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Review Article

CAR T-Cell Therapy for EBV-Associated Hematopoietic Malignancies

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SUMMARY

In recent years, cancer therapy has advanced from chemotherapy and targeted therapy to immunotherapy, thus resolving drug specificity-related problems pertaining to tumor cells and resistance. Chimeric antigen receptor (CAR) T cells are genetically modified T cells that express receptors for specific tumor cell antigens to induce T-cell-mediated cytotoxicity. Therefore, the identification of specific antigens on tumor cells is a key point that should be considered in CAR T-cell therapy. For instance, Epstein-Barr virus expresses the oncoprotein latent membrane protein 1 to induce various diseases such as nasopharyngeal carcinoma; posttransplant lymphoproliferative disorders; and diffuse large B-cell, Burkitt, and Hodgkin lymphomas.

1. Introduction

Cancer is a leading cause of death worldwide, and it ranks first among the top 10 causes of death in Taiwan. Present-day cancer therapies include surgical resection, chemotherapy, radiation therapy, and targeted therapy. However, these therapies are associated with several side effects and fail to eradicate the cancer. For instance, chemotherapy often leads to side effects such as nausea and vomiting;[1] furthermore, patients with cancer develop resistance to anticancer drugs during targeted therapy. Moreover, drug properties may lead to poor therapeutic efficacy.[2] Thus, most studies on cancer treatment have primarily been conducted focusing on the improvement of drug specificity for target cells and reduction of associated side effects. Recently, immunotherapy has garnered considerable attention. In this form of therapy, immune cells are cultured and activated in vitro or genetically engineered after activation; these activated cells are then administered into patients with cancer to kill cancer cells. Immunotherapy is more target specific and exerts better therapeutic effects than conventional therapies. Thus, immunotherapy provides a new treatment option for patients with cancer.

2. Epstein-Barr virus and cancer

Epstein-Barr virus (EBV) primarily infects lymphoid and epithelial cells.[3] Infection with EBV often leads to various diseases such as Burkitt, Hodgkin, and natural killer/T-cell lymphomas; nasopharyngeal carcinoma (NPC); leiomyosarcoma; and gastric carcinoma.[4] EBV exhibits 4 latency states in the host: 0, I, II, and III. EBV genes expressed during different incubation periods vary and also differ according to the tumor type (Table 1).[5]

Because prevention is better than cure, the prevention of EBV infection using vaccines is an important research topic. Vaccines targeting glycoprotein 350 (gp350) can prevent EBV from binding to the CR2 or CD35 receptors on B cells, thus preventing EBV's access to B cells.[7,8] Various targets on EBV can also be used to design effective vaccines (Table 2). Virus-like particles mimic the natural structure of viruses and thus effectively elicit immune responses that induce CD4+ and CD8+ T cells in vivo.[7]

The treatment can be classified into those involving therapeutic vaccination, adoptive T-cell therapy, EBV-specific T-cell-receptor-engineered therapy, and EBV-specific chimeric antigen receptor (CAR) T-cell therapy. Therapeutic vaccines can be divided into the following 2 categories: dendritic-cell-based EBV vaccines and recombinant viral vector vaccines.[9] Target and preventive vaccines vary, mainly those designed against Epstein-Barr nuclear antigen 1 (EBNA1), latent membrane protein 2 (LMP2), or LMP1.[9] However, studies on EBV vaccines are limited because of the lack of effective animal models.[10]

Adoptive cell transfer is a new approach in transfusion medicine that involves the infusion of lymphocytes to mediate antitumor, antiviral, or anti-inflammatory effects. The coculture of monocytes with EBV-transformed lymphoblastoid cell lines helps identify EBV proteins, such as BZLF1 and BMLF1, and some latent proteins, such as EBNA1 and EBNA2.[11] In some patients with NPC, disease symptoms are effectively alleviated after treatment.[12]

In CAR T-cell therapy, gene editing is performed to ensure that T cells express receptors against tumor-specific antigens to activate and remove cancer cells from the patient's body. The advantage of CAR T-cell therapy is that it does not require the interaction of major histocompatibility complexes and costimulatory molecules to

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Table 1
EBV latency gene expression and associated diseases.

	Latency 0	Latency 1	Latency 2	Latency 3
Gene		EBNA1	EBNA1 LMP1 LMP2 A/B	EBNA1 EBNA2 EBNA3 A/B/C LMP1 LMP2 A/B
Associated diseases		Burkitt (BL) Primary effusion lymphoma (PEL) Gastric carcinoma (GC)	Hodgkin (HL) Nasopharyngeal carcinoma (NPC)	Infectious mononucleosis (IM) Post-transplant lypjoptoliferative (PTLD)

EBNA: Epstein-Barr nuclear antigen, EBV: Epstein-Barr virus, LMP: latent membrane protein.
Latent gene expression in EBV is frequently designated as follows: Latency I (EBNA1 only), II (EBNA1, LMP1, and LMP2), or III (EBNA1, EBNA2, EBNA3A, EBNA3B, EBNA3C, EBNALP, LMP1, and LMP2).[6]

Table 2
EBV surface proteins used in the development of preventive vaccines.

Protein	References
gp340	Protection of 3/5 cottontop tamarins against malignant lymphoma.[33]
gp350	Rational design of an Epstein-Barr virus Vaccine targeting the receptor-binding site.[34]
gp350/220	A chimeric EBV gp350/220-based VLP replicates the virion B-cell attachment mechanism and elicits long-lasting neutralizing antibodies in mice.[35]
gH/gL/gB	Immunization with Epstein-Barr virus core fusion machinery envelope proteins elicit high titers of neutralizing activities and protect humanized mice from lethal dose EBV challenge.[32]

EBV: Epstein-Barr virus.

activate T cells, which is generally noted in the case of T cells without gene modification; instead, this therapy activates immune cells directly through genetically modified specific T-cell receptors. The structure of a CAR can be divided into 3 parts: an extracellular single-chain variable fragment (single-chain variable fragment) for the identification of cancer cell antigens, a transmembrane domain, and an intracellular structure responsible for the transmission of signal to the CD3ξ domain. Third-generation CARs incorporate 2 costimulatory molecules, CD28 and 4-1BB, to CD3ξ to enhance the effects of T cell activation.[13] Initially, CAR T-cell therapy was primarily used to clinically assess the safety and therapeutic efficacy of chronic and acute lymphocytic leukemias and B-cell lymphomas; this immunotherapy was made available under the brand name Kymriah (tisagenlecleucel) in 2017. The US Food and Drug Administration approved the use of anti-CD19 CAR T-cell therapy for the treatment of relapsed or refractory lymphoma and leukemia,[14] primarily diffuse large B-cell lymphoma (DLBCL) and primary mediastinal large B-cell lymphoma after ≥ 2 systemic therapies with other agents.[15]

Some solid and blood cancers are associated with EBV. Three major types of EBV-associated B-cell malignancies are Burkitt lymphoma, Hodgkin lymphoma, and DLBCL. CAR T-cell therapy is thus an attractive strategy for the treatment of EBV-associated malignancies.[6,16,17]

A study published in 2014 indicated that CAR T-cell therapy against LMP1 exhibits satisfactory results both *in vivo* (mouse model) and *in vitro*.[18] Therefore, several clinical trials on CAR T-cell therapy against cancer are currently being conducted worldwide. However, although this immunotherapy has certain advantages in the treatment of hematological malignancies, it is unsuitable for solid tumors. The therapeutic efficacy of CAR T-cell therapy against solid tumors is low.[19] The possible reasons are as follows. First, although solid tumors secrete chemokines such as CXCL1, CXCL12, and CXCL5, T cells lack the corresponding receptors. Therefore, these cells fail to effectively move to the site of tumor infiltration.[20–22] Moreover, the structure formed by fibroblasts and an extracellular matrix makes it difficult for T cells to constantly come in contact with and activate tumor cells.[23] Second, several factors inhibit the activity of CAR T cells in the tumor microenvironment, such as regulatory T cells that suppress immune responses[24] and bone marrow myeloid-derived suppressor cells[25] that secrete cytokines suppressing the immune response to prevent T cell activation. Finally, tumor cells express inhibitory immune checkpoint ligands, such as PD-L1 and immunoreceptor tyrosine-based inhibitor motif, to inhibit the activation of immune response.[26]

A study from the University of Pennsylvania reported a case in which a PD-1-blocking antibody was administered to a patient with refractory DLBCL and progressive lymphoma after the patient received CAR T-cell therapy against CD19.[27] Following PD-1 blockade, the patient exhibited clinically significant therapeutic responses; thus, the incorporation of PD-1 inhibitors into the treatment regimen may enhance the efficacy of CAR T-cell therapy.[27]

Thus far, over 1000 patients have received CD19-targeted CAR T-cell therapy in the United States alone. A key feature of the immune response elicited after CAR T-cell therapy is that the response is durable. To the best of our knowledge, few clinical trials have been conducted aiming at the head-to-head comparison of the designs of various CAR T-cell therapies; nevertheless, some inferences can be drawn based on the available data. For instance, the inclusion of mouse sequences may trigger the rejection of CAR T cells by the host immune system; various studies have suggested that the lack of immunogenicity, and hence the persistence of CAR T cells, is associated with improved relapse-free survival in patients with leukemia. Thus, CAR T-cell therapies that are designed based entirely on human sequences are recommended.[28]

Currently, CAR T-cell therapy has some limitations such as antigen escape; target nonspecificity; and CAR T-cell cytotoxicity, transport, and tumor infiltration; these challenges must be overcome in future studies (Table 3).[29]

Debottam Sinha's group demonstrated the allogeneic antigen-specific adoptive T-cell therapy for EBV-associated malignancies in vivo models. Through sequential infusion of two different allogeneic T-cell therapies restricted by different HLA alleles, they also override resistance to T-cell therapy using a 'restriction-switching' approach.

Table 3
Limitations of CAR T-cell therapy and current studies on potential strategies to overcome such limitations.

Limitations	Studies on potential strategies
Antigen escape	Multi-antigen-targeted CAR T cells for cancer therapy.[30]
On-target off-tumor effects	A rational mouse model to detect on-target, off-tumor CAR T-cell toxicity.[31]
Immunosuppressive microenvironment	CAR T cells hit the tumor microenvironment: strategies to overcome tumor escape.[32]
CAR-T-cell-associated toxicities	Recent advances in CAR T-cell toxicity: mechanisms, manifestations and management.[33]

CAR: chimeric antigen receptor.

As for the programmed death-ligand 1/programmed cell death protein-1 mechanism, the combination with EBV-specific T-cell therapy improved overall survival when compared with monotherapy in tumorbearing mice.[34]

3. Conclusions

The first step in combatting EBV infection is the administration of vaccines to prevent EBV from infecting lymphoid or epithelial cells. In addition, patients with EBV infection should receive appropriate treatment against EBV. The ultimate goal of CAR T-cell therapy is to cure cancer. Some markers present on EBV-associated cancer cells are identified by CAR T cells. Currently, preliminary progress in terms of treatment efficacy is noted for CAR T cells designed against some of the cancer cell markers. For instance, immunotherapies against gp350 and LMP1 exhibit satisfactory outcomes in mice. Thus, CAR T-cell therapy paves the way for standardized personalized cell therapies.

Conflicts of interest

We declare no conflicts of interest.

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