CP10*: Ultrasensitive platform for monitoring response to breast cancer therapy featuring hypermethylated DNA detection: Saraswati Sukumar, Johns Hopkins School of Medicine; Funding Source and Period: BC141315, 2015/04/01-2018/03/30, DOD/CDMRP
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Significance: The Sukumar laboratory has performed seminal studies to unravel the roles of members of the HOX gene family in breast cancer progression [1-4] and pioneered the application of intraductally administered therapeutic agents to treat preneoplastic ductal lesions [5-7]. Her lab’s discovery of novel methylated genes through first ever SAGE analysis of breast cancer followed by the development of multiplexed quantitative PCR assays set the foundation for monitoring tumor burden based on aberrantly methylated genes [8-13]. In the clinical management of breast cancer patients, hypermethylation in circulating cell-free DNA is of great interest in various contexts: first, when primary tumor or metastatic tissue samples are not available; second, for surveillance of asymptomatic cancer survivors; third, for evaluating response to systemic therapies in the neoadjuvant, adjuvant and metastatic settings; and finally, as potential diagnostic surrogates. Hence, methods that can quantitate multiple methylated gene markers at exceedingly low concentrations from small volumes with easily achievable sample processing requirements are highly desirable.

Approach: Because the levels of DNA shed are so low, especially in patients with no symptoms of metastatic disease, we now propose to develop an ultrasensitive and multiplexed platform featuring surface-enhanced Raman spectroscopy (SERS) and highly selective single base extension reaction [14]. Using DNA methylation analysis, the Sukumar lab has characterized a 10-gene panel composed of seven novel and three known breast cancer hypermethylated markers (Fig. 1). The panel displayed >90% sensitivity and 100% specificity when tested in sera of metastatic breast cancer patients [15]. A decrease in median serum DNA methylation levels was observed in the selected genes in patients with stable disease or a therapeutic response but not in patients with progressive disease. Leveraging this panel, the LBRC proposes to develop a SERS assay by extending their recently established breast tumor antigen detection method [16]. The benchmarks for the proposed assay development will be the sensitivity, specificity and reproducibility offered by the cMethDNA assay [15]. Working with the Sukumar lab, we will use the sera already acquired at different time points from the metastatic breast cancer patients undergoing chemotherapy to demonstrate the feasibility of our assay in monitoring response to therapy.

Push-Pull Relationship: To enable ultrasensitive and multiplexed detection of methylated markers, LBRC pushes rationally tailored plasmonic nanoprobe and a new method for sensing of gene-specific (rather than genome-wide) methylation. The Sukumar lab is also embarking on analyzing the prognostic and predictive utility of cMethDNA, CA 27.29 antigen and CTC, using prospectively collected serum. This effort pulls the LBRC to develop an integrated SERS-based platform for detecting circulating tumor antigens and methylated markers. The Sukumar lab is also investigating the sensitivity of microRNAs (miRNA) in revealing tumor-specific alterations thereby pulling the LBRC to design and engineer SERS nanoprobe constructs targeted for miRNA detection.

![Figure 1. Assessment of 10-gene methylated marker panel in sera acquired from recurrent metastatic stage IV breast cancer patients. A) Box plot showing that cancer sera display significantly higher median cumulative methylation than normal sera. B) Plot of cumulative methylation index (CMI) values for individual samples, where each colored segment represents the methylation index for an individual gene. C) Frequency of methylation for individual biomarkers in the 10-gene panel. Scatter plot depicts gene methylation intensity (y-axis, methylation index) for individual genes (x-axis) in the test set for normal and cancer sera. The Mann–Whitney P values are shown below each plot.](image-url)
Literature Cited: