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Selection of high affinity DNA-aptamer for activated protein C using capillary electrophoresis

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Background and Aims: Our study is the first report about selecting an aptamer that can target Activated Protein C (APC) efficiently by use of Capillary Electrophoresis (CE). Aptamers are ssDNA or RNA oligonucleotide strands that can bind specifically to their targets. They typically selected from random sequence nucleic acid library termed Selection of Ligands by Exponential Enrichment (SELEX) which involved repetitive rounds of partitioning and amplification. Capillary electrophoresis (CE) as an alternative SELEX procedure (CE-SELEX) has tremendous advantages such as increased separation power, reduced non specific binding and reduced amount of selection rounds in comparison to conventional aptamer selection.
Methods: A random nucleic acid library was incubated with target protein. Then mixture was injected into a capillary and separated under high voltage. Specific fraction was collected at proper time in the selection buffer from the outlet of capillary. The bound sequences are then amplified, purified and made single strand for the next rounds of selection. Final products were subjected to radioactive labeling with 32P, measuring their binding affinity with filter binding assay and the best products were cloned and sequenced.
Results: DNA molecules that bind to target protein move through the capillary with different migration time in comparison to protein or random pool alone producing a collection window containing target specific ssDNA conjugated to APC. Due to high resolution of CE, selected aptamer had a dissociation constant in nanomolar.
Conclusions: Herewith, we have found a new aptamer for APC with nanomolar dissociation constant. Moreover, this is the first report of using one fraction collection instead of gathering whole collection window in CE-SELEX. We also announced a new single-strand production method without any strong reagent like NaOH that potentially can introduce interfering unwanted targets for the next run of SELEX.

Keywords: Aptamer; Activated protein C; Capillary electrophoresis; SELEX