Efficacy of HSV-TK/GCV system suicide gene therapy using SHED expressing modified HSV-TK against lung cancer brain metastases

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1. 簡述論文的概要與重大發現

肺癌是最常見的惡性腫瘤之一，死亡率也是排名第一，約 20%的患者在診斷時會發現腦轉移的現象，其中以非小細胞肺癌（NSCLC）腦轉移患者的存活率大約不到一年。最常見的腦腫瘤治療為手術、放射、化療，可以改善對原發性和顱內轉移性病變的控制，並延長總存活率。但是，大約 50% ~ 60%的 NSCLC 腦轉移癌患者還是會死於中樞神經系統病變，因此，對於轉移性腦腫瘤需要有新的治療策略。所以作者使用 herpes simplex virus thymidine kinase (HSV- TK)/ganciclovir (GCV) 系統的 enzyme 前藥自殺基因療法，使用 Mesenchymal Stem Cells（MSC）通過旁觀者效應誘導惡性膠質瘤細胞凋亡。在這裡，作者使用人類脫落的乳牙 human exfoliated deciduous teeth (SHED) 的幹細胞做為 TK/GCV 系統的基因載體，由於 TK wild type 具有潛在毒性，作者將 A168H 突變株 TK (TKA168H) 引入 SHED 以建立治療細胞。SHED 表達 TKA168H (SHED-TK) 對 NSCLC 的 conditioned medium 表現出趨化性，在體外和體內都表現出強烈的旁觀者效應，並完全根除大腦中的 NSCLC。腫瘤內植入 SHED-TK 細胞，隨後施打 GCV 顯著抑制腫瘤的生長並提高存活時間。這些結果表明，TKA168H 突變株適用於建立治療性細胞，並且腫瘤內注射 SHED-TK 搭配 GCV 可能是有效的治療方法。

2. 對內容的疑問

腫瘤細胞會釋放種種細胞激素，導致 SHED 具有高遷移至腫瘤的能力，在臨床上沒辦法通過手術完全切除高侵襲性腫瘤細胞，雖然幹細胞具腫瘤趨向性，但對於尚未切除乾淨的癌細胞毒殺作用尚未確切證實。

3. 論文的缺點與評論

本研究的缺點是，在實驗中的小鼠模型是直接通過將腫瘤直接植入腦而不是使用肺癌轉移來產生轉移性腦腫瘤，自殺基因治療對於腦腫瘤仍處於動物實驗階段，臨床試驗需要進一步研究。

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Lung cancer is one of the most common cancers, and the number of patients with intracranial metastases is increasing. Previously, we developed an enzyme prodrug suicide gene therapy based on the herpes simplex virus thymidine kinase (HSV-TK)/ganciclovir (GCV) system using various mesenchymal stem cells to induce apoptosis in malignant gliomas through bystander killing effects. Here, we describe stem cells from human exfoliated deciduous teeth (SHED) as gene vehicles of the TK/GCV system against a brain metastasis model of non-small cell lung cancer (NSCLC). We introduced the A168H mutant TK (TK^{A168H}) into SHED to establish the therapeutic cells because of the latent toxicity of wild type. SHED expressing TK^{A168H} (SHED-TK) exhibited chemotaxis to the conditioned medium of NSCLC and migrated toward implanted NSCLC *in vivo*. SHED-TK demonstrated a strong bystander effect *in vitro* and *in vivo* and completely eradicated H1299 NSCLC in the brain. SHED-TK cells implanted intratumorally followed by GCV administration significantly suppressed the growth of H1299 and improved survival time. These results indicate that the TK^{A168H} variant is suitable for establishing therapeutic cells and that intratumoral injection of SHED-TK followed by GCV administration may be a useful strategy for therapeutic approaches.

INTRODUCTION

Lung cancer is the most common malignant tumor and the leading cause of death in cancer patients.[1] Brain metastasis is found in 20% of patients at diagnosis, and up to 80% of patients suffer from brain metastasis over the disease course. The median survival of patients with brain metastasis of non-small cell lung cancer (NSCLC) is estimated to be 3–15 months.[2,3] The development of local and systemic treatments, such as molecularly targeted drugs, immunotherapies, surgery, and radiotherapy, improved the control of primary and intracranial metastatic lesions and prolonged overall survival. However, approximately 10% of NSCLC patients with brain metastases die from lesions in the central nervous system,[4] and the neurological cause of death is expected to increase in the future.[5] Therefore, novel therapeutic strategies for metastatic brain tumors are required.

Suicide gene therapy was first reported in the 1990s.[6,7] The thymidine kinase (TK)/ganciclovir (GCV) system is a gene-directed enzyme prodrug therapy. The *herpes simplex virus 1 thymidine kinase (HSV-TK)* gene, called the suicide gene, introduced into cells phosphorylates a prodrug, GCV, to the monophosphate form in the introduced cells. After that, it is phosphorylated by intracellular TK to the triphosphorylated form, which inhibits DNA synthesis and causes cell apoptosis. In addition, phosphorylated GCV is passively transferred to surrounding HSV-TK-nonexpressing cells through gap junction intercellular communication and induces surrounding cell death. This is the so-called bystander effect and is important for enhancing the antitumor effect of the TK/GCV system.[8,9] Since the phosphorylated form of GCV inhibits DNA synthesis, suicide gene therapy is likely to be effective against cells with active DNA synthesis, such as tumor cells;[10] it is possible to kill the tumor cells superselectively. We introduced HSV-TK into several kinds of stem cells and reported the effectiveness of suicide gene therapy using stem cells as vehicles in previous reports.[8,9,11] It is advantageous to use stem cells as vehicles of the suicide gene to treat tumors because they have a strong migration ability to especially invasive tumors.

Mesenchymal stem cells (MSCs) have an excellent self-renewal ability and multipotency and are being actively studied mainly in regenerative medicine. Stem cells from human exfoliated deciduous teeth (SHED) and dental pulp stem cells (DPSCs) are stem cells that are

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