Archive of SID

چهارمین همایش ملی بیوتکنولوژی جمهوری اسلامی ایران
کرمان مردادماه ۱۳۸٤
International Center For Science & high Technology and Environmental Sciences
The 4th National Biotechnology congress
Islamic Republic of Iran
Kerman august 2005

Isolation and characterization of single-chain Fv genes encoding antibodies specific for p24 of HIV-1

Mohammadzadeh S.[*], Forouzandeh, M.[**], Rahbarizadeh F., Rasaee. M.J., Mohammadi. M., Khoddami Vishteh V., Saghfi. B., Gill. P.

Department of Medical Biotechnology, Faculty of Medical Sciences,
Tarbiat Modarres University, Tehran, I.R.IRAN

Abstract: By using phage display technology we generated recombinant mouse single chain antibody fragment directed against p24, the major capsid protein of human immunodeficiency virus type 1 (HIV-1). Using antibody variable-region (V) gene of B-cell derived from the spleen of an immunized mouse a library of single chain Fv fragments (scFv) was constructed. Following immunization of the mouse and preparation of scFv library, antibodies were selected by panning , using recombinant p24 protein which was expressed as a full length product in E.coli and purified by HPLC method. We isolated one single –chain antibody fragment which specifically recognizes p24 capsid protein of HIV-1. We showed the feasibility of the production of scFv in animal system (mice) by recombinant p24 cloning the repertoire of the heavy and light variable domains as a single chain Fv fragment (scFv), panning and selection, leading to the successful identification of a small antigen binder. It was observed that the antibody react specifically with p24 capsid protein which indicates that the Isolated mouse single-chain Fv fragment harbors the intact antigen binding site .Indeed specific antigen binder with good affinity was identified from this library, This antibody could be an ideal candidate for promising therapeutic applications and for HIV diagnosis. The isolated anti-p24 could be used in p24 antigen immunoassay system for detection of HIV-1 infection in an early phases . Indeed this antibody could be expressed as an intracellular antibody to aid treatment of HIV infection.

Keyword: HIV-1, p24, scFv, Recombinant, Antibody, Phage display

Introduction: P24 capsid protein forms the viral core containing a single RNA genome and is essential for viral assembly. With current enzyme-linked immunosorbent assays(ELISA),the presence of p24 antigen may be assessed 5 to 14 days earlier than could an Ab response measured by anti-HIV type1(anti-HIV-1) or anti-HIV type2 enzyme immunoassays .Recently ,the Food and Drug Administration recommended the implementation of p24 antigen test in donors screening, in addition, the capsid protein may be considered as a marker for virus replication therefore developing a sensitive immunoassay system for detection of p24 is very important .Using the produced recombinant antibody would offer a cheap and applicable alternative to the PCR-based assays for routine diagnosis and during treatment of HIV-1. Besides its diagnostic application, it may also be possible to use anti-p24 single-chain Fv fragments (scFv) for therapeutic purposes. The scFv format has many advantages over other antibody production methods, for example , library construction can be simplified by overlap extension PCR that effectively reduces the number of steps involved,the ability of scFv to multimerize can enhance avidity for antigen and facilitate selection against certain antigens such as cell surface molecules, and yields of the smaller scFv molecule in E,coli tend to be better than Fabs.

Materials & Method:

Immunization of mice : Balb/c mice were each immunized subcutaneously with 50mg of p24 with adjuvant ,after third immunization, the serum antibody titers were analyzed by ELISA and spleen was removed.

mRNA was prepared from the pooled splenocytes of positive mice using the Quick prep Micro mRNA purification Kit (Qiagen) .The resulting mRNA was then used for first-strand cDNA synthesis .VH and VL genes were amplified separately and assembled by PCR overlap extension .The PCR product was used for the production of mouse scFv phage Display Library.

www.SID.ir

Archive of SID

Using affinity chromatography the binder p24 was selected from scFv library by panning . In the following steps the soluble scFv antibodies produced in E.coli HB2151 and were characterized.

Results and Conclusion:

To augment the number of scFvs directed against p24 of HIV-1 , three mice were immunized with p24 within three periods of antigen injection after which one of the mice was sacrificed ,and the spleen was removed immediately then cDNA was generated by reverse transcription from RNA isolated from the spleen. The gene fragment corresponding to the variable regions of the heavy chain (VH) and light chains (VL) of the repertoire of antibody genes were amplified by PCR

scFv gene contracts were generated by randomly joining individual heavy-chain variable domains with light –chain variable domains through gene splicing by overlap junction , or SOE-PCR .

After purification the scFv was then digested by sfi1 and Not1 , and ligated into the phage display vector Pcantab5e the ligated DNAs were used to transform E.coli TG1 cells (amber suppression strain),in which the amber stop codon between the E tag DNA sequence and g3 was read through, allowing the production of scFv Etag-p3 fusion protein. From the resulting library $2x10^5$ clones were obtained, seventy percent of which were scFvs. Recombinant phage, expressing a library of scFv polypeptides on their surface, were produced by helper phage rescue and selectively were enriched by 6 rounds of panning on recombinant p24 . After 6 round of panning 200 individual clones were analyzed by ELISA . Forty percent of the tested scFv, produced high signals on p24 antigen, whereas no responses were found on BSA. To produce soluble scFv, E.coli HB2151 cells were infected with selected phage. Recognition of the amber stop codon between the scFv and the gene 3 protein of M13 resulted in the production of soluble scFv by HB2151. The affinity constant was calculated according to the Beaty et al. and found to be $2x10^9$, 10^9 and 10^8 in three selected clones.

Recombinant antibody against p24 was prepared in E.coli, these scFvs may be used in immunoassay systems and there could be potential application for the produced scFv andtibodies in immunotherapy.

References:

1- Hoogenboom H.R., de Bruine A.P., Hufton S.E., Hoet R.M., Arends J.W., Roovers R.C. Antibody phage display technology and its applications.Immunotechnology 4:1–20, 1998.
2- Haard H.D., Henderikx P and Hoogenboom R. Creating and engineering human antibodies for immunotherapy. Adv Drug Deliv Rew 31:5-31, 1998.
3- Winter G., Griffiths A.D., Hawkins R.E., Hoogenboom H.R. Making antibodies by phage display technology. Annu Rev Immunol 12:433–55 1994.
4- Burton D.R., Phage display. Immunotechnology 1:87–94 1995.

www.SID.ir

Archive of SID

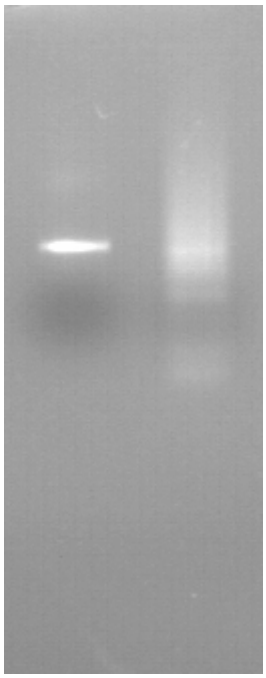

Fig.1 Single chain fragment of variable (scFv) (Lane1 750 bp DNA marker, lane2 750 bp scFv)

www.SID.ir