Human Fat-Derived Mesenchymal Stem Cells Bioengineered to Secrete BMP4 are Non-Oncogenic, Suppress Glioma, and Prolong Survival

Sara Ganaha MD; Rawan Al-Kharboosh; Alejandro Ruiz-Valls; Hugo Guerrero-Cazares; Alfredo Quinones-Hinojosa MD

Department of Neurosurgery, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

CNS 2016 ANNUAL MEETING San Diego CALIFORNIA SEPTEMBER 24-28, 2016

INTRODUCTION

Glioblastoma is malignant, aggressive, and resistant to treatment. We demonstrate the ability of human adipose-derived mesenchymal stem cells (hAMSCs) to home to and suppress brain tumor initiating cells (BTIC) implicated in glioblastoma progression. Bone morphogenetic protein 4 (BMP4) has anti-tumor effects; however a method to effectively deliver BMP4 to tumor sites yet needs to be investigated. In this study, we investigated the use of hAMSCs as a vehicle to deliver BMP4 to BTICs, by using bioengineered BMP4-secreting hAMSCs (BMP4 -hAMSC), for the treatment of glioblastoma.

OBJECTIVES

To investigate the therapeutic potential of bioengineered BMP4-secreting human adipose-derived mesenchymal stem cells (hAMSCs-BMP4) against glioblastoma.

METHODS

--hAMSCs were transduced to express BMP4; effects on BTIC proliferation, differentiation, and migration were assessed with state-of-the-art proprietary nanotechnology developed by us.

--We investigated the effect of BTICs on hAMSC proliferation, differentiation, and malignant transformation into tumor associated fibroblasts (TAFs) via western blot, immunofluorescence, and real-time RT-PCR.

--NOD/SCID mice were intracranially injected with BTICs derived from our own patient samples obtained from the operating room.

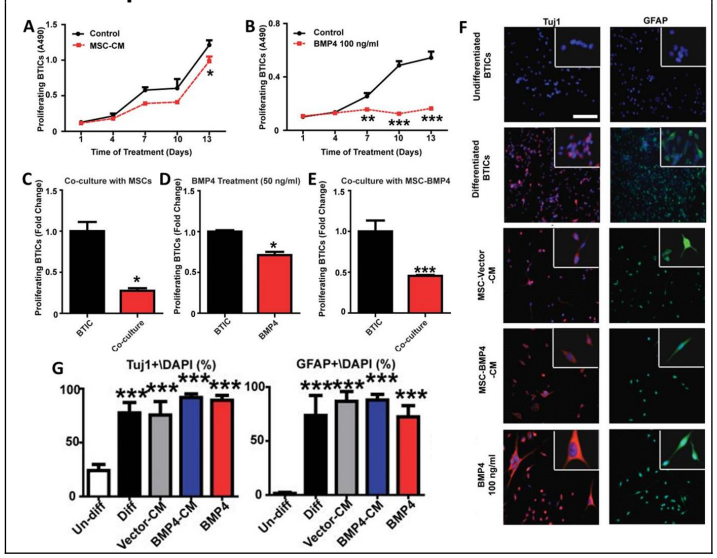
--Furthermore, mice underwent systemic injections of BMP4-hAMSCs to assess the safety of stem cell therapy, and their effect on GBM proliferation and migration.

Impact on survival was determined post-BMP4-hMSC treatment.

RESULTS

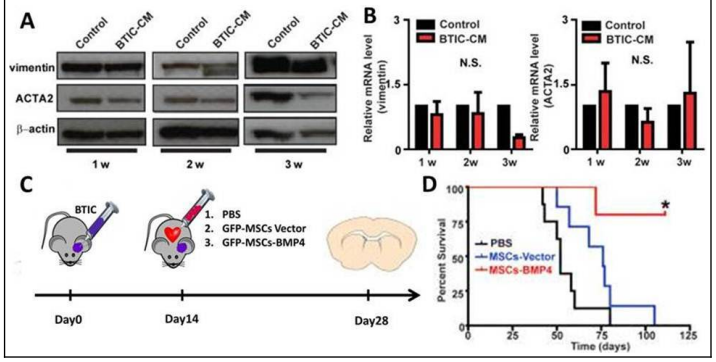
BMP4-hAMSCs decreased migration, proliferation, and induced differentiation of BTIC in vitro. In addition, hAMSCs remained multipotent upon exposure to BTIC-secreted factors, indicating retained stem-cell characteristics and integrity. In addition, BMP4-hAMSCs did not undergo oncogenic transformation upon exposure to BTICs in vitro and in vivo. Moreover, systemically delivered BMP4-hAMSCs significantly improved median survival in mice, whereby they significantly outlived controls.

hAMSCs-BMP4 decrease BTIC proliferation and promote BTIC differentiation in vitro.



BTICs were cultured in **(A)** hAMSC-CM or **(B)** BMP4 treated media for 2 weeks, and MTS assays were performed to measure BTIC proliferation. To determine BTIC proliferation, EdU assay of GFP-BTICs were **(C)** co-cultured with hAMSCs, **(D)** treated with recombinant BMP4 alone, or **(E)** co-cultured with hAMSCs-BMP4. Results were normalized and compared to BTICs condition. **(F)** BTICs were cultured in control media (stem cell media, undifferentiated BTICs), differentiation media (stem cell media+10%FBS, differentiated BTICs), hAMSC-Vector-CM, hAMSC-BMP4-CM, or recombinant BMP4 for 2 weeks and stained for Tuj1 and GFAP. **(G)** The percentages of Tuj1+/DAPI and GFAP+/DAPI were calculated from 5 random fields. **(*p<0.05, **p<0.01, ***p<0.001)**

hAMSCs do not transform into tumor associated fibroblasts (TAFs) in vitro, while hAMSCs-BMP4 increase the median survival of GBM bearing mice.



(A-B) hAMSCs were cultured in BTIC-CM or control media and (A) Western blots and (B) Real-time RTPCR were performed to quantify TAF markers. **(C)** In vivo experiment in which BTIC was intracranially injected into nude mice. At 2 weeks post-injection, GFP-hAMSCs-Vector, GFP-hAMSCs-BMP4, or PBS were injected intracardially. Mice were sacrificed 2 weeks later to assess hAMSC localization and homing properties to brain tumor. **(D)** U87 cells were intracranially injected into mice for survival. 10 days post-injection, GFP-hAMSCs-Vector, GFP-hAMSCs-BMP4, or PBS were injected intracardially and mice were followed for 125 days for survival. Kaplan-Meier analysis showed significantly longer survival in the treated group vs. controls; median survival of mice with hAMSCs-BMP4 was greater than that of mice treated with hAMSCs-Vector and control mice, with no significant difference between PBS and hAMSCs-Vector groups.

CONCLUSIONS

BMP4-hAMSCs are non-oncogenic, decrease tumor burden, and improve survival in mice with GBM.

FUTURE DIRECTIONS

We will explore the therapeutic efficacy of BMP4-secreting patient-derived hAMSCs against GBM.

REFERENCES

Secrete BMP4 Are Nononcogenic, Suppress Brain Cancer, and Prolong Survival. Li Q, Wijesekera O, Salas S, Wang J, Zhu M, Aprhys C, Chaichana K, Chesler D, Zhang H, Smith C, Guerrero-Cazares H, Levchenko A, Quinones-Hinojosa A.

ACKNOWLEDGEMENTS

Research reported in this poster was supported by two RO1 National Cancer Institute Grants of the NIH under award **R01CA183827** and **R01CA195503.** Disclaimer: The content is solely the responsibility of the authors and does not necessarily represent the official views of NIH.