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WARAPORN KASEKARN : IDENTIFICATION OF DISEASE SPECIFIC EPITOPES BY COMBINATORIAL PEPTIDE LIBRARIES. THESIS ADVISORS : WORACHART SIRAWARAPORN, Ph.D., YONGYUTH YUTHAVONG, D.Phil., PRAPON WILAIRAT, Ph.D. 116 p. ISBN 974-662-503-9

Phage display technology is a powerful method recently developed to screen selective repertories from large diversity of combinatorial peptide libraries displayed on the surface of filamentous bacteriophage. The screening of phage peptide libraries for specific ligands is performed based on the knowledge of specific antigen-antibody interaction. The present study aims at exploiting this technology for identification of specific epitopes. To demonstrate its potential power, monoclonal antibody (MAb) raised against a synthetic peptide of known sequence CYDVPDYASL previously shown to be an antigenic determinant of influenza virus hemagglutinin was used to screen a nanopeptide phage library. After three rounds of affinity purification process (biopanning), phages bearing peptide ligands recognized by mAb were selected and the DNA sequences corresponding to the peptide displayed were analyzed. Among 20 positive phage clones selected with mAb, all shared the common motif of DXPDYAS that have significant linear homology to the synthetic peptide.

A panel of monoclonal antibodies against dengue virus was investigated to identify their epitopes by using this technique. After two rounds of panning, five positive phages for each mAb were selected for analysis. The positive phages from mAb1B2 (group specific mAb) carried the sequences SAWGTAKET and SSWGRPRIE, corresponding to residue 924 to 928 of polyprotein and 118 to 122 of non-structural protein (NS-1) respectively in all dengue serotype. However, the selected phages from mAb1F1 (specific to dengue serotype 1) contained 5 different amino acid sequences. For mAb3H5 (specific to dengue serotype 2), all phages shared the sequence of KSHLSGQEW. The sera from dengue virus-infected patients with clear clinical diagnosis and normal healthy sera were also screened. The positive phages showed reactivity among patients' sera but did not show reactivity against normal sera after first and third round of biopanning. Four sequence motifs were found: RRPXF showed similar motif to amino acid residues 10 to 14 of C-protein in dengue virus 1; TTLXY partially resembled residues 915 to 920 of polyprotein in NS-1 of dengue virus 2 (NGC), residues 1630 to 1637 of polyprotein in NS-3 of dengue virus 1, and residues 3279 to 3290 of polyprotein in NS-5 of all dengue serotypes: LPL*MLRPT was similar to residue 496 to 501 of polyprotein or residues 216 to 221 of E-protein in all serotypes; and YHHNLMNQS resembled residues 91 to 95 of polyprotein or 77 to 81 of propeptide in C-protein in dengue virus 1. All sequence motifs were detected at high frequencies among sera from different patients. The studies demonstrated the potential applications of phage display technology for the identification of epitopes recognized by monoclonal and polyclonal antibody.