学位論文内容の要旨

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学 位 論 文 題 名

Studies about a therapeutic efficacy of EVA1-Antibody Drug Conjugate using glioblastoma-bearing mouse model
(膠芽腫モデルマウスを用いた EVA1-抗体薬物複合体の治療効果に関する研究)

Background and Purpose:

Glioblastoma (GBM) represents a highly aggressive and prevalent form of brain cancer. Although it has been thought that GBM originates from astrocytes, there are many evidence showing that neural stem cells (NSCs) and oligodendrocyte precursor cells (OPCs) are alternative cell-of-origin of GBM (Noble et al, 1995; Hide et al, 2011). The standard treatment for GBM is a combination of surgery, radiation, and chemotherapy. Despite of the extensive efforts, a median survival period, approximately 15 months, has not been changed (Stupp et al, 2005; Stupp et al, 2009). The identification of GBM-initiating cells (GICs) has redirected the focus of GBM research (Kondo, 2006; Singh et al, 2004; Vescovi, 2006). GICs have been demonstrated to be tumorigenic and be resistance to radiation and chemotherapy, suggesting that it is the cell-of-origin for the recurrence. Therefore, it is crucial to characterize GIC and identify the targets that are either specific or essential for the cells. Comparing the expression profiles of GICs and their potential cell-of-origins, NSCs and OPCs, we have shown that Epithelial V-like antigen 1 (EVA1) is a novel and functionally specific factor in GICs (Ohtsu et al, 2016).

Eva1 has been originally identified as a factor involved in T cell development in thymus (Guttinger et al, 1998). Eva1 is a type-1 transmembrane protein with a V-like structure in the extracellular domain, suggesting that Eva1 is involved in the cell-cell adhesion. However, it is yet unknown about its physiological function, except in our previous finding that EVA1 plays an essential role in GICs by activating non-canonical NF-κB signaling pathway (Ohtsu et al, 2016). Moreover, we found that EVA1 is upregulated in GBM and is associated with worse prognosis in patients with other cancers, such as breast cancer and pancreatic cancer, using public databases. Taken together, we started to develop a new antibody drug targeting EVA1 for GBM therapy.

Subjects and Methods:

Hybridoma cells were initially generated through the immunization of BALB/c mice with the EVA1-Fc fusion protein. Subsequently, the reactivity of the supernatant derived from these hybridoma cells was meticulously examined using EVA1-overexpressing cells and GICs. Candidate antibodies were then further screened using Biacore surface plasmon resonance analysis with antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). Among candidate antibodies, B2E5 was selected as it exhibited not only a high binding affinity but also robust ADCC and CDC activities *in vitro*. To achieve large-scale production of the B2E5 antibody, the hybridoma cells were injected into the abdominal cavity of NOD/SCID mice. The ascites fluid that had been generated was harvested, and following a purification process, the B2E5 monoclonal antibody was successfully obtained. To identify the epitope of B2E5, we generated a set of mutant EVA1, examined their binding with B2E5 and identified amino acid 72nd proline (P72) as the epitope. To explore the therapeutic potential of B2E5 as a therapeutic antibody for GBM, IgG-ADC and B2E5-ADC were generated by conjugating mouse IgG and B2E5, respectively, with a potent mitotic inhibitor, MMAE (Monomethyl Auristatin E). The cytotoxicity of the

B2E5-ADC was comprehensively evaluated by both adding it to cultured GICs and intracranially injecting it into GIC tumor-bearing brains.

Results:

We selected B2E5, as a potential candidate of the antibody drug, by its high affinity to EVA1 and the cytotoxic activities, ADCC and CDC. We determined P72 as the epitope of B2E5, by the binding assay with a set of mutant EVA1. I found that B2E5 was internalized into GICs over time and demonstrated that B2E5 conjugated with MMAE (B2E5-ADC) effectively killed GIC with its concentration-dependent manner in vitro. I established two Enhanced Luciferase-expressing GIC lines (ELuc-GICs, ELuc-E6 and ELuc-E16). Using a GBM-bearing mouse model that was transplanted with ELuc-GICs, I demonstrated that intracranial injection of B2E5-ADC effectively eliminated GICs with their death and the invasion of microglia/macrophages and significantly extended the survival time of the mice.

Discussion:

I successfully demonstrated that intracranial injection of B2E5-ADC killed GICs in brains of tumor-bearing mice and significantly extended their survival time. Considering the clinical setting, however, the intracranial injection is highly invasive and difficult to inject B2E5-ADC multiple times to the patients. Therefore, developing an effective and non-invasive method delivering B2E5-ADC into brain is crucial for the therapy. Alternatively, we may use smaller antibody-based molecules, such as Fab (fragment of antigen-binding)-ADC and scFv (single-chain fragment variable)-ADC, which can more easily traverse the blood-brain barrier (BBB).

In this study, I observed that B2E5-ADC extended the lifespan of GIC-bearing mice yet failed to achieve a complete cure as all mice succumbed 60 days post-tumor transplantation. Two potential reasons underlie this. Firstly, residual GIC tumors in the brain led to recurrence upon cessation of B2E5-ADC treatment, resulting in the mice's demise. To address this, we could consider increasing either the concentration or frequency of B2E5-ADC administration to ensure complete tumor elimination. Secondly, the severe and irreversible brain tissue damage caused by GBM is a contributing factor. Brain tumors can precipitate a host of serious consequences, including neurological dysfunction, cognitive impairment, epilepsy, elevated intracranial pressure, and associated complications.

I observed hemosiderin deposition in the brain that had been injected with B2E5-ADC, whereas no such deposition was observed in the brains injected with either IgG, B2E5, or IgG-ADC. This finding implies that the hemosiderin deposits likely originated from dead GBM cells (GICs), given that previous studies have demonstrated that GBM cells overexpress transferrin receptor and consequently accumulate iron within the cells. (Recht et al, 1990; Voth et al, 2015). Another possible explanation is that microglia or macrophages scavenged apoptotic GICs, which subsequently led to the formation of hemosiderin deposits. In either of these two scenarios, the occurrence of hemosiderin deposits presumably serves as an indicator or a characteristic feature of the elimination of GICs mediated by B2E5-ADC.

I demonstrated that the intracranial injection of B2E5-ADC was highly effective in impeding brain tumorigenesis in the E6 and E16 models. For clinical translation and application, it will be necessary in the future to conduct further evaluations regarding the efficacy of B2E5-ADC in other well-established brain tumor models.

Conclusion:

Our data indicate that B2E5-ADC is a new and promising therapeutic strategy for GBM.