SELEX FOR APTAMER SELECTION AGAINST SMALL MOLECULES ASSOCIATED WITH ACUTE-ON-CHRONIC LIVER FAILURE: DESIGN, OPTIMIZATION, AND DEVELOPMENT

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Acute-on-chronic liver failure (ACLF) is a life-threatening syndrome that may present in patients with underlying chronic liver disease, characterised by poor patient outcomes and high short-term mortality (28 days), culminating in different grades of multi-organ failure[1]. To address the need for early diagnosis and accurate prognosis, the MICROB-PREDICT Consortium has identified three small molecules as potential biomarkers of ACLF: Quinolinic Acid (QA), Kynurenic Acid (KA), and Phenylalanine (Phe). Aptamers, single-stranded DNA or RNA oligonucleotides with high specificity and affinity for their target molecules, have emerged as a promising and viable alternative to antibodies for small molecule detection, and may be identified through a well-established in vitro selection technique known as Systematic Evolution of Ligands by EXponential enrichment (SELEX)[2,3]. It is an iterative process of several selection rounds, where a nucleic acid library of up to 10^{60} random sequences is exposed to the target molecules, and target-bound aptamers are isolated, amplified by PCR, and purified[2,3]. Stringency is increased after each selection round, leading to a progressive enrichment of the initial library towards sequences with high affinity and specificity towards the target. Ultimately, the sequences with stronger affinities will have dominated the pool and can be sequenced to identify optimal aptamer candidate.

Herein, we report the design and optimization of two parallel independent SELEX strategies, introducing a novel variation of the One-pot SELEX to simultaneously isolate aptamers against QA, KA, Phe, speeding up aptamer selection for multiple targets[4]. Our novel approach was compared with a well-established conventional bead-based selection strategy, which was carried out to isolate aptamers against only one of the targets, phenylalanine (Phe).

References

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