Adaptive dynamic artificial poly-ligand targeting (ADAPT) enables plasma-based exosome profiling with potential diagnostic utility

Valery Domenyuk1, Zenyu Zhong1, Adam Stark1, Nianqing Xiao1, Heather O’Neill1, Jie Wang1, Xixi Wei1; Teresa Tinder1, Janet Duncan1, Andrew Hunter1, Mark R. Miglarese1, Joachim Schorr1, David Halbert1, John Quackenbush2, George Poste3, Günter Mayer1, Michael Famulok1,5, and David Spetzler1.

1 Caris Life Sciences, Phoenix, AZ, USA; 2 Department of Biostatistics and Computational Biology, Dana-Faber Cancer Institute and Department of Biostatistics, Harvard T. H. Chan School of Public Health, Boston, MA, USA; 3 Complex Adaptive Systems Initiative, Arizona State University, Scottsdale, AZ, USA; 4 LIMES Program Unit Chemical Biology & Medicinal Chemistry, University of Bonn, Germany; 5 Chemical Biology Max-Planck-Fellowship Group, Center of Advanced European Studies and Research (CAESAR), Bonn, Germany.

Introduction: Single stranded DNA (ssDNA) libraries consisting of several trillion oligodeoxynucleotides (ODNs) can adopt a nearly infinite number of three-dimensional structures. These structures can potentially bind any biomolecule and can be screened for specificity toward important biomarkers by employing suitable enrichment schemes. Since no prior knowledge on the binding partner is required, massively parallel biomarker identification is possible even on complex matrices like biological fluids and across a wide range of biological conditions. Here we present Adaptive Dynamic Artificial Poly-ligand Targeting (ADAPT) as a platform for biomarker and target discovery. We employed ADAPT for the molecular profiling of exosome-associated proteins in small volume plasma samples from women with breast cancer and healthy donors.

Enrichment of aptamer library for ADAPT

ADAPT workflow

Blood plasma collection

Enriched library library screening

Individual patients profiling analysis

Distribution of normalized counts of aptamers recovered from ADAPT enriched library L3 and L000 on technical replicates from the same sample (blue dots). Averaged counts from 3 replicates of two non-related samples (red dots). (B) Random Forest (RF) Out-of-Bag (OOB) ROC AUC from 323 clinical samples and permutation analysis of its reliability; the ROC AUC in the original dataset is 0.73, which is significantly higher compared to the majority of 1000 permutations (p=0.001). (C) ELONA analysis of C1Q-binding by the ssODNs H11 at indicated C1Q concentrations. H11RC, the reverse complement of H11, is used as control. H11RC as control shows low binding and no aptamer binding of 30 representative individual aptamers by qPCR.

Figure 2. ADAPT characterization and evaluation on 323 clinical samples. (A) Distribution of normalized counts of aptamers recovered from ADAPT enriched library L3 and L000 on technical replicates from the same sample (blue dots). Averaged counts from 3 replicates of two non-related samples (red dots). (B) Random Forest (RF) Out-of-Bag (OOB) ROC AUC from 323 clinical samples and permutation analysis of its reliability; the ROC AUC in the original dataset is 0.73, which is significantly higher compared to the majority of 1000 permutations (p=0.001). (C) ELONA analysis of C1Q-binding by the ssODNs H11 at indicated C1Q concentrations (0.625 nM). H11RC as control shows background of detector Streptavidin-HRP. H11 specifically binds C1Q (KD = 44.4 nM). (D) Filter retention analysis of C1Q-binding by the ssODNs H11 at indicated C1Q concentrations.

Figure 3. Oligonucleotides identified by ADAPT reveal aptamer-like characteristics. (A) Flow-cytometry measurement of exosome binding event with enriched library L3 and the starting library L0 or streptavidin / phycoerythrin (SA-PE). (B) Post-ADAPT quantification of binding of 30 representative individual aptamers by qPCR. The group of high copy number (H) ssODNs generally has higher recovery than the group of low copy number (L) from NGS data. (C) Cross-reactive-reducing SDS-PAGE gel of pulled down proteins from plasma with the indicated ssODNs (H1, H4, L4, L10) and the control without ssODN (NO). C1QA, C1QB, C1QC/IgMLC (IgM light chain) and the control (SA-PE) were incorporated into standard clinical practice.

Summary:

We have demonstrated the feasibility of aptamer library enrichment directly on blood plasma and have identified a set of 2000 aptamers that distinguish plasma from women with breast cancer from women without breast cancer.

This liquid biopsy approach requires only 200 microliters of plasma and is amenable to high-throughput processing.

By employing a number of statistical approaches including rigorous cross-validation, we consistently achieve ROC AUC values >0.6.

ADAPT-derived breast cancer test may serve as a vital diagnostic adjunct that can be easily incorporated into standard clinical practice.

This presentation is the intellectual property of Caris Life Sciences. Contact e-mail: vdomenyuk@carisls.com for permission to reprint and/or distribute.