Generation of DNA aptamers against envelope 2 (E2) protein of Chikungunya virus by *in vitro* systematic evolution of ligands for exponential enrichment (SELEX) for diagnostic application

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Abstract

Introduction: Chikungunya virus (CHIKV) causes febrile illnesses in human and these cases have rapidly expanded across the globe in recent years. The current antibody-based tests for CHIKV such as ELISA have a variety of limitations associated with the molecules such as batch-to-batch variation, high cost and less stable. Aptamers are single-stranded DNA or RNA that have high affinity and specificity against a wide variety of target molecules. Compared to antibodies, aptamers are cheaper, produced *in vitro*, no batch-to-batch variations and thus serve as a good molecular recognition element for the development of diagnostic tests for CHIKV. **Methods:** Cloning, expression and purification of the recombinant CHIKV E2 was carried out and its identity was verified with western blot analysis. The purified protein was subjected to 9 SELEX cycles, the resulting nucleic acid pools were cloned and sent for sequencing. The secondary structure of the aptamer was predicted using Mfold web server and the performance of the aptamer was determined by enzyme-linked aptamer assay (ELAA). **Result:** The 24kDa recombinant E2 proteins were successfully cloned and purified. The protein was reactive against anti-CHIKV positive sera and anti-CHIKV polyclonal antibody with no cross reactivity with anti-dengue positive pool sera. Sequencing result revealed there were 6 potential candidates of DNA aptamers. DNA aptamer candidate with the highest frequency (61.9%) showed two loops in their predicted secondary structures. ELAA analysis revealed a binding affinity (Kd) of 177.5 nM and limit of detection was 3.3 nM. **Conclusion:** DNA aptamers were successfully generated and it has great potential as a feasible tool in CHIKV detection.

Keywords: Chikungunya virus, E2 protein, DNA aptamer, SELEX

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