Title: Hypoxia generates cell-type-dependent responses in myeloid immune cell populations in diffuse astrocytomas

Authors:

Aliisa M. Tiihonen [1], Iida Salonen [1], Iina Koivisto [1], Anni Ritamäki [1,2], Serafiina Jaatinen [1], Tanja Hyvärinen [3], Joose Kreutzer [3,4,5], Maryam Mohammadlou [1], Masi Valkonen [1,6], Pekka Ruusuvuori [1,6], Seppo Parkkila [3], Pasi Kallio [3], Sanna Hagman [3], Juha Kesseli [1], Vidhya Ravi [7], Hannu Haapasalo [2,8], Arja Jukkola [2,3], Kevin Joseph [7], Kirsi J. Rautajoki [1]

1 Prostate Cancer Research Center, Faculty of Medicine and Health Technology, Tampere University and TAYS Cancer Centre, Tampere University Hospital, Tampere, Finland
2 TAYS Cancer Centre, Tampere University Hospital, Tampere, Finland
3 Faculty of Medicine and Health Technology, Tampere University, Tampere, Finland
4 Center of Excellence in Body-on-Chip Research, Tampere University
5 BioGenium Microsystems Oy, Tampere, Finland
6 Institute of Biomedicine, University of Turku, Turku, Finland
7 Department of Neurosurgery, Medical Center, University of Freiburg, Freiburg, Germany
8 Fimlab Laboratories Ltd., Tampere University Hospital, Tampere, Finland

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Abstract

Diffuse astrocytomas, especially glioblastomas (GBs), are aggressive brain tumors with a poor prognosis despite the efforts to improve their treatment. In diffuse astrocytomas, myeloid immune cells, notably monocyte-derived macrophages (MDM) and brain-resident microglia (MG) are highly abundant and pivotal in the immunoregulation of tumor microenvironment (TME). GBs, characterized by higher MDM frequencies, often exhibit hypoxia in the TME. In this work, we investigated the influence of hypoxia on the regulation of myeloid immune cells. We analyzed 222 diffuse astrocytoma patient samples using highly multiplex immunohistochemistry, 45 GBs with single-cell RNA sequencing (SCS), and 41 diffuse astrocytoma samples using spatial transcriptomics (GeoMx & Visium). Our findings reveal distinct spatial and gene expression responses of MDMs and MG to hypoxia. MG frequency decreases in highly hypoxic areas, while CD163- MDMs increase gradually compared to normoxia. Based on differentially expressed genes in SCS data, MDMs and MG respond to hypoxia differently and the responding genes are associated with different biological processes. Part of MG responses have been validated with cell culture models. Our results highlight the role of hypoxia in shaping the TME and immunosuppression in these devastating malignancies, and can help to explain the increased MDM/MG ratio typical for GBs.