Role of human--defensin 3 in Glioblastoma Multiforme Immunomodulation

Amber Kerstetter-Fogle; Peggy Harris; Alankrita Raghavan BS; Jill Barnholtz-Sloan; Marta Couce; Ge Jin; Aaron Weinberg; Andrew E. Sloan MD FACS

Departments of Neurological Surgery, Epidemiology and Biostatistics, Pathology, Department of Biological Sciences, University Hospitals Medical Center, Case Western Reserve School of Medicine, School of Dental Medicine, Cleveland, Ohio

CNS 2018 ANNUAL MEETING HOUSTON, TEXAS OCTOBER 6-10, 2018

Introduction

Glioblastoma Multiforme (GBM) ranks among the most lethal of all human cancers, with current therapy offering only palliation. The causes of treatment failure are myriad, but accumulating data support a subset of myeloid derived immune cells, specifically tumor-associate macrophages (TAMs) that contribute to worsening outcomes. Defensins, small molecules with chemotactic properties that play roles in innate immunity, have been proposed as one potential contributor of TAMs in GBM immunomodulation. One particular defensin, human-ß-defensin- 3 (HBD3) is dysregulated in head and neck cancers and is found to be elevated in GBM samples. Based on the immunomodulatory role of HBD3 outside the CNS, we hypothesized that HBD3 affects tumor-associated macrophages and microglia and contributes to immune-evasion in GBM.

Methods

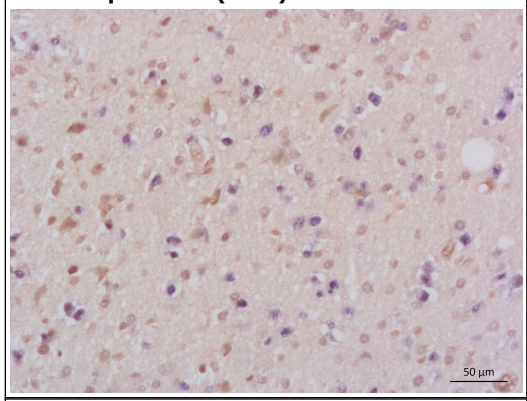
Immunohistochemistry (IHC) of primary tumor sections revealed the presence of HBD-3 in the tumor periphery. Colocalization of HBD-3 with stem cell markers, SOX2 and Nestin, demonstrates that HBD3 expressing cells were glioma stem cells (GSCs). IHC also revealed the presence of CD68+ macrophages alongside HBD-3 expressing SOX2+ GSCs. GSCs were isolated from GBM tumors by FACS. QT-PCR revealed a 7-fold increase in the expression of HBD3 in GSCs compared to control neural stem cells (NSCs). Chemotaxis assays demonstrate that recombinant HBD3 chemoattracts macrophages and microglia associated with GBM.

Learning Objectives

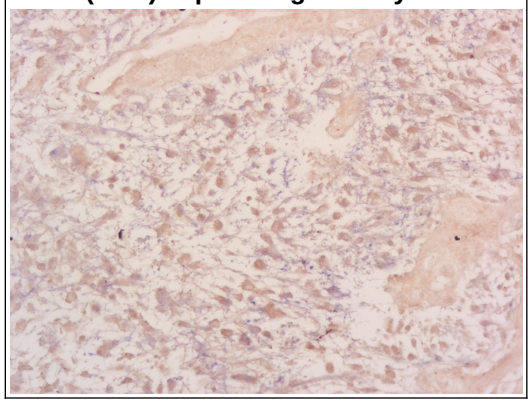
- Understand the physiology of h-BD3;
- Understand the physiology of M1 and M2 macrophages
- Understand the role of immunotherapy

HBD3 expression (brown) expression next to the high expression GFAP+ (blue) gliosis

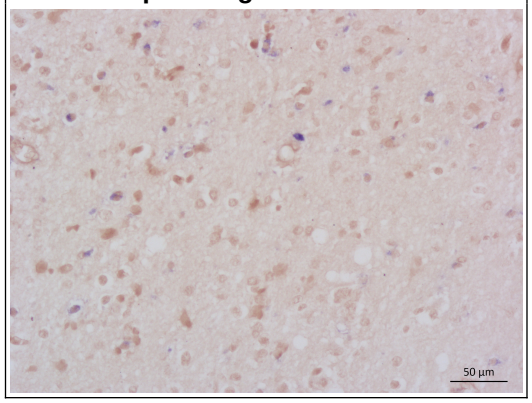
HBD3 (brown) expression in leading edge of tumor stains a subset of Sox2 positive (blue) stem cells



HBD3 (brown) cells interact with GFAP (blue) expressing astrocytes



CD68 (blue) positive cells surrond HBD3 expressing cell in tumor



Conclusions

- HBD3 is secreted by cancer stem cells expressing CD133 or CD15 (qPCR; data not shown).
- CD68 positive macrophages in the tumor microenvironment respond to HBD3
- Normal microglia migrate chemotactically in response to HBD3 (migration assay: data not shown)
- **The ability of hBD3 to attract macrophages suggests it may play a role in the M1 to M2 phenotype switch of macrophages, thereby allowing for immune evasion.**

Acknowledgements

Extension of gratitude to Jonathon Karm for the gift of immortalized microglia.

This research was supported by the Cytometry and Imaging Microscopy shared resource of the Case Comprehensive Cancer Center (P30CA043703), and the Kimble Foundation to AES.