NON-ENZYME IDO1 ACTIVITY AND ITS IMMUNOSUPPRESSIVE EFFECTS IN GliOBLASTOMA

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INTRODUCTION

• Glioblastoma (GBM) is the most common primary malignant brain tumor in adults with a median survival of 15-20 months.
• GBM-induced immunosuppression is a significant obstacle that prevents patients from immunotherapy-related survival benefit.
• IDO1 is a type 2 enzyme (IDO2 is not detectable in GBM) that inhibits T-cell function and downregulates the immune response.
• Our previous work demonstrates that syngeneic, immunocompetent mice genetically knocked down for IDO1 in mouse GBM cells, ablates tumor-infiltrating Treg accumulation and increases overall survival.

AIMS & SIGNIFICANCE

Create wild-type and enzyme null IDO1-expressing glioma cell lines to re-evaluate the requirement for IDO1 enzyme activity in GBM-induced immunosuppression.

METHODS

• Transgenic mouse that spontaneously develop glioma (tGBM); G93A(SERT2+>Cre+); pIRES2-eGFP; Rb10; p53+ were provided by Dr. Suzanne Baker, PhD (St. Jude Children’s Research Hospital, Memphis, TN). IDO1 mice were initially mated with the IDO1 founder line to obtain IDO1-tGBM mice.
• The spontaneous mouse model of GBM was induced by 2 intracranial injections of tamoxifen (2.5 mg/kg body weight) at +21, +42 days postnatal into (G93A(SERT2+>Cre+); pIRES2-eGFP; Rb10; p53+ IDO1) j mice.
• The orthotopic mouse model of GBM was performed by intracranial (i.c.) injection of GBM cells (2x10^6 cells) into syngeneic mice.
• Wild-type mIDO1-GFP encoding lentiviral plasmid was purchased from GeneCopoeia. Amino acid mutagenesis was performed using QuickChange II XL site-mutagenesis kit (Agilent).
• Cell proliferation was measured by MTT Cell Proliferation Assay Kit (Trevigen).
• Plasma and tissue Tryp and Kyn were analyzed by high-performance liquid chromatography (HPLC) using an ESA Coulombel II detector with a 5uM Enhanced Analytical column containing a glassy carbon electrode (+600 mV).

CONCLUSIONS

• Novel mouse GBM cell lines expressing GFP-tagged mIDO1 WT, enzyme-null (HA), and ITIM-null (YF) were created, for syngeneic, intracranial xenografting into IDO1-null immunocompetent mice.
• The GFP-tag fused to mIDO1 does not interfere with Trp metabolism in IDO1-null GBM cells.
• Substitution for mIDO1 histidine 350 to alanine disabled enzyme conversion of tryptophan into kynurenine.
• mIDO1 has no effect on tGBM cell growth.
• Mouse GBM cell IDO1 increased Treg accumulation in brain tumors independent of Trp metabolism, in vivo.

FUTURE PLAN

• Evaluate the in vivo metabolism of mIDO1 WT, enzyme-null (HA), and ITIM-null (YF) tumors in IDO1-null, syngeneic immunocompetent mice.
• Evaluate overall survival in syngeneic immunocompetent mice with intracranial mIDO1 WT, enzyme-null (HA), and ITIM-null (YF) tumors.
• Interrogate the effect(s) of WT, enzyme-null (HA), and ITIM-null (YF) tGBM cells on Treg differentiation in an in vitro co-culture system.

REFERENCES


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