SOLID TUMORS

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Improved Outcomes Following Drug-Resistant Immunotherapy in a Hunan Xenograft Model of Temozolomide-Resistant Glioblastoma Multiforme

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Introduction: Conventional treatment strategies for Temozolomide (TMZ)-resistant high-grade gliomas have been uniformly dismal. Our previous studies have shown that TMZ-resistant tumors upregulate stress-associated NKG2D ligands (NKG2DL) during the first several hours following exposure to TMZ, thereby creating an opportunity for NKG2DL-directed cell therapy, particularly *ex vivo* expanded/activated γδ T cells and NK cells that directly recognize these stress-associated antigens. Using a human/mouse primary xenograft model, we report improved survival using a combination of TMZ chemotherapy and gene modified TMZ-resistant γδ T cells + NK cells which we term Drug Resistant Immunotherapy (DRI). Drug resistance is conferred by lentivector transfer of methylguanine methyltransferase (MGMT), enabling cytotoxic lymphocyte function in a chemotherapy-rich environment at a time when the tumor is maximally stressed and regulatory T cells are depleted.

Methods: Tumor NKG2DL expression and cytotoxicity of DRI were assessed using flow cytometry. Intracranial (IC) glioma xenografts were established in immunodeficient mice using either an unmodified (P) or a TMZ-resistant clone (T) of human GBM-X12 primary explants. Tumor-bearing mice were treated with an intraperitoneal 60mg/kg dose of TMZ on days 6, 8,13, and 15 post-tumor placement and received IC injection of 1.5 x 10^6DRI 1x/week x 2weeks on post-injection days 7 and 14 or 2x/week x 2 weeks following each TMZ dose by 4h. Control mice received non-modified cells, TMZ alone or no therapy. Survival was assessed using Kaplan-Meier analysis.

Results: Both GBM-X12P and X12T express stress antigens MIC-A, MIC-B, and ULBP-4. DRI cells tested for potency *in vitro* killed both tumors at approximately 80% at an effector: target ratio of 20:1 and showed no evidence of toxicity against cultured human astrocytes. Cell therapy alone did not improve survival beyond that of untreated mice for either tumor. For the unmodified parent tumor X12P, both TMZ therapy and TMZ + DRI significantly improved survival over untreated controls ($p < 0.0001$), and the combined therapy increased median survival from 57 to 75 days over TMZ alone. Combined therapy with TMZ + DRI also marginally improved survival for X12T ($p = 0.0147$) extending median survival over TMZ alone from 22 to 27 days ($p = 0.0966$). Keeping the TMZ dosing schedule constant, intensification of DRI therapy from 1x/week to 2x/week increased median survival from 22 days to 38 days over untreated animals ($p = 0.0004$) and significantly improved median survival over TMZ-treated animals from 27 to 38 days ($p = 0.0017$) with 10% of animals showing long-term survival.

Conclusions: The combination of chemotherapy-induced tumor stress and targeted DRI significantly increases time to progression and improves survival in immunodeficient mice bearing otherwise impervious temozolomide-resistant human xenograft tumors.

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Upregulation of Class I MHC on Neuroblastoma Cells By NK Cell Exposure for Enhanced CTL Reactivity

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Neuroblastoma is the most common solid tumor in pediatric patients, with a clear unmet need as survival rates are <20% for stage IV disease. As adjuvant immunotherapy is considered to have additional anti-tumor activity we aim to increase cytotoxic T lymphocyte (CTL)-mediated immune surveillance in neuroblastoma patients by administration of a dendritic cell-based vaccine. Neuroblastoma has developed mechanisms to circumvent CTL recognition; therefore it is our aim to find clinical strategies to make neuroblastoma susceptible for CTL killing. PRAME is highly expressed in stage IV primary neuroblastoma and PRAME-derived antigenic peptide can be presented as peptide/MHC I complexes to reactive T cells. Low MHC I expression levels on neuroblastoma cells enable them to escape CTL recognition, but MHC I levels can be increased by interferon-gamma (IFNg). Because of the clinical toxic effects of IFNg administration, we attempted to deliver IFNg to neuroblastoma via alternative routes. We added natural killer (NK) cells which recognize target cells by their lack of MHC I and found that NK cell recognition of neuroblastoma cells upregulated class I MHC surface expression. This effect was contact-dependent, however could thereafter be transferred to “naïve” neuroblastoma cells through replacement of culture supernatant. Interestingly, IFNg was not detected in the supernatants, suggesting a different factor that triggers the MHC I upregulation in neuroblastoma. Importantly, PRAME-specific T cells were specifically activated by neuroblastoma cells after exposure to NK cells. In conclusion; we showed that NK cells can regulate MHC I levels on neuroblastoma cells which transforms them into CTL targets.

STEM CELL BIOLOGY

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Rescue of the Mucocutaneous Manifestations in a Mouse Model of Recessive Dystrophic Epidermolysis Bullosa (RDEB) By Human Cord Blood Derived Unrestricted Somatic Stem Cells (USSCs)

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