1371 IDENTIFICATION OF VASOACTIVE INTESTINAL PEPTIDE (VIP)-SPECIFIC SINGLE-CHAIN ANTIBODY FRAGMENTS (SCFVS) VIA YEAST SURFACE DISPLAY

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Background Vasoactive intestinal peptide (VIP) is a 28-amino acid neuropeptide expressed in various tissues including the pancreas, intestines and central nervous system.[1, 2] The over-expression of VIP and its receptors is associated with increased growth and metastasis of breast, prostate, and lung malignancies.[3] In addition, the interaction of VIP with its receptors on activated T cells results in immune suppression which further supports tumor growth.[4, 5] Furthermore, tumor-supporting regulatory T cells have been found to be promoted by VIP-dependent mechanisms.[6] Altogether, prior literature implies that blockade of VIP signaling may inhibit tumor-mediated immune suppression and augment antitumor immune responses. Recent preclinical studies in acute myeloid leukemia and T lymphoblastic leukemia demonstrated that VIP receptor antagonists increase T cell-dependent anti-tumor responses.[2]. Unfortunately, the short-half lives of peptide antagonists limit their clinical utility. A more translatable approach is the development of long circulating antibodies that bind VIP and inhibit its immunosuppressive activities.

Methods In this study, we utilized a yeast display of a non-immune human single-chain variable fragment (scFv) library to identify VIP-binding scFvs.[7-9] VIP binders were screened by several rounds of selection using magnetic-activated cell sorting (MACS) and fluorescence-activated cell sorting (FACS). The enriched binder population was cloned into single colonies of yeast cells by limited dilution. The binding affinities of VIP-binding clones were evaluated via flow cytometry by titrating fluorescence-labeled VIP. Clones with high binding affinity (Kd < 500 nM) were selected for sequencing (figures 1-5).

Results Sequences of the isolated scFv revealed that a unique section of complementarity-determining region 3 (CDR3) of the heavy chain played an important role in VIP binding. Multiple clones with similar but distinct CDR3 sequences produced a useful range of binding affinities for further development (figure 6).

Conclusions Yeast display is an effective technology for identifying human scFvs that bind to the immunosuppressive neuropeptide, VIP. CDR3 of scFv heavy chains were influential in VIP recognition. Ongoing studies are focused on the production, purification, and validation of novel anti-VIP human antibodies.

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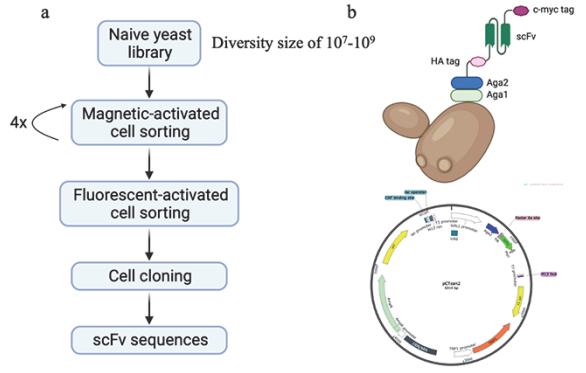
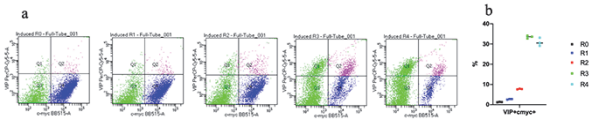


Abstract 1371 Figure 1 isolation process of anti-VIP scFv utilizing yeast display a) Workflow illustrating the process for isolating protein binders from large combinatorial libraries on yeast using magnetic bead separation followed by flow cytometry-based selection. (b) Illustration of protein scaffolds displayed on the surface of yeast and a related plasmid map.



Abstract 1371 Figure 2 Four enrichment rounds of VIP binders using MACS selection
(a) Dot plots show the binding events of VIP to yeast library analyzed by flow cytometry. VIP binders were selected by MACS in four rounds. Selections include a brief incubation of yeast library and VIP-magnetic beads, then binders were pulled out using a magnet. Selected yeasts were expanded and induced by specific culture media before analyzed by flow cytometry. Flow cytometry experiments were performed as staining each round of yeast library by anti-c-myc antibody-FITC (RMYC45FZ, Immunology Consultants), VIP-biotin (P001353, Aapptec), and Streptavidin PerCP-Cyanine5.5 Conjugate (45431782, Invitrogen). Stained cells were analyzed by BD FACSCelesta flow cytometer. (b) Dot plot shows representative binding percentages of VIP+c-myc+ events from 4 rounds of enrichment.

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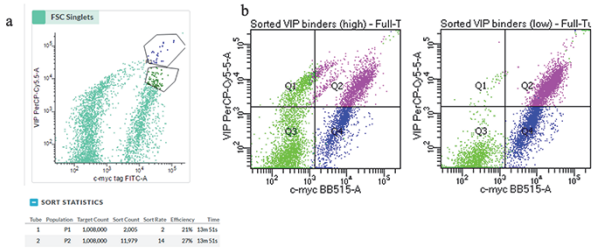
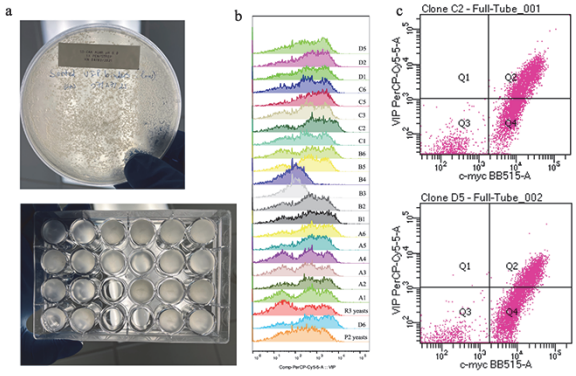


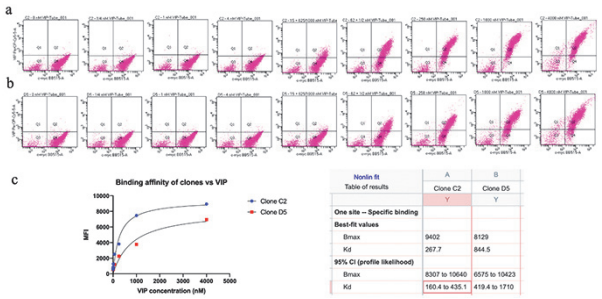
Abstract 1371 Figure 3 Isolation of VIP binders (round 3) by FACS sorting

(a) VIP binders after 3 rounds of selection (R3 yeasts) were subjected to a FACS sorting. Cells were stained with anti-c-myc antibody-FITC (RMYC45FZ, Immunology Consultants), VIP-biotin (P001353, Aapptec), and Streptavidin PerCP-Cyanine5.5 Conjugate (45431782, Invitrogen). FACS sorting was performed on a BD FACSMelody Cell Sorter. Two populations of VIP high signal and low signal were collected. Sorted yeasts were expanded and induced by specific culture media before analyzed by flow cytometry. (b). Comparison of VIP binding between VIP high signal (left) and low signal(right) populations. Flow cytometry experiments were performed as staining yeast cells by anti-c-myc antibody-FITC (RMYC45FZ, Immunology Consultants), VIP-biotin (P001353, Aapptec), and Streptavidin PerCP-Cyanine5.5 Conjugate (45431782, Invitrogen). Stained cells were analyzed by a BD FACSCelesta flow cytometer.



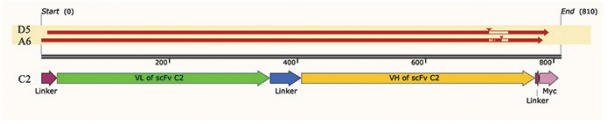
Abstract 1371 Figure 4 Cloning of VIP binders using limited dilutions

(a.top) Yeast cells from low signal population (P2 yeasts) were streaked onto an agar plate using limited dilutions. Single colonies were then picked and cultured in single wells of a 24 well plate (a.bottom). Cells were then expanded and induced by specific culture media. (b). VIP+ signals of different clones analyzed by flow cytometry. (c). Comparison of VIP binding between clone C2 (top) and clone D5 (bottom). Flow cytometry experiments were performed as staining yeast cells by anti-c-myc antibody-FITC (RMYC45FZ, Immunology Consultants), VIP-biotin (P001353, Aapptec), and Streptavidin PerCP-Cyanine5.5 Conjugate (45431782, Invitrogen). Stained cells were analyzed by a BD FACSCelesta flow cytometer.



Abstract 1371 Figure 5 Comparison of VIP-binding affinity between clones C2 vs D5

Cells of clone C2 (a) and D5 (b) were incubated with a gradient of VIP concentrations before stained and analyzed by flow cytometry. (c) Mean fluorescent intensity (MFI) of VIP+c-myc+ events were analyzed and used to calculate the Kd values of each clone by Prism 9.0. Flow cytometry experiments were performed as staining yeast cells by anti-c-myc antibody-FITC (RMYC45FZ, Immunology Consultants), VIP-biotin (P001353, Aapptec), and Streptavidin PerCP-Cyanine5.5 Conjugate (45431782, Invitrogen). Stained cells were analyzed by a BD FACSCelesta flow cytometer.



Abstract 1371 Figure 6 Comparison of DNA sequences of different VIP binder clones

DNA plasmids of 3 clones (C2, A6, and D5) were purified from yeast cells, then they were transformed into E. coli for expansion. Plasmids were purified from E. coli, subjected to Sanger sequencing, and analyzed by SnapGene. The DNA sequence of scFv clone C2 was used as a template to compare with clone D5 and A6. The isolated scFv sequences indicated that a specific region in the heavy chain's complementarity-determining region 3 (CDR3) played a key role in VIP binding.

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