Bio Summit & Molecular Biology 2016: Development of DNA aptamers against human heart type fatty acid binding protein for early detection of acute myocardial infarction - Pranab Goswami and Ankana Kakoti - Indian Institute of Technology Guwahati, India

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Abstract

Cardiovascular diseases are the single greatest cause of adult mortality globally, constituting about 31% of all global deaths. Detection of cardiovascular diseases has thus emerged as not only a social and clinical issue but also as an economic one. The current investigation is centered on the development of specific aptamers against human heart type fatty acid binding protein (FABP3), a novel early marker for detection of acute myocardial infarction (AMI). It also encompasses the detection of FABP3 using the developed aptamers on a specially designed paper based microfluidic device (μPAD). Two ssDNA aptamers, N13 and N53 were isolated through Systematic Evolution of Ligands by Exponential Enrichment (SELEX) against human heart-type fatty acid binding-protein (FABP3). The aptamers bound to FABP3 with dissociation constants 0.0743±0.0142 μM and 0.3337±0.1485 μM, respectively. The aptamers displayed stable behavior at different pH, temperature and ionic strength. Considering the large sizes of the aptamers, limited proteolysis of the aptamer-protein complex was performed to map the amino acids involved in binding, which was then used to screen docked structures.

The N13 led interaction with stronger affinity, involving more salt bridges and fewer hydrogen bonds, whereas N53 had less number of salt bridges with higher number of hydrogen and hydrophobic interactions. The greater footprint of N53 incited synergistic conformational changes in N53 and FABP3 leading to decrease in binding affinity during the recognition. The aptamers so developed and characterized were then used to detect FABP3 on a paper based microfluidic device designed for the same with leak proof property and low cost. An aptamer modified gold nanoparticle aggregation assay was used as the Yes/No format for the detection of FABP3 with a minimum detection limit of 54 ng per ml.

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