Application of Next-Generation Sequencing in Monitoring Ribosome Display Selection Against Doxorubicin

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Abstract

The selection of peptide ligands for small molecule drugs presents unique challenges due to their limited structural epitopes and the prevalence of non-specific interactions during screening. This study employs ribosome display coupled with next-generation sequencing (NGS) to investigate the enrichment trajectory of a random 50-amino acid peptide library against the small molecule chemotherapeutic agent doxorubicin. The library was subjected to five rounds of selection using doxorubicin-coated magnetic beads, with mRNA recovery and NGS analysis performed after each round to monitor sequence convergence and population dynamics. NGS data revealed progressive library enrichment across successive selection rounds, indicating effective selection pressure. The most highly enriched peptide candidates were synthesized and evaluated for binding affinity to doxorubicin using isothermal titration calorimetry (ITC). However, no detectable binding was observed under the tested conditions. These findings demonstrate that while NGS provides valuable insights into library evolution during ribosome display selections, sequence enrichment alone does not guarantee specific target binding, particularly for small molecule targets. The observed discrepancy between NGS data and biophysical validation underscores the importance of incorporating counter-selection strategies and orthogonal validation methods in screening workflows. This study contributes to the methodological understanding of peptide library screening against small molecules and offers practical considerations for optimizing future selection campaigns.

Keywords

Ribosome Display, Next-generation Sequencing, Peptide Library, Doxorubicin, Small Molecule