestin (Chemicon©), and glial fibrillary acidic protein (GFAP) (Dako©). Propidium iodide (PI) was used for cell cycle analysis. Flow cytometry was performed using the BD© (New Jersey, USA) LSR II. Analysis was conducted using Flow Jo (Tree Star, Inc.©).

The tumor cells were also grown in culture in 96 well plates with and without BMP 4. After 5-14 days, the cells were fixed with paraformaldehyde (PFA) and the nuclei were stained with 4'6-diamidino-2-phenylindole (DAPI). Cells per well were counted using a fluorescent microscope.



Results

A. GBM

Cell culture

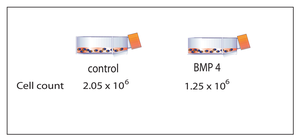


Fig 1. BMP4 results in reduces proliferation of GBM cells in culture.

Flow cytometry

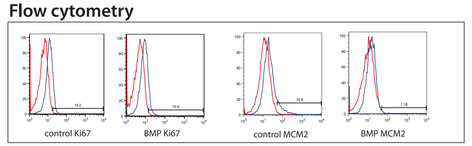


Fig 2. BMP reduces proliferative markers in GBM tumor cells.

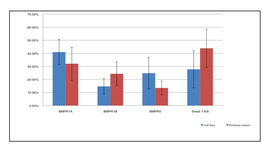


Fig 3. BMPR and pathway activation (Smad 1/5/8) in GBM primary tumor cells and established cell lines (L0, L1, L2).

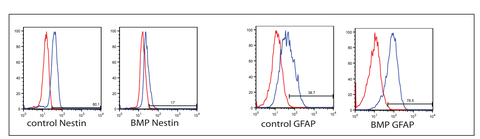


Fig 4. Cells treated with BMP 4 have decreased staining for Nestin and increased staining for GFAP.

B. Anaplastic PXA

Cell culture

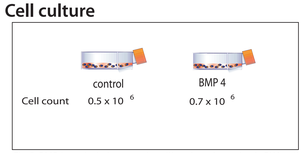


Fig 5. BMP4 results in increased proliferation of anaplastic PXA cells in culture.

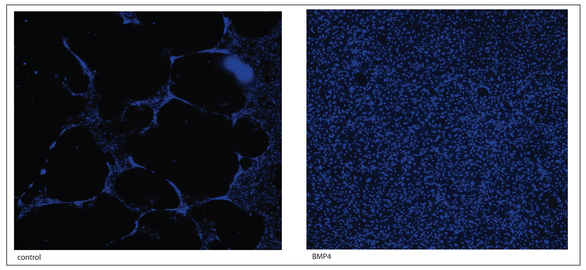


Fig 6. Cells were grown with and without BMP4. Cells treated with BMP4 had increased growth. (n=3)

C. Ependymoma

Cell culture

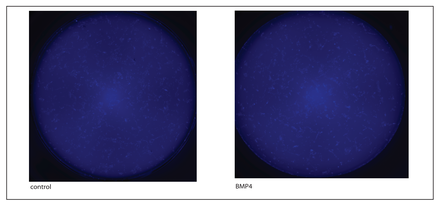


Fig 7. Cells grown in 96 well plates with and without BMP4. No difference in cell proliferation was noted. (n=10)

D. Pilomyxoid astrocytoma

Cell culture

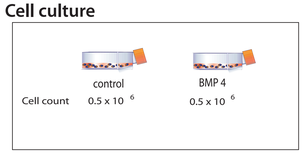


Fig 8. BMP4 does not affect proliferation of pilomyxoid astrocytoma cells in culture.

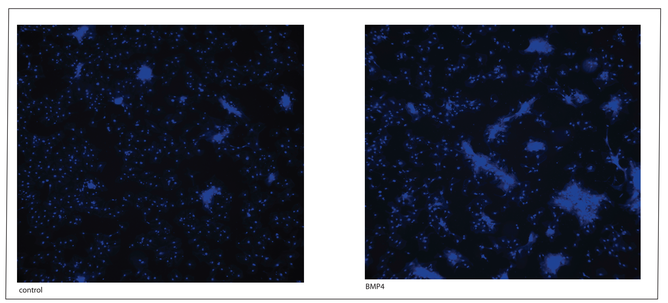


Fig 9. Cells grown in culture with and without BMP4. Fixed and stained with DAPI. BMP4 did not result in a significant difference in cell growth. (n=3)

E. JPA

Cell culture

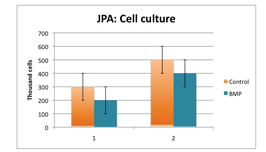


Fig 10. Cells grown in culture with and without BMP4. BMP 4 decreased the number of cells at the time of cell passage.

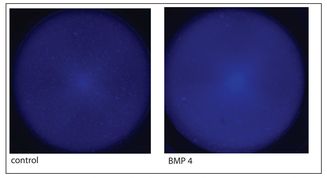


Fig 11. Cells grown in 96 well plate with and without BMP 4. Cells grown with BMP 4 did not fill the wells compared to the control cells that filled all of their wells. (n=10, p < 0.001)

Flow cytometry

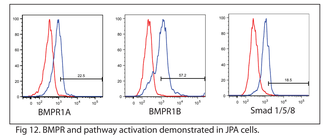


Fig 12. BMPR and pathway activation demonstrated in JPA cells.

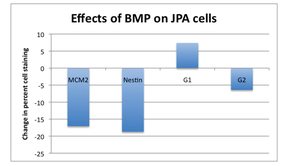


Fig 13. BMP 4 decreases MCM2 and Nestin staining in JPA cells. Cells treated with BMP 4 have a larger percentage of cells in G1 and less in G2 of the cell cycle.

Conclusion

BMPR and pathway activation is evident in various human glial tumors. BMP decreased cell proliferation inducing differentiation (increased GFAP, decreased Nestin) in the GBM and JPA cell lines. BMP4 increased cell proliferation in the PXA cell line. The ependymoma and anaplastic PXA cell lines did not demonstrate cell proliferation changes with BMP4 treatment.

References

1. Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, Dewhirst MW, Bigner DD, Rich JN: Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature 444:756-760, 2006.
2. Dirks PB: Cancer: stem cells and brain tumours. Nature 444:687-688, 2006.
3. Galli R, Binda E, Orfanelli U, Cipelletti B, Gritti A, De Vitis S, Fiocco R, Foroni C, Dimeco F, Vescovi A: Isolation and characterization of tumorigenic, stem-like neural precursors from human glioblastoma. Cancer Res 64:7011-7021, 2004.
4. Gomes WA, Mehler MF, Kessler JA: Transgenic overexpression of BMP4 increases astroglial and decreases oligodendroglial lineage commitment. Dev Biol 255:164-177, 2003.
5. Huntly BJ, Gilliland DG: Leukaemia stem cells and the evolution of cancer-stem-cell research. Nat Rev Cancer 5:311-321, 2005.
6. Ignatova TN, Kukekov VG, Laywell ED, Suslov ON, Vrionis FD, Steindler DA: Human cortical glial tumors contain neural stem-like cells expressing astroglial and neuronal markers in vitro. Glia 39:193-206, 2002.
7. Lim DA, Tramontin AD, Trevejo JM, Herrera DG, Garcia-Verdugo JM, Alvarez-Buylla A: Noggin antagonizes BMP signaling to create a niche for adult neurogenesis. Neuron 28:713-726, 2000.
8. Liu SY, Zhang ZY, Song YC, Qiu KJ, Zhang XC, An N, Zhou Z, Cai WQ, Yang H: SVZa neural stem cells differentiate into distinct lineages in response to BMP4. Exp Neurol 190:109-121, 2004.
9. Mongan NP, Gudas LJ: Diverse actions of retinoid receptors in cancer prevention and treatment. Differentiation 75:853-870, 2007.
10. Nasr R, Guillemin MC, Ferhi O, Soilihi H, Peres L, Berthier C, Rousselot P, Robledo-Sarmiento M, Lallemand-Breitenbach V, Gourmel B, Vitoux D, Pandolfi PP, Rochette-Egly C, Zhu J, de The H: Eradication of acute promyelocytic leukemia-initiating cells through PML-RARA degradation. Nat Med 14:1333-1342, 2008.
11. Panchision DM, Pickel JM, Studer L, Lee SH, Turner PA, Hazel TG, McKay RD: Sequential actions of BMP receptors control neural precursor cell production and fate. Genes Dev 15:2094-2110, 2001.
12. Piccirillo SG, Reynolds BA, Zanetti N, Lamorte G, Binda E, Broggi G, Brem H, Olivi A, Dimeco F, Vescovi AL: Bone morphogenetic proteins inhibit the tumorigenic potential of human brain tumour-initiating cells. Nature 444:761-765, 2006.
13. Reya T, Morrison SJ, Clarke MF, Weissman IL: Stem cells, cancer, and cancer stem cells. Nature 414:105-111, 2001.
14. Rich JN: Cancer stem cells in radiation resistance. Cancer Res 67:8980-8984, 2007.
15. Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD, Dirks PB: Identification of human brain tumour initiating cells. Nature 432:396-401, 2004.
16. Varga AC, Wrana JL: The disparate role of BMP in stem cell biology. Oncogene 24:5713-5721, 2005.
17. Vescovi AL, Galli R, Reynolds BA: Brain tumour stem cells. Nat Rev Cancer 6:425-436, 2006.
18. Wang ZY, Chen Z: Acute promyelocytic leukemia: from highly fatal to highly curable. Blood 111:2505-2515, 2008.

* This work was funded by a generous donation by the Musella Foundation and the Grey Ribbon Crusade.℠