Global quantitative measures using next-generation sequencing for breast cancer presence
outperform individual tumor markers in plasma

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1 Cancer Care Associates, TMPN, Redondo Beach, CA, Genomic Health, Inc., Redwood City, CA.

Introduction & Objectives

Next-generation technologies have many advantages and will improve our understanding of tumor biology and disease management for patients. Many groups have successfully sequenced a small portion of the human genome, using a wide variety of technologies, including next-generation sequencing (NGS). We performed a series of studies at a small number of patients to examine the value of individual tumor markers, both in the absence and presence of a tumor-derived cell free DNA (cfDNA) in plasma. Three classes of tumor-derived markers were studied: single nucleotide variants (SNV), copy number alteration (CNA) and differentially methylated regions (DMR).

Methods

Whole Genome Biallelic Sequencing

Samples from two patients, including primary tumor (grafted into grafted mice, TCGA) and normal blood (grafted into normal mice, TCGA) were sequenced. SNVs were identified in both primary and normal tissues, and found to be present in the plasma of both patients. Whole genome Methylations were sequenced and sequenced on a Illumina (Bisulfite sequencing with ~7 million reads per sample) of DNA methylated of coverage. Data were analyzed to identify copy number and methylation status.

Single-Nucleotide Variants Sequencing

Breast tissues were prepared from the DNA isolated from four patient samples using direct and primary tumor samples. A single nucleotide sequencing technology was used to generate DNA samples. DNA sequencing was performed on the Illumina (Bisulfite sequencing with ~7 million reads per sample) of DNA methylated of coverage. Data were analyzed to identify copy number and methylation status.

Methylation-Specific Quantitative PCR

DNA samples (FETP and blood) were collected from patients at various disease stages. DNA was isolated from samples by proteinase K digestion followed by purification with the QIAamp DNA Blood Mini Kit. DNA methylation analysis was performed by methylation specific quantitative PCR analysis. DNA was isolated from both primary tumor and control samples to identify methylation status.

Target Enriched Bisulfite Sequencing

Tissue samples were collected from patients at various disease stages. DNA was isolated from samples by proteinase K digestion followed by purification with the QIAamp DNA Blood Mini Kit. DNA methylation analysis was performed by target enriched bisulfite sequencing. DNA was isolated from both primary tumor and control samples to identify methylation status.

Enriched Methylation

(cytosine enrichment analysis)

An estimated copy number for all regions of interest was derived from the methylation enrichment analysis.

Differentially methylated regions were identified by comparing methylation status in 1965 loci between tumor and blood. The patient’s plasma samples were subsequently analyzed for methylation status at sites identified by the TCGA Research Network.

Differential Methylated Regions (NGS – WGBS)

The tumor status of individual and/or patients was analyzed in different classes of methylation status.

Enriched Methylation (NGS)

Hybridization Capture

Buffy coat DNA was sequenced by Next Generation Sequencing from 13 patients to evaluate whether the DNA methylation status differed between patients.

Targeted Methylation (PC-MS)

Representative methylation-specific PCR results were shown in top 3 patients. PCR was used to evaluate DNA methylation status at specific regions.

References


WGBS Results Summary:

- WGBS data can be used to detect both CNA and methylation-derived tumor signal in cfDNA
- Two different approaches were used to identify these sites: (1) analysis of the copy number signal, and (2) analysis of the methylation signal
- These methods give consistent, but not identical, estimates for tumor DNA in plasma
- Visualization of the CNA results indicates that some CNA are only observed in the plasma sample

Methyl-Specific PCR Results Summary:

- Methyl-PCR assays identify differential methylation in all tumor normal pairs
- This is the first study to evaluate a panel of tumor markers for detection of tumor tissue in metastatic disease
- Further, a more sensitive assay was not required for early stage disease

Conclusions

- Reduced detection of tumor in plasma from patients with active disease requires sensitivity, digital detection methods, such as NGS
- Liquid biopsy is an effective tool for treatment selection, copy number and tumor markers may be more appropriate for detection and monitoring of tumor-derived DNA.
- Copy number and methylation-based tumor estimates provide similar, but not identical, estimates of tumor burden using a variety of DNA fraction.
- Profiling many tumor specific markers in both the primary tumor and cfDNA may be useful for staging, predicting response and/or survival.
- Much more work is required to determine what relationships exist between tumor fraction estimates and clinical pathology measures.

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