

CANCER

Constitutive and TNF α -inducible expression of chondroitin sulfate proteoglycan 4 in glioblastoma and neurospheres: Implications for CAR-T cell therapy

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The heterogeneous expression of tumor-associated antigens limits the efficacy of chimeric antigen receptor (CAR)-redirected T cells (CAR-Ts) for the treatment of glioblastoma (GBM). We have found that chondroitin sulfate proteoglycan 4 (CSPG4) is highly expressed in 67% of the GBM specimens with limited heterogeneity. CSPG4 is also expressed on primary GBM-derived cells, grown in vitro as neurospheres (GBM-NS), which recapitulate the histopathology and molecular characteristics of primary GBM. CSPG4.CAR-Ts efficiently controlled the growth of GBM-NS in vitro and in vivo upon intracranial tumor inoculation. Moreover, CSPG4.CAR-Ts were also effective against GBM-NS with moderate to low expression of CSPG4. This effect was mediated by the in vivo up-regulation of CSPG4 on tumor cells, induced by tumor necrosis factor- α (TNF α) released by the microglia surrounding the tumor. Overall, the constitutive and TNF α -inducible expression of CSPG4 in GBM may greatly reduce the risk of tumor cell escape observed when targeted antigens are heterogeneously expressed on tumor cells.

INTRODUCTION

Glioblastoma (GBM) is the most lethal primary brain cancer, with standard treatment based on surgery, radiotherapy, and chemotherapy promoting an overall survival of about 15 months (1, 2). Immunotherapy may complement such treatments, and considerable optimism is presently given to T cells with redirected specificity via expression of chimeric antigen receptors (CAR-Ts). CARs are fusion proteins in which the binding moiety derived from a monoclonal antibody (mAb) is fused with a signaling molecule of the CD3/T cell receptor complex and costimulatory endodomains (3). Upon insertion in T cells, CARs confer human lymphocyte antigen (HLA)-independent cytotoxic activity to T cells and promote T cell proliferation and survival (4).

Crucial to the successful application of CAR-Ts in malignancies including GBM are the restricted expression pattern and the amount of expression of the targeted tumor-associated antigens (TAAs). In this regard, epidermal growth factor receptor variant III (EGFRvIII) is an attractive target antigen for GBM, because its expression is restricted to tumor cells (5–7). In contrast, typical GBM-associated antigens for CAR targeting, such as interleukin-13 receptor subunit α -2 (IL-13R α 2), HER2, and EphA2, are not only generally overexpressed by GBM tumor cells but also detectable in some normal tissues (8–11). Another common feature of the GBM-associated antigens so far targeted with CAR-Ts is the marked intratumoral heterogeneity of expression, which promotes tumor immune escape due to antigen loss (12–15). Finally, although they are frequently used to assess the

efficacy of new therapies, established GBM cell lines do not mirror the heterogeneity of the histopathology and molecular characteristics of primary GBM (16, 17).

To overcome these limitations, we have selected to target chondroitin sulfate proteoglycan 4 (CSPG4) in GBM and to use primary glioma-derived cells grown as neurospheres (GBM-NS) as a tumor model. CSPG4 is a cell surface type I transmembrane protein critical for tumor progression and metastasis (18–20). CSPG4 is overexpressed, with limited intra- and intertumoral heterogeneity, by many types of solid tumors (21–25). Notably, the CSPG4 protein is not or barely detectable in normal tissues (18, 22, 25). We have now found that CSPG4 is expressed in considerable amounts not only in GBM specimens but also in GBM-NS, which are particularly relevant for assessing the efficacy of potential therapies because they recapitulate the molecular properties of the primary GBM when expanded in vitro or engrafted in immunodeficient mice (16, 17).

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Acknowledgments: This work is dedicated to the memory of Viola Kraus. We thank D. Lecis (Department of Experimental Oncology and Molecular Medicine, AmadeoLab—Fondazione IRCCS Istituto Nazionale dei Tumori) for providing reagents, help, and advice with TNFα-blocking experiment. **Funding:** This work was supported by University Cancer Research Fund at University of North Carolina, Lineberger Comprehensive Cancer Center. S.P. was supported by "Il Fondo di Gio Onlus." **Author contributions:** S.P. designed and performed all the in vitro and in vivo experiments. B.S. designed and performed the in vivo experiments. N.D.I. performed immunohistochemistry and immunofluorescence staining. C.C. performed in vitro and in vivo experiment testing sorted cells. Y.C. performed in vivo experiments and in vivo imaging. M.P. performed immunohistochemistry staining. C.S. performed in vivo imaging. S.F. provided critical reagents. F.D. performed surgery and follow-up of the patients. G.F. provided clinical and genetic information of the patients. G.D. designed all the experiments. S.P., B.S., and G.D. wrote the manuscript. All the authors reviewed, edited, and approved the manuscript. **Competing interests:** G.D. is an inventor on patent application (WO2015080981A1) held and submitted by Baylor College of Medicine that covers the CSPG4.CAR. All other authors declare that they have no competing interests. **Data and materials availability:** GBM-NS are available from G.F. under a material transfer agreement with Istituto Neurologico Besta, Milan, Italy.

Submitted 5 July 2017
Resubmitted 31 October 2017
Accepted 13 December 2017
Published 28 February 2018
10.1126/scitranslmed.aao2731

Citation: S. Pellegatta, B. Savoldo, N. Di Ianni, C. Corbetta, Y. Chen, M. Patané, C. Sun, B. Pollo, S. Ferrone, F. DiMeco, G. Finocchiaro, G. Dotti, Constitutive and TNFα-inducible expression of chondroitin sulfate proteoglycan 4 in glioblastoma and neurospheres: Implications for CAR-T cell therapy. *Sci. Transl. Med.* **10**, eaao2731 (2018).