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Review Article

Prophylactic and Therapeutic EBV Vaccines of rBCG

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ABSTRACT

Based on the research of recombinant BCG (rBCG) vector vaccine for many years, we recombined *BZLF1* and *LMP2* gene into BCG vector and studied its immunological mechanism and function *in vivo* and *in vitro*, which laid a solid foundation for the application of recombinant BCG vector vaccine, in this paper, the reason of using *BZLF1* and *LMP2* gene and the function of BCG vector are summarized.

Keywords: BCG; *BZLF1*; *LMP2*; Vaccine

INTRODUCTION

On the basis of previous studies, we transformed the *BZLF1* and *LMP2* fusion genes of EBV specific antigen into BCG vector and expressed them efficiently in BCG to obtain rBCG with stable secretory expression, then we studied the effect of rBCG secreted Epstein-Barr Virus (EBV) fusion protein on the proliferation of tumor cells and the antitumor activity of effector cells *in vitro*, and established the tumor-bearing mouse model, studied the different preventive and therapeutic effects of rBCG on EBV positive tumors and its immune mechanism, a rBCG vaccine that can effectively prevent or treat EBV positive tumors was gotted, in order to prevent and treat EB virus infection and tumor.

EBV is a 184 kb lymphocyte Herpesvirus that was discovered in 1964 by Epstein and Barr in the study of Malignant Lymphoma in African children. EBV latent infection has been found to be associated with a variety of human cancers, including Nasopharyngeal Carcinoma, Burkitts Lymphoma, Hodgkins Lymphoma, and Gastric Cancer, so in 1997 the International Agency for Research on Cancer (IARC) classified EBV as a first-class carcinogen. Since the discovery of EBV, great progress has been made in the study of EBV-related vaccines. However, because of the complexity of EBV life cycle, there is no mature vaccine on the market. It is urgent to develop a safe and effective vaccine to prevent many diseases and tumors caused by EBV. The potential carcinogenicity of EBV, live attenuated vaccine of EBV is not suitable for prevention in healthy people. The research of EBV vaccine based on EBV subunit vaccine has become a hot spot. But there is no literature and patent report around the world and no report of clinical application also. With the development of molecular biology and tumor immunology, people pay more and more attention to using immunotherapy to prevent the recurrence and distant metastasis of NPC.

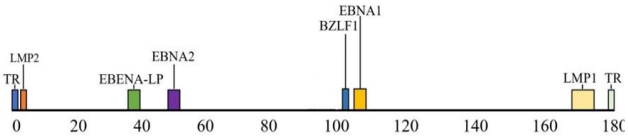

Figure 1: Epstein-barr virus sequence.

LITERATURE REVIEW

LMP2 including *LMP2A* and *LMP2B*, only the first exon of N-terminal is different from each other. *LMP2* has been proved to induce stronger specific cellular immune response, and many CTL epitopes have been identified [1]. In recent years, the expression of *LMP2* gene in all B cells of EBV latent infection *in vivo* and *in vitro* has been demonstrated, moreover, *LMP2* is one of the few conservative antigens that are stably expressed in nasopharyngeal carcinoma, lymphoma and other tumor cells. It has potential T cell activation epitope and can mediate the function of killer T cells. Therefore, *LMP2* may be an ideal target antigen for EBV related tumor immunotherapy. If the gene is transferred into the expression vector alone or in combination, it may express this target antigen and induce

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specific cellular and humoral immunity, prevention of EBV infection and killing of EBV positive tumor cells [2,3].

In this study, we used another key Epstein Barr virus gene, the immediate early gene (*BZLF1*), the immediate early gene expression can induce the virus from latent period to lytic period, the encoded protein is a transcription activator. EBV infects host cells including latent period and lytic period. In EBV positive tumor cells, EBV is usually in latent period, and its latent existence and DNA integration with host cells are the important reasons leading to the occurrence of tumor, artificial induction of EBV from latent phase to lytic phase, leading to the death of tumor cells, is expected to be a new method to treat EBV-associated tumors. *BZLF1* of EBV encodes a transcriptional activator, and the expression of *BZLF1* can induce EBV to enter the viral replication cycle from incubation period. It can induce latent EBV in tumor cells to enter the lytic phase, and open the mechanism of virus replication, EBV proliferation leads to the lytic and death of EBV positive tumor cells, which can specifically kill tumor cells. In this study, we propose to fuse *BZLF1* coding protein expression. The expression of *BZLF1* coding protein immediately causes the latent EBV to enter the lytic phase, the immunogenicity of the protein encoded by the early gene *BZLF1* can also induce specific cellular and humoral immunity, which has been confirmed in our previous studies.

We linked the two genes *BZLF1* and *LMP2* together by the DNA sequence of the peptide junction (Gly4Ser)3, which was composed of 15 amino acids. By constructing the fusion gene, *LMP2* induced specific CTL and *BZLF1*-induced latent EBV replication into the lytic stage, thus killing EBV positive tumor cells. How to make the fusion gene play an effective and safe anti-tumor role in nude mice model of nasopharyngeal carcinoma? It provides an objective basis for further clinical research and treatment of patients with nasopharyngeal carcinoma.

DISCUSSION

The molecular biology modification of BCG can make its therapeutic effect on tumor more obvious. Therefore, if BCG is used as the expression vector of these cytokine genes, the effect may be better if it is combined with adjuvant and vector. This genetic engineering vaccine may play the following roles: BCG and cytokines which it stimulates the secretion and expression of the body can activate and promote the proliferation of immune cells and exert their function of killing tumor cells, increase the activity of killing tumor cells. The cytokines secreted by BCG after direct intratumoral injection directly affect the immunocytes and tumor cells, which can avoid the side effects of systemic administration of cytokines according to the specificity of the treated tumor. The antigen protein produced by the rBCG can induce tumor-specific CTL.

The molecular modifications of BCG maybe make its therapeutic effect on tumor more obvious. Therefore, if BCG is used as the expression vector of some important proteins, it may be more ideal to combine adjuvant and vector together. These kind of genetic engineering vaccines may play the following role: BCG and the cytokines can activate and promote the proliferation of immune cells, and these would play the role of killing tumor cells, improve the activity of killing tumor cells. The proteins secreted by BCG after direct intratumoral injection directly affect tumor immune cells and tumor cells, it can completely avoid the side effects of systemic use with cytokines on the whole body. The relevant surface antigen protein produced by rBCG can be introduced into the tumor according to the specificity of the tumor to induce the production of tumor-specific CTL. The rBCG can enter into the tumor cells and persist in these cells, then the tumor-specific CTL can be induced by the secreted antigen protein, with tumor cells proliferate, they divide and pass on.

The aim gene was transferred into BCG. We used the pMV261 secretory expression plasmid which was constructed by Stover et al. This plasmid can make exogenous gene express in BCG secretively and stably, which will promote the research of rBCG greatly.

The plasmid pMV-BFP2 was transformed into BCG vaccine by electroporation to obtain rBCG which is resistant to EBV virus and can eliminate EBV positive tumor cells, to produce an immune response superior to that of a single gene? *In vitro* and *in vivo* anti-tumor experiments were used to examine the effects of target gene expression on tumor cell apoptosis and proliferation, and the inhibition of tumor growth in tumor-bearing mice, the effect of fusion protein on apoptosis of tumor cells was analyzed at cellular and molecular levels to prepare subunit vaccine with high immunity to EBV positive tumor cells [4-7].

CONCLUSION

In the future, the other EBV specific gene fragments *EBNA1*, *LMP1* will be fused with *BZLF1* respectively, and the fused gene will be transferred into BCG expression vector, to study the anti-tumor effect of rBCG expressing EBV fusion protein *in vitro* and the anti-tumor activity of effector cells, then we will study the different therapeutic effects of rBCG on EBV positive tumors and their immune mechanisms, to evaluate their anti-tumor effects and safety, in aim to construct BCG vector vaccine that can effectively treat EBV positive tumors, in order to prevent and treat EBV infection and tumor, and benefit EBV positive tumor patients, especially nasopharyngeal carcinoma patients, lay the theoretical and experimental foundation. *In vitro* and *in vivo* anti-tumor experiments were carried out to investigate the effect of target gene expression on apoptosis and proliferation of tumor cells, the effect of the fusion protein on the apoptosis of tumor cells was analyzed at the cellular and molecular levels. Then a subunit vaccine was prepared which could produce high immunity to EB virus positive tumor cells. If the experiment can achieve the expected results, it will bring great economic and social benefits; it will lay a theoretical and experimental foundation for further clinical research and treatment of EBV positive tumor patients, ideally, volunteers will be recruited for the pre-clinical phase of the trial.

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