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ساخت آنتی بادی تک دومنی با تمایل بیشتر علیه MUC1 با استفاده از تکنیک PCR مستعد به خطا

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خلاصه:
دراین تحقیق تکنیک ایجاد موتاسیون و نمایش فاژ برای بهبود افینیتی یک آنتی بادی تک دومنی (RRB7) که پروتئین MUC1 را هدف قرار می دهد مورد بررسی قرار گرفته است. آنتی بادی تک دومنی نوترکیب دومن VH از آنتی بادیهای زنجیره سنگین موجود در شتر و لاما می باشد. MUC1 یک گلیکوپروتئین با وزن مولکولی بالاست که در بسیاری از بدخیمی ها بیان بالا دارد یک کتابخانه فاژی که 3.4×10^6 عضو مستقل دارد با استفاده از روش PCR مستعد به خطا ساخته شد. فاژهای با تمایل بالا به آنتی ژن با استفاده از غلظت کاهشی آنتی ژن متصل به بیدهای پوشیده با استرپتواویدین انتخاب شد و به وسیله PCR و ELISA (برای تعیین فنوتیپ و ژنوتیپ) ارزیابی گردید. سپس فاژ ها به میزبان HB 2151 منتقل شدند و محصول نوترکیب از مایع رویی جمع آوری شد. تمایل آنتی MUC1 از کلون RRB7 و کلون موتانت با استفاده از ELISA مقایسه شد. با استفاده از چندین بار انتخاب به واسطه تمایل جهش- یافته های با تمایل بیشتر و حاوی ژن VHH انتخاب شدند. تمایل آنتی بادی VHH از دو کلون جهش یافته و غیر جهش یافته نشان می دهد که PCR مستعد به خطا می تواند به عنوان یک فرایند ساده و سریع به منظور بهبود تمایل آنتی بادیهای تک دومنی باشد.

Generating improved single domain antibody against MUC1 using error-prone PCR

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Abstract:
We have employed random mutagenesis and phage display to improve the affinity of an anti MUC1 single domain antibody (RRB7) that target MUC1 protein. Recombinant single domain antibodies are V_H domain of heavy chain antibody found in camels and llama.MUC1 is a high molecular weight glycoprotein with an aberrant expression profile in various malignancies. A phage library of 3.4×10^6 independent mutants was generated following an error prone PCR procedure. Phages with higher affinity for antigen were selected following decreasing antigen concentration using streptavidin-coated beads. Higher affinity phages were separated and detected by PCR and ELISA (for genotype and phenotype detection).The phages were transferred to a non-suppressor host (HB2151) and the product was collected from the supernatant. The affinity of anti MUC1 from clone RRB7 and mutated clone was compared following ELISA procedure.
Using several rounds of affinity selection, the mutants with improved affinity and V_{HH} gene content were selected. The affinity of the V_{HH} antibody from two clone showed that controlled error prone PCR may be used as a rapid and simple procedure to improve affinities of the single domain antibody.

Keywords: single domain antibody; MUC1; error prone PCR; phage display

Introduction
Single-domain antibodies (sdAbs), in particular those based on camelid heavy-chain antibody variable domains (VHHs), have emerged as an important class of recombinant antibodies, rivaling Fabs and single-chain Fvs (scFvs) in many diagnostic, therapeutic, and consumer product applications (Hamers-Casterman et al., 1993; Nguyen et al., 2001). The principle of molecular evolution consisting of cycles of diversification, selection, and amplification has been extensively applied to generating antibodies with improved affinities in vitro. In the non-targeted random mutagenesis approach, error-prone polymerase chain reaction (PCR), a mutator strain of *Escherichia coli* and indiscriminate randomization of CDR residues have been employed to construct mutant antibody fragment display libraries from which species with improved affinity have been selected. From this library higher-affinity species are isolated through cycles of selection and amplification. Display technologies such as phage display, yeast display, and ribosome display form an integral part of this approach and are the key to its success. (Yang et al., 1995; Boder et al., 2000). In an attempt to increase the affinity of RR-B7,

a MUC1 peptide-specific VHH isolated from a two-humped camel VHH phage display library, we constructed a mutant phage display library based on an random mutagenesis strategy.

Materials and Methods

Plasmid, phagemid ,cells and media:

The single domain antibody gene was cloned into the phagemid pCANTAB5E, using the NotI and SfiI sites for the insertion of the gene. Phagemid rescue was carried out with the helper phage M13KO7 (Kan^r,1×10^{10} pfu). The host strains were E.coli TG1 and E.coli HB2151. Several standard media such as LB,2XYT and SOC medium were used in this study.

Error-prone PCR:

The error prone PCR conditions were adopted from Leung et. al. (1989) and Cadwell and Joyce(1992).The single domain antibody gene from clone RR-B7 was amplified using conventional PCR reaction and then used as template in the error prone PCR reaction. The reaction mix (100µl) contained 10 mM Tris-HCl(PH 9.0), 50 mm KCl,7mMMgCl_2,0.5 mMMnCl_2, 1mMdCTP and dTTP,0.2 mM dGTP and dATP, 0.01%(w/v) gelatin,0.1%(v/v)Triton x-100,5 U Taq polymerase and 0.3 µM each of the primers. The DNA was amplified in the DNA termal cycler. The amplified DNA fragment was gel purified and subjected to another round of error prone PCR. The products of second PCR were cloned back into the vector using the NotI and SfiI restriction site.

Preparation of phage display library:

The error prone PCR products and plasmid pCANTAB 5E and the plasmid pCANTAB 5E were cut with NotI and SfiI and fragments after Gel purification were ligated at a vector/insert ratio of 1/5. The ligation mixture was transformed into TG1 E.coli strain. The size of phage library was estimated about 3.4×10^6 mutants. The transformants were panned against decreasing concentration of biotinylated PDTRPAP peptide. A relatively high antigen concentration (1000 nM) was used for the first round to capture rare or poorly expressed phage antibodies. The antigen concentration was then decreased to 500 nM and 100 nM for the next rounds of selection. After each round of selection dilution of harvested phage particles were transferred to TG1 cells.

Screening for high-affinity antibodies:

ELISA procedure was applied to detect the high affinity binding phages as described before (Rahbarizadeh et al. 2004).

Results:

Fig.1 shows the PCR results of our experiments.
Fig.2 shows the purified pCANTAB 5E vector before and after digestion.
Fig.3 shows the colony-PCR result of selected colons after 3 round of panning
After screening with ELISA procedure 4 mature high-affinity colons were selected.

Discussion:

We have isolated some peptide-specific VHHs from a mutant VHH phage display library, confirming the previous finding that the camelid VHH repertoire is a good source of peptide- specific VHHs. Furthermore, we have demonstrated that the affinity of such binders

could be significantly improved by in vitro affinity maturation experiments involving a random mutation strategy and phage display. We succeeded in isolating the affinity improved VHHs.

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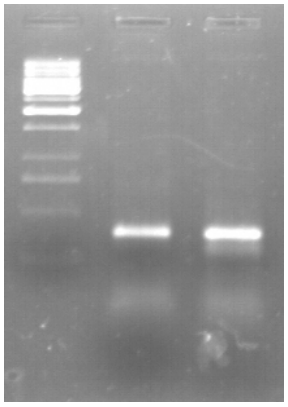

Fig1.

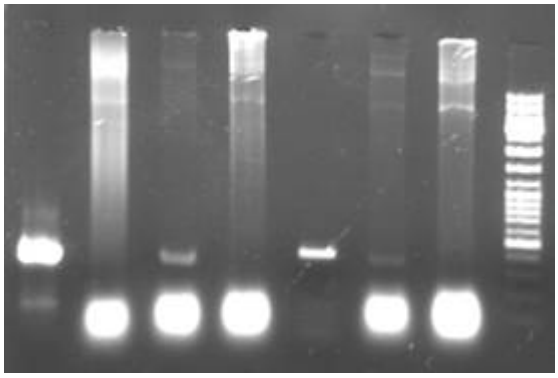

Fig3.

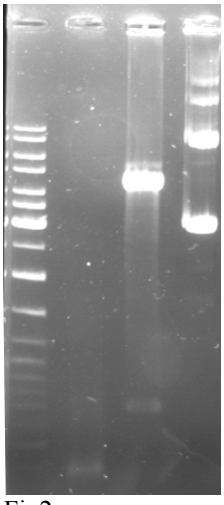

Fig2.