Innovative Simultaneous Aptamer Selection Strategy for ACLF-Associated Small Molecules

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Decompensation of liver cirrhosis and its further progression to ACLF causes 1.2 million yearly deaths. The deregulation of the intestinal microbiota triggers the development of cirrhosis, i.e., altering the gut microbiome composition would have an inherent effect on the concentration of several metabolites, including kynurenic acid (KA), quinolinic acid (QA) and phenylalanine (Phe), which have caught attention as potential biomarkers for diagnosis and prognosis [1]. A well-known in vitro selection method called Systematic Evolution of Ligands by EXponential enrichment (SELEX) can be used to isolate aptamers —single-stranded DNA (ssDNA) or RNA oligonucleotides with high specificity and affinity for their target molecules— which have emerged as a promising alternative to antibodies [2]. SELEX involves multiple selection rounds where a ssDNA library with up to 10^{60} random sequences interacts with target molecules. Aptamers bound to the target are isolated, amplified via PCR, and purified. Stringency increases with each round, gradually enriching the library for sequences with high target affinity and specificity. Ultimately, sequences with the strongest affinities dominate and can be sequenced to identify optimal aptamer candidates [3].

In our research, we conducted two parallel and independent SELEX approaches, including a novel Joint One-Pot method to simultaneously isolate aptamers for QA, KA, and Phe, thus expediting aptamer selection for multiple targets. We compared this novel approach with a conventional bead-based selection strategy, which focused solely on isolating aptamers for Phenylalanine (Phe). Our findings aim to validate the feasibility of our proposed method while highlighting the importance of various optimizations and considerations to enhance the likelihood of successful aptamer selection.

References

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