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Generation of HBsAg DNA aptamer using modified cell based SELEX strategy

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Aptamers as potential alternatives for antibodies could be employed against hepatitis B surface antigen (HBsAg), the great hallmark and first serological marker in HBV, for further theragnostic applications. Therefore, isolation HBsAg specific aptamer was performed in this study with a modified Cell-SELEX method. HEK293T overexpressing HBsAg and HEK293Tas target and control cells respectively, were incubated with single-stranded rounds of DNA library during six SELEX and Counter SELEX rounds. Here, we introduced the new modified Cell-SELEX using deoxyribonuclease I digestion to separate single stranded DNA aptamers against the HBsAg. Characterization and evaluation of selected sequences were performed using flow cytometery analysis. The results led to isolation of 15 different ssDNA clones in six rounds of selection which were categorized to four clusters based on common structural motifs. The evaluation of SELEX progress showed growth in aptamer affinity with increasing in the cycle number. Taken together, the application of modified cell-SELEX demonstrated the isolation of HBsAg-specific ssDNA aptamers with proper affinity. Modified cell-SELEX as an efficient method can shorten the selection procedure and increase the success rate while the benefits of cell-based SELEX will be retained. Selected aptamers could be applied in purification columns, diagnostic kits, and drug delivery system against HBV-related liver cancer.

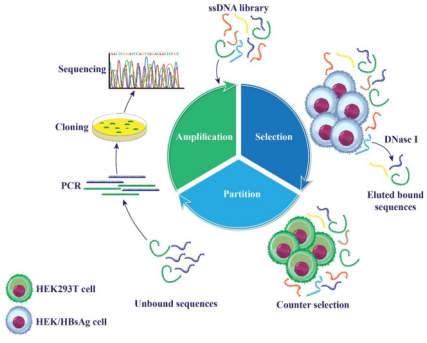

Figure 1 Schematic of modified Cell-based SELEX method for selection of HBsAg ssDNA aptamers

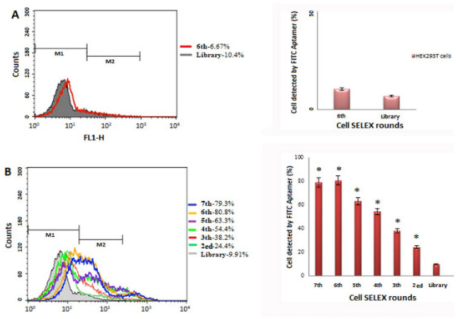

Figure 2 Affinity monitoring of the cell SELEX process to confirm enrichment of ssDNA aptamers using flow cytometery.

A) Flow cytometery results showed no affinity difference between 6th round selected aptamers and initial library on untransfected HEK293T cells (negative control)). **B)** Comparison of seven round selected aptamers cell-based with initial library on HEK293T/HBsAg cells indicated significant affinity progression of enriched aptamers (mean±SD, n =3),*significantly different from library p-value < 0.05).

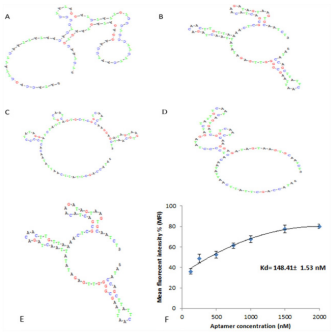

Figure 3 Secondary structure prediction of aptamers and Kd estimation of Cm-4 aptamer.

The secondary structure of individual aptamer was predicted with DNAMAN software which the representative aptamers of family 1–4 with lowest free Gibbs energy are shown as **A-D)**, respectively. **E)** The structure of Cm-4. **F)** Correlation of aptamer-ligand binding affinity and different concentrations of Cm-

Biography

Mina Mirian, assistant professor and experienced researcher with a demonstrated history of working in the hospital & health care industry and university. Skilled in design of molecular tools such as aptamer, CRISPR, MicroRNA and their evaluation in 2D and 3D Cell Culture, Flow Cytometry, ELISA, Real-Time Polymerase Chain Reaction (qPCR), Cyto and genotoxicity assay, Systems biology, Life Sciences, and Genetics. Strong research professional with a Doctor of Philosophy (Ph.D.) focused in Molecular Medicine from Isfahan University of Medical Sciences. University of Health Sciences, USA.