Selection of aptamers for fluorescent detection of alpha-methylacyl – CoA racemase by single-bead SELEX

Deng-Kai Yang, Lin-Chi Chen, Ming-Ying Lee, Chun-Hua Hsu, Chun-Shen Chen
Department of Bio-Industrial Mechatronics Engineering, National Taiwan University, Taipei 10617, Taiwan
Department of Agricultural Chemistry, National Taiwan University, Taipei 10617, Taiwan

Presenter: Cha-Hung Chi Date/Time: 2014/12/18 16:10~17:00
Commentator: Shainn-Wei Wang Location: Room 602, Med college Building

Background: Prostate cancer is one of the most frequently diagnosed cancers and is the first-leading cause of new cancer cases and the second-leading cause of cancer death in males in the United States in 2013. Alpha-methylacyl-CoA racemase (AMACR) has been proven to be highly expressed in prostate cancer cells and has become a novel protein biomarker for prostate cancer diagnosis. However, AMACR assay or other sensory tools are costly and with limited shelf-life. Since DNA aptamers are promising class of synthetic affinity ligands that are chemically stable and easy to generate with high affinity and specificity, they may be an alternative probes for biosensor development. For assay development using fluorescent AMACR detection, in vitro selection of AMACR-specific DNA aptamer by a new Systematic Evolution of Ligands by Exponential Enrichment (SELEX) protocol and ELAA (enzyme-linked aptamer assay) assessment of the affinity and specificity of selected anti-AMACR aptamers were carried out in this study.

Objectives: To develop a novel single-bead SELEX approach for selection of highly sensitive and specific anti-AMACR DNA aptamers.

Results: The aptamers were in vitro selected using a new single-bead SELEX approach, which was rapid and consumed only minute amounts of AMACR. Before SELEX, silane chemistry was used to prepare epoxide-functionalized glass microbeads for recombinant AMACR coating. During SELEX, the ligand evolution was assured by a differential real-time quantitative PCR assay. After SELEX, the aptamers were identified by the alignment analysis and 2nd structure prediction from the selected cloned sequences. The circular dichroism (CD) analysis revealed that the aptamers formed stable B-form with stem–loop conformation. The fluorescent ELAA method confirmed the affinity and high specificity of the aptamers against AMACR. Finally, an aptamer-based fluorescent AMACR assay was demonstrated.

Conclusion: a novel, microscope-independent single-bead SELEX protocol was
capable of tackling the aptamer selection against a precious protein biomarker, AMACR.

References: