Isolating Protein Kinase Inhibitors from a Phage-displayed Library of Conformationally Constrained Loop Peptides

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Protein Kianse plays a fundamental role in defining and maintaining signal transduction pathways, and the aberrant activity is linked to human diseases [1]. Thus, selective inhibition of protein kinases has the potential to provide useful reagents and therapeutic leads [2]. Despite intense interest in this area, the design of highly selective protein kianse inhibitors remains a challenge due to the highly structural similarity among kinases [3]. Peptide inhibitors have successfully isolated through site-directed molecular evolution experiments, but showed low affinity and low specificity [4].

Displaying peptides or proteins on filamentous phage is an in vitro selection technique that enables polypeptides with desired properties to be isolated from a large collection of variants [5]. As a novel class of phage-displayed peptide libraries, we have previously constructed a library of de novo designed helix-loop-helix peptides on major coat protein (p Ⅷ) of M13 filamentous phage. In this work, we have randomized the loop region of the helix-loop-helix peptides to construct a new loop library (AELAALEAELAALEGXXXX XXXXXG-KLAALKAKLAALKA: X is the randomized position). The loop library was screened against Aurora-A, a protein kinase overexpressed in a number of tumor cells, to obtain selective peptide inhibitors. Repeated selections resulted in an enrichment of phage particles capable of binding to Aurora-A. The phage ELISA experiments showed that a number of the displayed peptides bound to Aurora-A. We prepared the selected peptides by solid phase synthesis to examine binding affinity to Aurora-A. As a result, the peptides potentially inhibited the kinase activity of Aurora-A in the mid-micromolar range.

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