Identification of a Binding Motif of Epigallocatechin Gallate Using a Phage Display Random Peptide Library

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Epigallocatechin gallate (EGCG) is known as a component of green tea. It has been shown that EGCG has various activities including anticancer and antiviral activities. However, its cellular target molecules and mechanism of action still remain obscure. To identify the cellular targets of EGCG, I sought the binding motif of EGCG using a phage display random peptide library (PD-RPL). PD-RPL is widely used for mapping of epitopes of monoclonal and polyclonal antibodies, identifying of peptide ligands, and mapping substrate sites for proteases and kinases. Appropriate length of peptide with random sequence is expressed as a fusion protein with N-terminal end of pIII (minor coat protein) of M13 filamentous phage. Several rounds of panning of PD-RPL against target molecule result in increase of phage titer, that means the enrichment of target molecule binding peptide-expressing phages. Screening of phage clones with binding activity followed by the sequencing of the region coding for the random peptide can easily elucidate the binding motif of target molecule. Searching of database for proteins having the identical or similar sequence of binding motif may be helpful for identification of target molecules

In this study, EGCG derivative (kindly provided by Professor Toshiyuki Kan at the University of Shizuoka) was used as a target of heptapeptide PD-RPL. EGCG derivative-Affigel-10 (Bio-Rad) matrix was prepared by coupling of primary amine of EGCG derivative and N-hydroxysuccinimidyl ester of Affigel-10. Coupling was monitored by UV absorption derived from aromatic rings of EGCG. Decreased UV absorption after the completion of reaction implicates the successful conjugation of EGCG-derivative and Affigel-10. Four rounds of panning of heptapeptide PD-RPL against EGCG-Affigen-10 revealed that phage clones possessing Leucine-Proline or Isoleucine-Proline in the heptapeptide sequence were retrieved as a major population. This motif was corresponding to the partial sequence (A_{142}-A_{143} or A_{161}-A_{162}) of 67kDa laminine receptor that may constitute the putative EGCG binding site.

Keywords- Phage display random peptide library, Epigallocatechin gallate