Bone Morphogenetic Protein in the Treatment of Glial Tumors

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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 tumors contain this relatively rare subpopulation of cells that exhibit stem cell characteristics.(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 85% by forcing leukemic stem cells to differentiate.(10) 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 late choice that mimics the in vivo developmental differentiation process.(11, 12, 16) BMPs inhibit neurogenesis and promote astrocytic 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 NSCs in human neural stem cell (NSC) tumors by forcing them to differentiate.

Having previously 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, anaplastic pleomorphic xanthoastrocytoma (PXA), ependymoma, pilocytic 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 100% methanol at -20C for 15 minutes. Immunohistochemistry was performed with antibodies against BMPR (BMPR1A, BMPR1B) (Santa Cruz), N-Cadherin and vimentin (Abcam). Smad 1/5/8 (Cell Signal), K67, Nestin (Nestin) and Ki67 (Cell Signaling), MC2 (San Cruz Inc.), and glioblastoma acidic protein (GAP) (Dako). Propidium iodide (PI) was used for cell cycle analysis. Flow cytometry was performed using the BDI (New Jersey, USA). LSR II Analysis was conducted using FlowJo (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

Flow cytometry

B. Anaplastic PXA

Cell culture

Flow cytometry

C. Ependymoma

Cell culture

D. Pilocytic astrocytoma

Cell culture

E. JPA

Cell culture

Flow cytometry

Conclusion

BMPR and pathway activation is evident in various human glial tumors. BMP decreased cell proliferation (increased GFAP, decreased Nestin) in the GBM and JPA cell lines. BMP 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


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