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DEVELOPMENT OF GENETICALLY ENGINEERED MOUSE/ HUMAN CHIMERIC AND SINGLE CHAIN ANTIBODIES AGAINST HUMAN BRAIN GLIOMA: A PRELIMINARY REPORT

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In order to improve the clinical usefulness of mAb of mouse origin in targeting diagnosis and therapy for human brain glioma, it is necessary to humanize it and reduce its molecular size. By means of RT-PCR technique, a 348 bp heavy chain variable domain (VH), and a 318 bp light chain variable domain (VL) cDNA fragments were cloned from mouse hybridoma cell line SZ_{39} secreting mAb against human brain glioma. By recombinant DNA technique, the two cDNA fragments were linked to human IgG1 heavy chain CH1 and light chain κ constant regions, respectively, to form a mouse/human chimeric gene which was then inserted into an expression vector pHEN1-SZ_{39} Fab/Hu. In addition, the two cDNA fragments were linked directly with a universal linker and inserted into an expression vector pHEN1-SZ_{39}ScFv. The two expression vectors were separately introduced into non-suppressor E.coli HB2151 to secrete chimeric antibodies and single-chain antibodies, respectively. On ELISA and Western blot assays, the two genetically engineered antibodies were bound specifically to the same 180 kD cell surface membrane antigen on human brain glioma cell line SHG_{44} as did the parental mAb SZ_{39}.

Key words: Monoclonal antibody, Glioma, Genetic recombination.

The targeting diagnosis and therapy of malignant tumor by means of tumor monoclonal antibody (mAb) is one of the highly popular research subjects in recent years. The murine origin mAb SZ_{39}[1] against malignant human brain glioma generated in our laboratory in 1988 has been conjugated with adriamycin and shown to possess an increased targeting activity both *in vitro* and in animal models. In addition, it has been labeled with isotopes and used as targeting agent for the diagnostic purpose in human brain glioma patients.[2] However, the murine origin mAb possessed the drawback of potential immunogenicity large molecular weight and hard to penetrate blood brain barrier, thus limits its value in clinical application. In order to eliminate those aforesaid defects of murine mAb and retain the advantage of its high affinity for the relevant antigen, from mid-1980s on, the scholars at home and abroad have made great effort on the study of genetic engineering technology to convert murine originated mAb into humanized one and to reduce the molecular size of the antibody fragment. Great attention has been paid to these research work. So far, quite a few chimeric antibodies[3] and single-chain antibodies have been achieved.

In this paper, we report the preliminary result of converting the whole murine mAb SZ_{39} into mouse/ human chimeric and single chain antibodies against human brain glioma.

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MATERIALS AND METHODS

Human glioma cell lines SHG_{44}[4] and hybridoma cell lines SZ_{39} were produced in our laboratory. The primers were designed according to reference 5: VH Back: 5'-AGGTSMARCTGCAGSAGTCWGG-3'; VHFor: 5'-TGAGGAGACGGTGACCGTGGTCC-CTTGGCCCCAG-3' (S=C/G, M=A/C, R=A/G, W=A/T); VLBack: 5'-GACATTCAGCTGACCCA-GTCTCCA-3'; VLFor: 5'-GTTAGATCTCCAGCTT-GGTCCC-3', were synthesized by Shanghai Institute of Cell Biology.

Murine *myc* mAb was generously provided by Dr. Hu Chuanmin of The Fourth Military Medical University. All expression vectors pHEN1, pSV2Fab, pSV2ScFv and non-suppressor E.coli HB2151 were kind gifts sent by Dr. Greg Winter of Cambridge University U.K.. The expression vectors pSV2Fab and pSV2ScFv contain the necessary construct genes and modifying components for expression of chimeric and single-chain immunoglobulin between the restriction site *Hind*III and *Eco*RI. The rest of the reagents were bought from the market of correlated companies.

Single-step method of RNA isolation[6] described by Chomczynski and Saachi was applied with some modifications. Total RNA isolated from the freshly cultured hybridoma SZ_{39} cells was subjected to appraise its quality and undergone oligo (dT) cellulose chromatography to get mRNA. The obtained mRNA of SZ_{39} was used to amplify the heavy and light chain variable domain genes by means of reserve transcription-polymerase chain reaction (RT-PCR). The PCR products were recovered and purified by glass beads purification method, then subcloned into plasmid pUC18 for sequence analysis which demonstrated that the obtained cDNA fragments were actually the heavy (VH) and light (VL) chain variable domain genes of SZ_{39}. Consequently, expression vectors pHEN1-SZ_{39}Fab/Hu and pHEN1-SZ_{39}ScFv were constructed, respectively, then transfected into non-suppressor E.coli HB2151 for expression. The expressed products were examined by SDS-PAGE and Western Blot assay to determine their protein characteristics and also by enzyme-linked immunoabsorbent assay (ELISA) to determine their activities.

RESULTS

Sequence Analysis

Four positive recombinants of each heavy and light chain variable domain genes of SZ_{39} were selected randomly for sequence analysis. It revealed the identical results of each one i.e. the heavy chain variable domain genes containing 348 base pair (bp), encode 116 amino acids, and light chain containing 318 bp, 106 amino acids (Figure 1).

Construction of Expression Vectors of Chimeric and Single Chain Antibodies

Fab/Hu fragment was amplified by PCR using pSV2Fab as the template, 5'-d(ACAAACCTTGCAT-GCAAA)-3' as the upstream primer and 5'-d(AATG-CGGCCGCTTACTATAGCTC)-3' as the downstream primer. PCR products were digested with *Hind*III and *Not*I and ligated with the phagemid pHEN1 which had been digested with the same restriction enzyme. The resulting expression vector pHEN1-Fab/Hu was transfected into E.coli TG1 and screened by the relevant restriction enzyme digestion. The positive clones were verified by sequencing. Then expression vector pHEN1-Fab/Hu was digested with *Pst* I and *BstE* II and ligated with SZ_{39}VH genes which was generated by digesting the pUC18-SZ_{39} VH with *Pst* I and *BstE* II, then expression vector pHEN1-SZ_{39}VH Fab was successfully constructed.

Furthermore, pHEN1-VHFab and pUC18-SZ_{39} VL were digested with *Sst* I and *Xho* I respectively and followed by ligation. Thus the SZ_{39}VL was inserted into pHEN1-SZ_{39}VH Fab for generation of pHEN1-SZ_{39}Fab/Hu. Likewise we constructed the pHEN1-SZ_{39}ScFv according to above mentioned method with example of using pSV2ScFv as the template instead of pSV2Fab. Following the sequence analysis the both vectors were in good assembly and their reading-frames were correct.

Assay of Prokaryotic Expression and its Products[7]

The above mentioned two expression vectors were transfected into non-suppressor E.coli HB2151. The soluble Fab/Hu and ScFv fragments were expressed by induction with isopropyl-1-thio-β-D-galactopyramoside (IPTG). MAb SZ_{39} could specifically recognize the 180 kD glycoprotein[1] expressed in the membrane surface of human brain glioma cell line SHG_{44}. Using SHG_{44} as the antigen and mAbSZ_{39} as the positive control, Western Blot assay revealed that the genetically engineered fragments SZ_{39}Fab/Hu produced in the expression

supernatant were bound specifically to the relevant glycoprotein as did the intact mAb, and manifested obvious expression band (Figure 2). Quantitative analysis using ELISA also showed that both SZ_{39}Fab/Hu and SZ_{39}ScFv yielded 200 μg/L in expression supernatants, and had the similar capacity of specific binding to 180 kD membrane antigen on SHG_{44}.

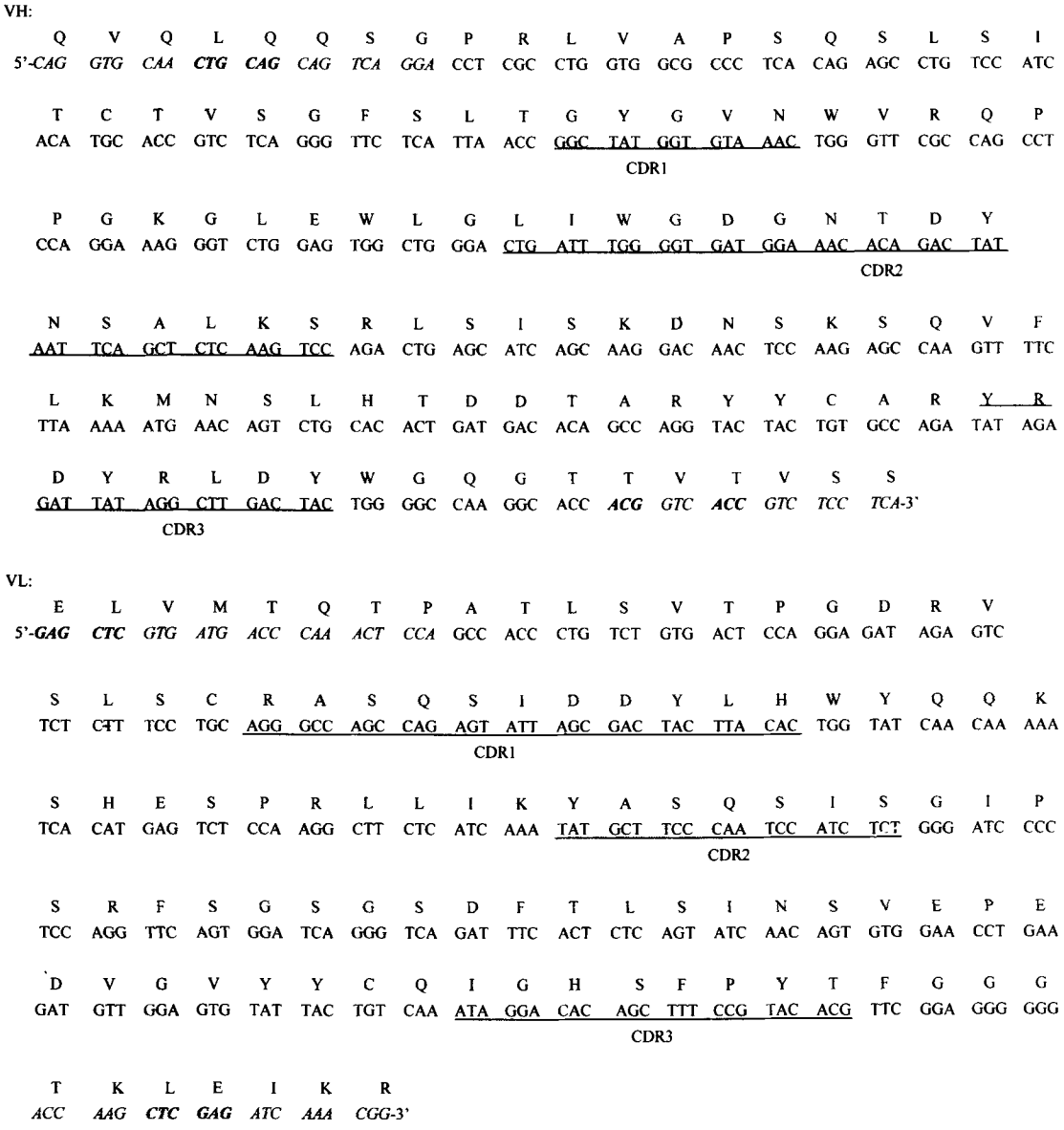

Fig. 1. Sequences of the heavy (VH) and light (VL) chain variable domain genes (lower line) and deduced amino acids of SZ_{39} mAb (upper line).

VH: Top: boldface type: *Pst* I; italic type: 5'upstream primer;
Down: boldface type: *BstE* II; italic type: 3'downstream primer.

VL: Top: boldface type: *Sst* I; italic type: 5'upstream primer;
Down: boldface type: *Xho* I; italic type: 3'downstream primer

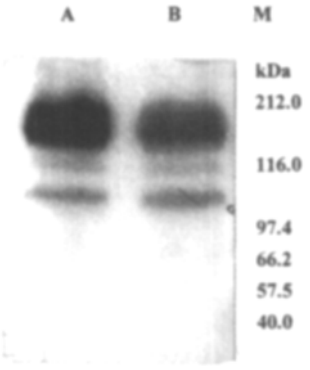

Fig. 2. Assay of activity of SZ_{39}Fab/Hu.
A. mAbSZ_{39} (positive control)
B. expression supernatants
M. standard molecular weight

DISCUSSION

Cloning the heavy and light chain variable domain genes from mAb, the RT-PCR method provided an excellent means to avoid some disadvantages, for instance, certain unnecessary mutation sites might be created following the multiple amplifications. From the PCR products, we picked out several positive recombinants and expressed them directly then screened out the genes with good expression activity for sequence so to rule out those with loss of activity due to gene mutation.

Comparing SZ_{39}Fab/Hu generated in this experiment with intact murine mAb SZ_{39}, its major structure Fv terminus for recognition of antigen doesn't change while the murine Fc terminus is replaced by human immunoglobulin CH1. The resulting mouse/human chimeric antibody has small molecular weight and low immunogenicity which offer advantages of avoiding human anti-mouse antibody reaction (HAMA) and easily penetrating blood brain barrier in further clinical applications. In addition, SZ_{39} heavy and light chain variable domain genes were linked by a universal linker to prepare SZ_{39}ScFv, which is in lack of Fc terminus, so its molecular weight is smaller than the relevant Fab. ScFv can be used to prepare bispecific antibodies and immunotoxins for targeting treatment. It possesses much more flexibility than relative chimeric antibodies.[8] However, this sort of antibody composed of only one single chain, its space configuration would, as a drawback, affect the capacity of binding to the relevant antigen.

At present, using phage display expression system[9] to express genetically engineered mAb is a quite popular practice. It allows the recombination of variable domain genes of cloned antibody and the fusion of them with micro coat protein gene III (gIII) of linear phage to construct phage antibody expression vector, which has great benefits to construct antibody libraries and to screen the genetic engineering mAb with high affinity. Accordingly we adopted this set of expression system with the expression vector pHEN1 containing the constructor of phage coat protein gIII and incorporating amber mutation codon UAG. In the present study, recombinant vectors pHEN1-SZ_{39}Fab/Hu and pHEN1-SZ_{39}ScFv were transfected into non-suppressor E.coli HB2151 for expression. Because the host bacteria could recognize amber mutation, so gIII protein couldn't be expressed, and Fab/Hu and ScFv could fold correctly in the periplasm of E.coli and be secreted into culture supernatants with the form of soluble product. This treatment not only enables us to acquire directly the aforementioned two kinds of mAb possessing activity, to eliminate the difficulties in downstream processing of expressed proteins but also facilitates the screening identification of the positive clones.[10]

SZ_{39} chimeric antibody fragment Fab/Hu and single chain ScFv fragment expressed by pHEN1 expression system, exhibits the similar capacity of murine parental mAb to bind with the relevant antigen. This is fairly significant for further clinical applications. However, the yields of this set of expression system were not high, only 200 μg/L in culture supernatants, so without practical value. This might be correlative with the inappropriation of modifying sequence of this expression vectors. How to further raise its yields is under our study.

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