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Cloning and expression of recombinant scFV monoclonal antibody against carcinoembryonic antigen

M. Salavatifar[1]

1- Department of aerospace physiology, Aerospace Research Institute, Ministry of Science Research and Technology, Tehran, Iran salavati@ari.ac.ir

Introduction

Monoclonal antibodies have a high ability to diagnose, treat, and target cancer cells, but due to their large size, their use is limited. The emergence of recombinant antibody technology has revolutionized the production and use of monoclonal antibodies and has removed some of the limitations of hybridoma technology. Hybridoma is the cells capable of continuously producing monoclonal antibodies that recognize a specific antigenic marker. Despite the widespread use of monoclonal antibodies, using these molecules as therapeutic agents has encountered some limitations so far. The special structure of variable domains of the antibody provides the possibility of dividing them into efficient subunits. This possibility can create new molecules with efficient properties by combining and connecting variable domains in different manners. The single-chain FV (scFV) antibody fragments are a group of these molecules. In terms of size, scFV is about one-fifth of the primary IgG molecule and therefore has advantages such as rapid discharge from plasma and, as a result, higher tumor absorption than normal antibodies. Another advantage of these molecules compared to complete mouse IgG antibodies is the lack of constant antibody parts; thus, the immunological responses are reduced. In this research, the *scFv* gene fragment was made against the carcinoembryonic tumor marker, which is widely expressed in all types of carcinoma cancers. After cloning and expressing that in *E.coli* bacteria, its specificity was confirmed by the western blotting test.

Materials & Methods

In this study, total RNA was extracted from cultured hybridoma cells that secreted specific IgG antibodies against carcinoembryonic tumor marker. The RNA integrity was investigated with agarose gel. The complementary DNA (cDNA) was made from the RNA template. In the next step, the gene coding variable regions of the monoclonal antibody against the carcinoembryonic tumor marker were amplified by PCR reaction with special primers and connected to each other by a linker fragment. After making the *scFV* gene fragment, it was purified from agarose gel.

Then, both ends of the fragment were cut by *SfiI* and *NotI* restriction enzymes. The pCANTAB 5 E plasmid was also cut by the same enzymes, and then both fragments were assembled by the ligase enzyme. Then, the binding reaction product was introduced into *E. coli* HB2151 competent cells, and its expression was induced by IPTG. The specific anti-Etag HRP antibody checked the expressed protein specificity to carcinoembryonic tumor marker antigen in the western blotting analysis following desired protein fragment observed on SDS-PAGE with the right size.

Results & discussion

Carcinoembryonic tumor marker is one of the most important oncofetal markers, and its blood levels are significantly different between healthy and cancer people. Carcinoembryonic tumor marker is located on the cell surface of clone tumors and other cells. This antigen can act as a homotypic adhesive molecule, which leads to the accumulation of cells expressing that. Although the expression of carcinoembryonic tumor marker in normal epithelial cells of the clone is minimized in adults, its accumulation pattern is different in most tumor cells of the clone from the developing fetal clone. The aim of the current research was to construct the recombinant scFV fragment against the carcinoembryonic tumor marker for use in the diagnosis, treatment process monitoring, as well as the specific drug delivery to the cancer cells that express that. The majority of mRNAs in hybridoma cells cytoplasm are the RNA encoding the target antibody. So the specific hybridoma cells secreting specific IgG antibodies against carcinoembryonic tumor markers were cultivated. In order to amplify variable antibody fragments (VH and VL), total RNA was successfully extracted from hybridoma cells. After confirming the extracted RNA on agarose gel, complementary DNA was synthesized from it. The variable fragments were amplified using the polymerase chain reaction (PCR) method. Then, the scFV fragment was successfully constructed through PCR reaction with specific primers by connecting the variable fragments through a linker segment. The constructed scFV fragment was confirmed by observed the 800 bp DNA size band. In order to obtain high amounts of scFV fragment, cleavage reactions and subsequent enzyme ligation of scFV and pCANTAB 5 E plasmid were done. The plasmid selected for the expression of the scFV molecule had dual efficiency. It could display the scFV molecule as a fusion on the phage surface. As well as, it was able to express the protein in the periplasmic space in a soluble form if the appropriate strain of bacteria was applied. Due to the presence of disulfide bonds inside the VH and VL antibody fragments, a suitable space was needed to form these bonds. The periplasmic space of bacteria provided this possibility. On the other hand, the amount of protein accumulation in the cytoplasm of bacteria was lower by transferring to the periplasmic space. Since high levels of foreign proteins in the cytoplasm of the cell can result in the death of the bacteria, this transfer is beneficial to the growth and production of recombinant proteins by bacteria.

After cloning confirmation, the recombinant plasmid was successfully introduced into the bacterial expression host. After protein expression induction, a band with a 32-kilodalton protein size was observed on the SDS-Page gel. The protein size was consistent with the actual calculated size.

In addition, the specificity of the recombinant antibody against carcinoembryonic tumor marker was confirmed through western blotting analysis.

Conclusion

The scFV recombinant antibody expressed in this research was specifically capable of carcinoembryonic tumor marker recognition. Therefore, it can be applied to diagnosing and monitoring some types of cancers. In addition, this method

can be used to synthesize scFV-type recombinant antibodies against other antigens. So that they can be used in various diagnostic tests, monitoring, and treatment of diseases.

Keywords: *Cloning, Hybridoma, Tumor marker****.***

Declaration of conflict of interest: *The authors declare that they have no conflicts of interest*