

Identification of HLA-A2- and A24-restricted T-cell epitopes derived from SOX6 expressed in glioma stem cells for immunotherapy

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Malignant gliomas are the most aggressive human primary brain tumors and are currently incurable. Immunotherapies have the potential to target glioma and glioma stem cells (GSCs) that are resistant to conventional therapies. We previously identified SOX6 as a human glioma antigen and demonstrated that vaccination with SOX6 DNA induced cytotoxic T lymphocytes (CTLs) specific for glioma, thereby exerting therapeutic antitumor responses in glioma-bearing mice. In this study, we attempted to identify SOX6-derived peptides as specific targets for effective and safe T-cell-mediated immunotherapy targeting SOX6-positive glioma and GSCs. *In vitro* stimulation with human leukocyte antigen (HLA)-A*2402 (A24)-restricted peptides, RFENLGPQL (SOX6₅₀₄) and PYEEQARL (SOX6₆₂₈) or the HLA-A*0201 (A2)-restricted peptide, ALFGDQDTV (SOX6₄₄₇) was capable of inducing SOX6 peptide-specific CTLs in peripheral blood mononuclear cells derived from healthy donors and glioma patients. These CTLs were able to lyse a majority of glioma cell lines and a GSC line derived from human glioblastoma in an HLA Class I-restricted and an antigen-dependent manner. Furthermore, peptide vaccines of SOX6₆₂₈, which was conserved in the murine SOX6 protein and expected to bind to major histocompatibility complex (MHC) H-2^d, induced CTLs specific for SOX6₆₂₈ in H-2^d mice. Normal autologous cells from mice, in which SOX6-specific immune responses were generated, were not destroyed. These results suggest that these SOX6 peptides are potentially immunogenic in HLA-A24 or -A2 positive glioma patients and should be considered as a promising strategy for safe and effective T-cell-based immunotherapy of patients with gliomas.

Key words: tumor antigen, SOX6, CTL epitope, immunotherapy, glioma stem cells

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Malignant gliomas, such as glioblastoma multiforme (GB), represent the most common primary brain tumors and patients have a dismal prognosis.¹ Over 12,000 new cases are diagnosed annually in the U.S.² with a median survival rate of ~15 months.³ Development of novel, molecularly targeted, multimodal therapeutic approaches is critical to further improve the outcomes of patients with these deadly tumors. The recent identification of chemotherapy and radiotherapy-resistant glioma stem cells (GSCs) in GBs^{4,5} may help explain why conventional therapies are ineffective. Although the exact mechanism of tumor stem cell resistance to conventional therapies remains elusive, their quiescent state and increased capacity to eliminate cytotoxic drugs⁵ and repair damaged DNA are thought to be key contributing factors.^{4,6-8} Immunotherapy might offer some benefit to GB patients, because immune-mediated killing relies neither on tumor cell proliferation nor the aforementioned cytotoxic pathways. Indeed, dendritic cell-based vaccine therapy in a GSC-bearing mouse model showed an efficient anti-tumor immune response against GSCs derived from malignant glioma cells.⁹

The effectiveness of T-cell-mediated immunotherapy for cancer depends on both an optimal immuno-stimulatory context of the therapy and the proper selection with respect to