GenPro and Helen F. Graham Cancer Center Present A Novel Epigenetic Biomarker Discovery Approach to a Potential Prognostic Marker for Triple Negative Breast Cancer at NAACR Conference in Washington DC on April 1-4, 2017

The work will be presented in a poster session at the NAACR Conference in Washington DC on Tuesday, April 4, 2017 from 8:00AM - Noon in the poster area in Halls A-C of the Convention Center. It is Section 15, Poster #30, Abstract #3369 which is part of the Epigenetics 3 Section of the Molecular and Cellular Biology & Genetics Session. The project is titled, "Epigenetic DNA Methylation Profiling of Triple Negative Breast Cancer: A Quantitative NGS Approach".

The lead author on the study who will be presenting it is Dr. Kimberly Arnold from Department of Medical Sciences at the University of Delaware and the Center of Transnational Cancer Research at the Helen F. Graham Cancer Center in Newark, Delaware. The poster, which describes the under-served need for a prognostic marker in triple negative breast cancer, the approach used by GenPro in discovering the novel epigenetic marker (EpiMarker), new molecular insights into genes and pathways that were illuminated by GenPro, and plans to verify and translate the new EpiMaker into a fast, low-cost, high-throughput qPCR assay are discussed. The poster can be viewed at: NAACR 2017 Poster

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Abstract #3369

Epigenetic DNA methylation profiling of triple negative breast cancer: A quantitative NGS approach

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Introduction

Triple negative breast cancer (TNBC) is an aggressive disease with a high degree of genomic instability. TNBC patients have poor prognosis and the risk of metastasis and death is increased for women who relapse within four years of treatment compared to other breast cancer subtypes. Currently, there are no reliable prognostic markers to identify which population is at risk for early relapse and the molecular mechanism for disease recurrence is not well understood. Epigenetic changes, especially DNA methylation, are common in breast cancer and have been found to be associated with increased metastatic capability. Therefore, the methylation states of genes in TNBC tumors compared to non-tumor breast tissue was examined in order to identify potential biomarkers and therapeutic targets in TNBC. Millions of CpG sites exist within the human genome and many of these are altered with tumor formation and progression. Therefore, changes in methylation profiles of CpG sites may be useful as a diagnostic and/or prognostic biomarker in TNBC patients.

Patient Cohort & Methods

•Matched tumor and adjacent non-tumor tissue from four patients with TNBC were obtained following surgical resection

•Epigenetic profiling of TNBC tumors and non-tumor tissue was performed using a highly sensitive and quantitative analytics platform, which utilizes methylation sensitive restriction endonucleases to detect changes in methylation of CpG sites

•Validation studies were performed on tissue isolated from 36 TNBC patients

Figure 1: Overview of the process of genomic DNA extraction and determination of methylation patterns among the cohorts is described

Epigenetic discrimination of tissue type

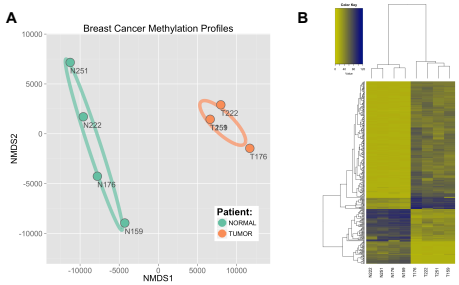

Figure 2: (A) Tumor discrimination derived from the most significant quantitative CpG methylation state changes. An integrated analysis across all CpG sites within the genome was performed using an ordinate analysis technique of non-metric multidimensional scaling (NMDS). CpG methylation profiles were compared among individuals to isolate patterns conserved within tissue type while also differing between tumor and non-tumor tissues. NMDS of the CpG sites revealed highly distinct methylation patterns between tumor and non-tumor tissue. Ellipses drawn represent 95% confidence intervals around the position of the true group means. Bootstrap analysis estimates indicate that the probability of the observed separation arising by random chance is $p < 0.0001$. **(B) Differential methylation profiles in adjacent non-tumor and tumor tissues.** Dendogram heatmap of CpG sites showing significant differences between tumor and non-tumor samples. The resolution between tumor and normal samples revealed quantitative separation between distinct subgroups of CpG sites. Approximately 326 sites had a significant methylation score difference ($p<0.0025$, after false discovery rate correction) between tumor and non-tumor tissue, with most of the CpG sites having greater than 2-fold change in methylation status.

Summation of differential methylation load across genes within defined pathways

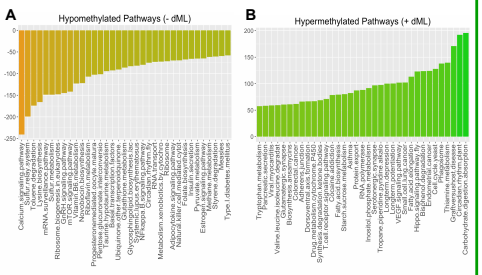

Figure 3: Summation and normalization of differential methylation levels among KEGG pathways as presented as a rank ordered histogram (one bar = one pathway). The distribution is sigmoidal with sharp exponential tails at each end. The load score is a difference of non-tumor minus tumor tissue, where positive values reflect higher methylation within pathways in non-tumor tissue and a negative value reflects higher methylation within pathways in tumor tissue. **(A)** Lowest negative pathway scores in distribution represent the pathways where tumors are hypermethylated. **(B)** Highest positive pathway scores in distribution represent pathways where non-tumor are hypermethylated (tumors are hypomethylated).

Summation of differential methylation load across CpG sites within genes

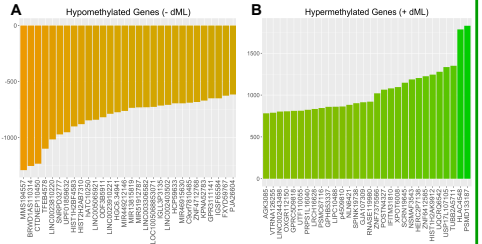

Figure 4: Summation and normalization of differential methylation levels among all genes as presented as a rank ordered histogram (one bar = one gene). The distribution is sigmoidal with sharp exponential tails at each end. The load score is a difference of non-tumor minus tumor tissue, where positive values reflect higher methylation within genes in non-tumor tissue and a negative value reflects higher methylation within genes in tumor tissue. **(A)** Lowest negative gene scores in distribution represent genes that have lower methylation scores in non-tumor tissue relative to tumor tissue (genes are hypermethylated in tumors). **(B)** Highest positive gene scores in distribution represent genes that have higher methylation scores in non-tumor tissue relative to tumor tissue (genes are hypomethylated in tumors).

Differential methylation load between tumor and adjacent non-tumor tissue and linked CpG sites within the genome

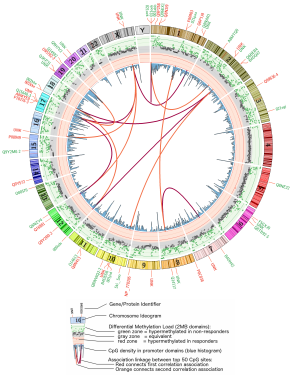

Figure 5: Integrated epigenome profile presenting the choromosomal locations of the top 10 discriminating CpG sites between tumor and adjacent non-tumor tissue. In addition, the highest correlation in among CpG sites, the domain methylation load between non-tumor and tumor tissues, the genes containing the top 40 CpG sites, and the CpG density in gene promoter domains are also represented. A mean subtraction of CpG methylation scores (non-tumor minus tumor) was used to calculate a summation methylation load score across genomic domains. These data are presented as a scatterplot with gray and green background to accentuate areas where they are most different. Blue histogram bars represent CpG densities in gene promoter domains. The correlative association between top 10 CpG sites are shown as red arcs that track the first highest correlation for each CpG site while orange arcs show the second highest. Gene labels indicate loci positions of the top 40 discriminating CpG sites, with red indicating higher methylation in tumor tissue and green indicating higher methylation in adjacent non-tumor tissue. This integrated plot helps to pin-point associations not evident in the massive data tables to suggest associations, correlations and parallel function and response mechanisms to explore further.

Expression of Gli in triple negative breast cancer patients correlates with overall and recurrence-free survival

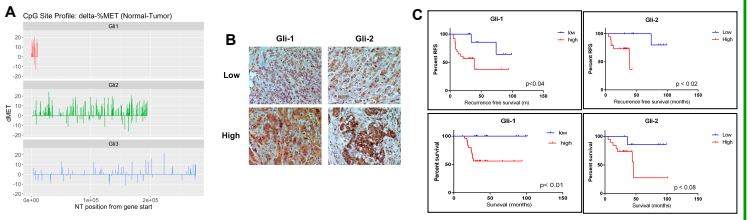

Figure 6: Gli was found to be one of the top hypomethylated genes in tumor tissue. **(A)** CpG site profiles for Gli-1, Gli-2, and Gli-3 gene. A positive result is indicative of the CpG site being more methylated in non-tumor tissue where as a negative result is indicative of the CpG site being more methylated in tumor tissue. The height is the difference in methylation between non-tumor and tumor tissue. **(B)** Immunohistochemical staining revealed differences in Gli-1 and Gli-2 expression in 36 TNBC tumors. **(C)** Expression levels of Gli-1 and Gli-2 in TNBC tumors correlated with poor recurrence free ($p<0.04$, $p<0.02$, respectively) and overall survival ($p<0.01$, $p<0.08$, respectively).

Summary

•Analysis of CpG methylated sites in TNBC tumors and adjacent non-tumor tissue revealed differences in epigenetic profiles allowing for distinction between tumor and non-tumor tissue

•The resolution between tumor and non-tumor tissue reveals quantitative separation among distinct subgroups of CpG sites that could be utilized in developing a targeted assay for clinical discrimination

•Gli was one of the top hypomethylated genes in tumor tissue. Overexpression of Gli in TNBC tumors is correlated with poor recurrence free and overall survival