

The anti-tumor research of rBCG with the fusion gene GM-CSF and BZLF1

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Objective: To investigate the construction and expression of rBCG combining human granulocyte macrophage colony stimulating factor (*GM-CSF*) and EB virus (EBV) encoding immediate early gene (*BZLF1*). Then the antitumor activity and immunological mechanisms of rBCG including fusion genes *GM-CSF* and *BZLF1* were researched.

Methods: The purified *GM-CSF* and *BZLF1* were amplified by PCR and inserted into plasmid pMV261, then transformed them into *Escherichia coli* DH5 α (*E. coli* DH5 α). In LB culture medium containing kanamycin, the positive colony was selected, the correct sequence of the *GM-CSF* and *BZLF1* were connected by the polypeptide (Gly4Ser)₃ series with the splicing overlap extension technology. The fusion gene *GCBF* was constructed and cloned into pMV261, transformed competent bacteria and selected on LB culture medium flat containing kanamycin. Plasmid pMV*GCBF* extracted from positive clones were transformed into competent BCG. Western-blot was employed for determination of expression of *GCBF*. C57BL/6 mice were used to build animal models of EB virus positive tumors. Antitumor activity was analyzed by the formation time of tumors, survival time and tumor weight. The specific antibodies of mice stimulated with rBCG was detected by ELISA, lactate dehydrogenase assay was used to detect the mouse cellular immunity, HE staining of tumor tissue to assay lymphocyte infiltration in tumor of mice, so the rBCG immune effect was researched. At last, we used statistical methods to analyze the immunization of rBCG.

Results: The RT-PCR product sizes of objective gene *GM-CSF* and *BZLF1* were 461 bp and 788 bp, consistent with expected values. The recombinant plasmid was confirmed by restriction double enzymes, amplification and sequencing, then the fusion gene (1209 bp) was correctly inserted into the vector. pMV*GCBF* were correctly transformed into competent BCG. The expression of fusion protein *GCBF* was detected in rBCG. Tumor formation time was delayed in mice immunized by rBCG. Tumor growth was slow and survived time of mice was prolonged. The mice immunized by rBCG could produce specific IgG antibodies of *GM-CSF* and *BZLF1*. At the same time, specific CTL activity was detected in mice and tumor tissue infiltration of lymphocytes was found by microscopy.

Conclusion: rBCG encoding *GCBF* fusion gene was successfully constructed to have provided basis for further study of the function of rBCG. The mice immunized by rBCG could produce humoral and cellular immune responses. The mice produced a strong antitumor effect. The EB virus-positive tumors were significantly inhibited in mice.

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