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Gene recombinant bone marrow mesenchymal stem cells as a tumor-targeted suicide gene delivery vehicle in pulmonary metastasis therapy using non-viral transfection

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Abstract

One of the main limitations of anti-tumor gene therapy is the lack of an effective way to deliver therapeutic genes to tumor sites. Bone marrow mesenchymal stem cells (BMSCs) have been proposed as cellular delivery vehicles to tumor sites in tumor-targeted cancer gene therapy. Here, we investigated the therapeutic effects of cytomegalovirus-thymidine kinase expressing BMSCs (TK-BMSCs) on pulmonary melanoma metastasis combined with prodrug ganciclovir. BMSCs were successfully engineered through a non-viral gene vector. The gene recombinant BMSCs migrated to the pulmonary area and were found to have the tendency to target tumor nodules after systemic delivery. In vitro results demonstrate that the engineered BMSCs have significant suicide effects in the presence of ganciclovir in a dose-dependent manner and can exert a sufficient bystander effect on B16F10 tumor cells in co-culture experiments. In vivo studies confirmed the therapeutic effects of TK-BMSCs/ganciclovir on the metastasis tumor model.

From the Clinical Editor: This study investigates the possibility of gene transfer via bone marrow mesenchymal stem cells in anti-cancer gene therapy using a metastatic melanoma model and cytomegalovirus-thymidine kinase expressing stem cells, demonstrating clear therapeutic effects.

Key words: BMSCs; Non-viral gene vector; CMV-TK/GCV; Pulmonary melanoma metastasis; Suicide gene therapy

In the past several years, researchers have shown great enthusiasm in developing target drug/gene delivery systems (TDDS) for tumor treatment, and some pioneers have explored the possibility of using cells as vehicles in TDDS. The results are inspiring, as one of the examples shows that stem cells or progenitor cells can be exploited to selectively deliver therapeutic genes to metastatic solid tumors and that the expression of an appropriate transgene occurs at tumor loci.[1] Among the numerous kinds of cells, bone marrow-derived mesenchymal stem cells (BMSCs) are regarded as one of the promising candidates because of their capability of self-renewal, relative ease of isolation and expansion in vitro, and homing capacity, enabling them to migrate toward and engraft into the tumor sites.[2] According to some reports, BMSCs have the ability to migrate to and incorporate within the connective tissue stroma of tumors,[3,4] and they can be used as a tool to track tumor sites and target-deliver anti-tumor agents to tumor cells and their micro-metastases. Moreover, BMSCs lack the major histocompatibility complex MHC-II and show only minimal MHC-I expression.[5,6] These properties make the allogeneic BMSCs able to substitute autologous stem cells in tumor therapy and for multiple dosing administrations. Therefore, BMSCs are considered to have the potential to solve inherent gene therapy delivery problems.[7]

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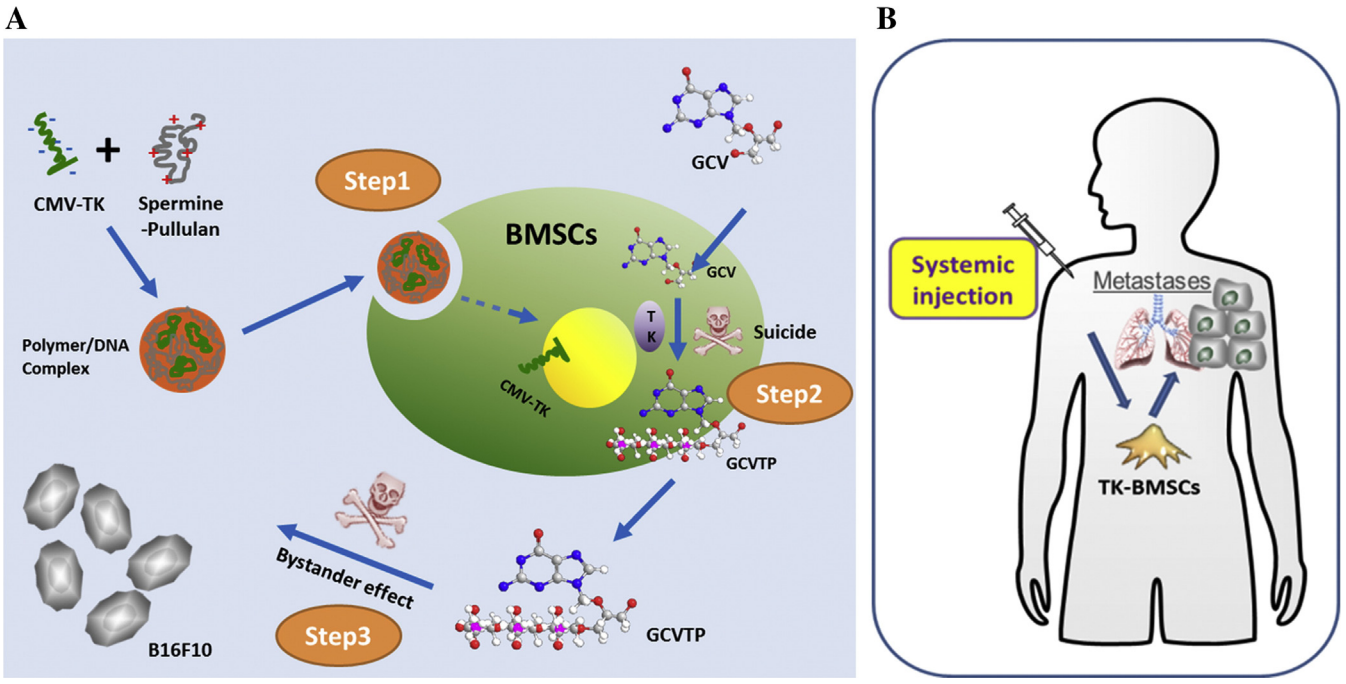

Figure 1. **(A)** Mechanism of CMV-TK expressing BMSCs through a non-viral carrier to kill tumor cells in the presence of GCV. First, BMSCs are transfected to express CMV-TK through the SP. Second, the TK-BMSCs activate the GCV to its toxicity form (GCVTP) and kill the BMSCs themselves, the so-called suicide effect. Finally, the toxic agents kill the tumor cells around the BMSCs through the bystander effect. **(B)** BMSCs-based target drug delivery system for metastases gene therapy. TK-BMSCs migrate to the pulmonary and metastatic nodules after intravenous infusion. Then, GCV is given and converted to toxicity agents by TK-BMSCs in the lung to kill tumor cells.

Metastasis to the lung is a lethal attribute of sarcomas and several other cancers.[8] However, the success of conventional therapy, such as chemotherapy or irradiation, is still limited by several drawbacks, including insufficient drug concentrations in tumors, systemic toxicity and lack of selectivity for tumor cells over normal cells.[9-13] Surgical resection of the metastasis is also not the best choice for most cancer patients with lung metastasis.[14] Therefore, alternative and complementary strategies for metastasis need to be developed because of this limited success in the available treatment modalities for metastatic cancer.

Cancer gene therapy using the suicide gene(s) or known as gene-directed enzyme prodrug cancer therapy has been suggested as an alternative approach to battle metastases.[8] The main concept of this therapy is tumor-specific targeting and selectively eradicating tumor cells with therapeutic gene expression.[15] Nevertheless, suicide gene therapy is limited by the delivery methods currently available.[7] Antibodies and virus are most often applied in this therapy to deliver suicide genes. However, antibodies have many obstacles for clinical application, especially the immunogenicity of the antibody–enzyme conjugate;[16] and for virus, there is also some limitations like insertional mutagenesis, anti-DNA antibody formation, local infection, and tumor nodule ulceration that restrict its use.[17] Thus, how to deliver suicide genes to tumor sites effectively and safely is critical for the successful therapeutic strategies for tumor.

Considering the aforementioned advantages of BMSCs, their use as efficient tumor-targeting gene delivery vehicles can be a promising therapeutic option for cancer gene therapy. Mesenchymal stem cells (MSCs) carried on suicide genes such as cytosine deaminase (CD)[18] and herpes simplex virus thymidine kinase (HSV-TK)[19] have been proved to exert cytotoxic effect on tumor cells. In most studies, MSCs were transduced through viral gene vectors, such as adenovirus,[20] lentivirus[7] and baculovirus,[21] but only a limited number of studies have been conducted using non-viral vectors. For viral vectors, risks such as unexpected adverse effects, toxicities, immunogenicity, and oncogenicity have limited their clinical applications and have led to the termination of many clinical trials.[22-24] Therefore, non-viral vectors including cationic liposomes[25] and cationic polymers[26] have been developed as an alternative. These vectors have the advantages of ease of synthesis, low immune response, unrestricted plasmid size,[27,28] and lower risks than viruses for clinical application. In our previous studies, we have investigated some non-viral gene vectors including chitosan-linked-PEI series (CP and CPT),[29,30] spermine-pullulan (SP),[31,32] PEI-cyclodextrin,[2] and others on tumor cells or stem cells. We additionally established a three-dimensional cell culture system that can further enhance DNA expression and prolong the time of transgene expression to 21 days on BMSCs.[32,33] Therefore, we consider that non-viral gene vectors, like SP, can be applied for BMSCs engineering, and have numerous benefits and a promising future for gene recombination.

In a previous study, we also proved that BMSCs could carry the therapeutic gene for tumor targeted gene therapy.[34] In this study, we further explored the feasibility and efficacy of BMSCs