MULTIPLEXED DETECTION OF CANCER BIOMARKERS USING AN OPTICAL BIOSENSOR

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Introduction. Early detection of cancer is important in administering timely treatment and increasing cancer survival rates. For early cancer detection one can use biomarkers, which are characteristics that can be objectively measured or evaluated as indicators of normal or pathogenic processes. In our study we study three protein biomarkers: carcinoembryonic antigen (CEA), interleukin-6 (IL-6) and extracellular protein kinase A (ECPKA), which have been implicated in various types of human cancer. The main objective of this project is to develop a biosensor for detection of multiple cancer biomarkers. To detect these protein biomarkers high affinity ssDNA aptamers are being selected. Aptamers are short single stranded DNAs with an ability to bind to various targets with high affinity and specificity which selected by SELEX (Systemic Evolution of Ligands through Exponential enrichment) [2]. Ultimately, aptamers against each of the biomarker will be conjugated to magnetic nanoparticles to capture biomarkers from biological fluids. Another aptamer is proposed to be conjugated to quantum dots for quantitation of biomarkers when analyzed on spectrometer.

Materials and methods. Aptamers against protein biomarkers were selected using SELEX: incubation of protein targets and DNA library consisting of central 40nt random region flanked by primer regions; elution of sequences that bind target; amplification of eluted sequences by PCR; preparation of ssDNA from PCR products. Biacore X100 surface plasmon resonance (SPR) instrument was used to analyze binding of sequences from round 12 and protein-target. Biotinylated sequences were immobilized on streptavidin coated chip and CEA used as an analyte in different concentrations (10, 20, 50, 50 and 100nM).

Results and discussion. We have conducted 12 rounds of SELEX for CEA protein and one for ECPKA. Analysis of PCR products from all rounds of SELEX showed a correct product size of 80bp. Preliminary SPR analysis showed binding of SELEX round 12 products with the target protein. Products of CEA SELEX are being sequenced using next-generation sequencing and are being analyzed using bioinformatics tools.

Conclusions. In total, twelve rounds of *in vitro* selection of aptamers against CEA were performed and products are being sequenced. Preliminary SPR analysis demonstrated positive results. For ECPKA protein one round of SELEX was performed.

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References.

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