Glioblastoma Multiforme Lipid Rafts Enhance Antigen Cross-presentation by Dendritic CDells

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Introduction

Glioblastoma multiforme (GBM) is the most common malignant primary brain tumor, and remains nearly universally fatal within two years despite aggressive treatment with surgery, radiation, and chemotherapy. Immunotherapy has been shown to be a promising approach in the treatment of GBM due its highly specific targeting of tumor cells while also inducing long-term immune protection. Current GBM immunotherapeutic vaccines rely largely on bulk tumor-associated antigen (TAA) sources from limited patient tumor samples or specific GBM peptide antigens susceptible to immunoediting. This study examined the ability to exploit allogeneic GBM cell line lipid rafts, enriched in specific TAAs as well as immunogenic chaperone proteins, as a renewable antigen source for dendritic cell (DC) vaccines for GBM.

Methods

Whole cell lysates, total membrane fractions, lipid raft fractions, or non-lipid raft membrane fractions were isolated from a syngeneic murine glioma cell line transfected with model antigen ovalbumin (GL261-OVA). For each membrane fraction, we measured levels of protein heterogeneity, TAA expression, and immunogenic chaperone protein by Western blot. DCs cultured from C57BL/6 mice were pulsed with the various lysate fractions, and DC maturity was assessed by CD83 staining. Stimulation of TAA-specific CD8+ T-cells by pulsed DCs was determined by Kb-OVA-specific tetramer staining and interferon-gamma (IFNg) ELISA.

Western Blot Analysis of TAA Expression in Membrane Fractions

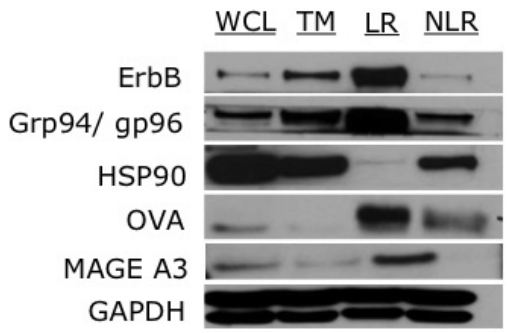

FIGURE 1. Western blot demonstrates increased levels of specific TAAs (ErbB, gp96, OVA, MAGE A3) in lipid raft fractions isolated from a syngeneic murine glioma cell line transfected with model antigen ovalbumin (GL261-OVA). 10ug of protein lysates were loaded for each sample of the whole cell lysate (WCL), total membrane (TM) fraction, lipid raft (LR) fraction, and non-lipid raft (NLR) fractions. Lipid rafts had markedly reduced overall protein heterogeneity, but were enriched for multiple TAA antigens, including the chaperone protein gp96, compared with other membrane fractions.

Flow Cytometry Analysis of OVA-specific CD8+T cells

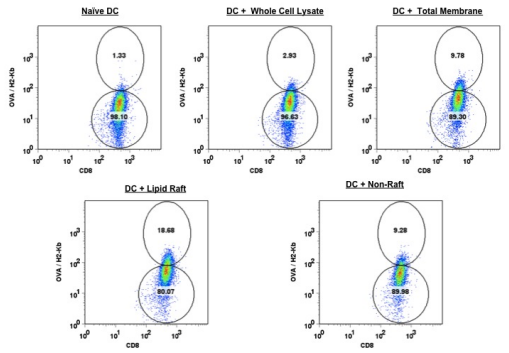

FIGURE 2A, 2B. DCs pulsed with LR fractions demonstrated greater stimulation of DC maturity (CD83+) and OVA model antigen cross-presentation to CD8+ T-cells. DCs were cultured from C57BL/6 mouse bone marrow precursors and pulsed with isolated lysate fractions from G261-OVA glioma cells. DCs treated with LR fractions enhanced stimulation of murine H-2Kb, increasing Kb-OVA tetramer expression of OVA-specific CD8+T cells by flow cytometry as compared to DCs pulsed with standard WCL (13.11%+/-2.93% vs. 2.44%+/-0.58%; p=0.02) or other membrane fractions.

IFNg Released by DC-T Cell Co-Culture Pulsed with Fractions

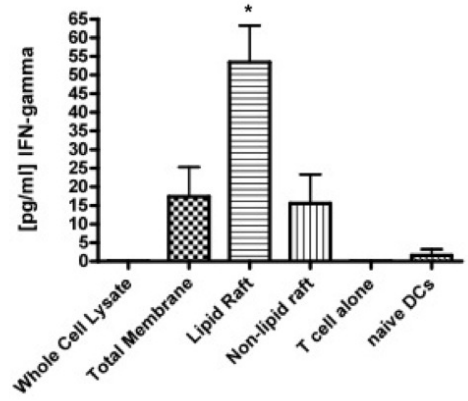

FIGURE 3. DCs pulsed with LR fractions enhance T-cell recognition of glioma cells as measured by IFNg release ELISA. To assess functional anti-tumor recognition, CD8+ T cells were co-cultured with DCs (1 DC : 5 T-cells), and pulsed with different fractions. Co-incubation with LR-pulsed DCs induced substantially elevated levels of IFNg secreted by T cells as compared to those pulsed with WCL (53.42 pg/mL vs. unmeasurable; p=0.0015) or other membrane fractions.

Conclusions

Our findings suggest that GBM lipid rafts from GL-261 glioma cells are enriched for multiple TAAs, and may stimulate more effective DC activation than standard whole cell lysates. Lipid rafts isolated from established glioma cell lines may be a potent and renewable antigen source for glioma vaccines. Further studies are underway characterizing vaccine efficacy in mouse studies, as well as stimulating HLA-A2+ human T-cells by autologous DCs pulsed with lipid rafts from allogeneic human GBM cell lines.