Application of Whole Genome Microarrays in Cancer: You should be doing this test!!

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Disclosures

› Clinical Laboratory Director and Employee, Medical University of South Carolina
› No financial or conflicts of interest to disclose

Evolution of Clinical Genomic Assessment

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<th>Year</th>
<th>1956</th>
<th>1972</th>
<th>2001</th>
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<td>46</td>
<td>600</td>
<td>1,000,000</td>
<td>3,000,000,000</td>
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Tools for Genomics Studies

Cellular level – context of single cell abnormality

- **Karyotype**
  - Evaluation of entire genome
  - Limited resolution (5–10Mb)
  - Highly subjective
- **FISH (Fluorescence In Situ Hybridization)**
  - Probe-specific areas
  - Higher resolution
  - Higher objectivity

Molecular level – result is based upon all cells in sample

- **Genomic Microarrays**
- **DNA sequencing**

Current Best Way to Study Copy Number
High Resolution Genomic Microarrays

- **Whole Genome Microarray**
  - DNA
  - Deletions, duplications, amplifications, loss of heterozygosity (LOH)
  - Two kinds
    - Array Comparative Genomic Hybridization (CGH)
    - Single Nucleotide Polymorphism (SNP) arrays

Basic Microarray Technologies

- Detection of copy number changes using array technology
- array CGH or SNP microarray
- Allows for detection of small copy number changes (deletions/duplications)
Why is Copy Number So Important?

- Copy number variants comprise at least 3X total number SNPs
  - On average, 2 human differ by 4 – 24 Mb of DNA by CNV; 2.5 Mb due to SNP
- Often encompass genes
- Important role in human disease and in drug response

Main Classes of Cancer Genes

- Diagnosis: Genes contribute to cellular transformation/classification
- Prognosis: Genes -> progression
- Therapy: Rational approach to drug development for targeted therapies
- Characterization of inherited variation that contributes to cancer susceptibility

Cancer Genetics

Importance of finding copy number changes
Genetic Complexity

- Simple
  Chronic Myeloid Leukemia

- Complex
  Most solid tumors

Understanding the Data: Constitutional Microarray
100% of cells abnormal in a child with multiple congenital abnormalities

Whole Genome View of Microarray:
Cancer clones not 100% of cells

- Normal
  - Loss skews line down
  - Gain skews line up

- Chromosomes 1 2 22, X, Y

- Genotype track
  - Chronic lymphocytic leukemia
  - Oligodendroglioma
SNP Microarray in Cancer: Detect copy number and LOH

Loss of heterozygosity
80% of cells

Loss
40% of cells

How can we use microarrays in clinical cancer studies?

› Diagnosis
  – Renal cell carcinoma
  – Glioblastoma
  – Fatty tumors

› Prognosis/Disease monitor
  – Chronic lymphocytic leukemia
  – Acute myeloid leukemia/MDS
  – Plasma cell dysplasias
  – Renal cell carcinoma
  – Glioblastoma

› Therapy
  – Acute lymphoblastic leukemia
  – Acute myeloid leukemia

Hematologic Malignancy
MUSC First Cancer Microarray Test
Chronic Lymphocytic Leukemia: Perfect target

- Genetic lesions = losses or gains
- Copy number variation stratifies cases
- Fresh samples readily available
- Tumor burden determined by Flow cytometry

Correlation of FISH and Microarray – Overall 98%

<table>
<thead>
<tr>
<th></th>
<th>Chrom 4</th>
<th>Chrom 12</th>
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<th>6q</th>
<th>P53</th>
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<td>Abn</td>
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<td>Abn</td>
<td>Abn</td>
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<tr>
<td>Nor</td>
<td>13</td>
<td>17</td>
<td>7</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Vec</td>
<td>0</td>
<td>16</td>
<td>17</td>
<td>6</td>
<td>3</td>
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</table>

Overall correlation
25/25 (100%) 25/25 (100%) 25/25 (100%) 25/25 (100%) 25/25 (100%)

Microarray replaced FISH for clinical study.

SNP Microarray: Clonal Diversity
Clinical Significance: Clonal Diversity

- Findings of study
  - 12/25 cases exhibited clonal diversity
  - 77 genetic aberrations (52 not covered by FISH)
  - 3X more likely to be CD38+
  - 10 cases were stage III/IV
  - Clonal diversity correlated with need for therapy

![Graph showing clonal diversity](image)

Clinical implementation of microarray

- Results of pilot project shared with clinical team; microarray replaced FISH in 2014
  - Cheaper than FISH panel
  - NOT faster; test is batched and run once per week
  - Better
    - Whole genome coverage; other aberrations associated with prognosis
    - Follow clone over time; at time of Richter transformation, can determine if original clone progressed (bad prognosis) or new diffuse large B-cell clone (better prognosis)
  - Microarray performed at diagnosis, disease progression, change in therapy

Acute Myeloid Leukemia Prognosis

- Standard Cytogenetic Testing
  - 25% Good prognosis: balanced rearrangements [t(8;21), t(15;17), inv(16)]
  - 25% Intermediate prognosis: -8 (10%), NORMAL cytogenetics (40%)
  - 50% Unfavorable prognosis: deletions 5q, 7q, 17p, KMT2A (MLL) rearrangement, complex karyotypes (>4 abn)

- Microarray Analysis
  - Provides exact breakpoints for known cytogenetic aberrations
  - Reveals cryptic abnormalities
  - Copy number neutral loss of heterozygosity (15-20% of cases of normal cytogenetics cases; like LOH for 7q)
    - LOH regions often harbors genes with homozygous mutations

- MUSC algorithm
  - Chromosome analysis, rapid FISH for t(15;17), microarray and myeloid gene mutation panel
  - Challenges integration of reports/data

<table>
<thead>
<tr>
<th>Region of LOH</th>
<th>Associated Gene</th>
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<tr>
<td>5p</td>
<td>NRAS</td>
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<tr>
<td>4q</td>
<td>TET2</td>
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<tr>
<td>7q</td>
<td>EZH2</td>
</tr>
<tr>
<td>9p</td>
<td>JAK2, CDKN2A, PAX5</td>
</tr>
<tr>
<td>11p</td>
<td>MYC, PAI1</td>
</tr>
<tr>
<td>11q</td>
<td>CBL</td>
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<tr>
<td>13q</td>
<td>FLT3</td>
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<tr>
<td>17p</td>
<td>TP53</td>
</tr>
<tr>
<td>18q</td>
<td>CEBPA</td>
</tr>
<tr>
<td>21q</td>
<td>RUNX1</td>
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Among FLT3-ITD patients, 13qLOH associated with poor prognosis

Min Fang, personal communication; Cancer. 2015 Sep 1;121(17):2900-8.

Acute Lymphoblastic Leukemia

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Frequency</th>
<th>Clinical significance</th>
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<tr>
<td>Hyperdiploid (&gt;50 chroms)</td>
<td></td>
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<tr>
<td>Hypodiploid (&lt;44 chroms)</td>
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<td></td>
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<tr>
<td>t(1;19)/ TCF3-PBX1</td>
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<td></td>
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<tr>
<td>ERG deletion</td>
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<tr>
<td>iAMP21</td>
<td></td>
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<tr>
<td>CRLF2 rearrangement</td>
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<tr>
<td>BAKOS deletion</td>
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<tr>
<td>PAX5 deletion</td>
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Genetic Abnormalities of known prognostic significance in B-ALL
Approximately 40% of ALL patients had abnormalities of genes involved in the B-cell development and differentiation pathway, including: PAX5, TCF3, EBF1, LEF1, IKZF1 and IKZF3. Other genes frequently affected were those controlling cell cycle progression including: CDKN2A, CDKN1B, and RB1.

Implication for therapy: 23 year old male with Philadelphia-like B-cell Acute Lymphoblastic Leukemia; Cytogenetics and FISH testing negative.
Molecular Mechanism

EBF1 -PDGFRB

Activates a tryrosine kinase that can be targeted by imatinib

Solid Tumors

Renal Cell Neoplasms

Challenging Cases
- Eosinophilic variants
- Mixed architecture
- Small biopsies

84 MUSC cases
- 2 diagnoses changed
  - ccRCC -> chromophobe
  - papRCC -> oncocytoma
- 6 cases did not have typical microarray result; could be another entity
<table>
<thead>
<tr>
<th>Type Renal Tumor</th>
<th>%</th>
<th>Chromosome Abnormality</th>
<th>Microarray Result</th>
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<tbody>
<tr>
<td>Clear Cell Renal Cell Carcinoma</td>
<td>~70</td>
<td>Loss of 3p</td>
<td><img src="image" alt="result" /></td>
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<tr>
<td>Papillary Renal Cell Carcinoma</td>
<td>15-20</td>
<td>Extra copies of 7 and 17</td>
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<tr>
<td>Chromophobe Renal Cell Carcinoma</td>
<td>5</td>
<td>Loss of chromosomes 1, 2, 6, 10, 15, 17, 21</td>
<td><img src="image" alt="result" /></td>
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<tr>
<td>Oncocytoma</td>
<td>&lt;5</td>
<td>Normal or loss of 1p</td>
<td><img src="image" alt="result" /></td>
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Other useful information: prognosis

- Clear Cell RCC with 3p-
  - Better prognosis
- Clear Cell RCC with 3p- Plus other genetic abnormalities
  - Worse prognosis

Aberrations Associated with Adverse Prognosis Clear Cell Renal Cell Carcinoma

- Loss
- Gain
- Low risk
- High risk
Other Challenges that Microarray Analysis Provides Clinical Information For

New primary or met?
- 84 years old male with history of poorly differentiated lung squamous cell carcinoma
- Follow up studies at 3 months detected a gastric polyp with features of poorly differentiated carcinoma on histology; tumor was negative for sarcoma, melanoma, angiosarcoma, and leiomyosarcoma markers per immunohistochemistry
- Microarray data for lung mass (A) and gastric polyp (B) tissue samples. Green arrows indicate abnormalities shared between the specimens, red arrows – abnormalities unique to each specimen.

SNP-CMA diagnosis: Metastatic Lung Cancer

Same tumor or what?
- 32 years old female with posterior mediastinal tumor with two areas of distinct morphology
- Block E: bland histology with foci of schwannoma morphology
- Block F: histology consistent with MPNST (malignant peripheral nerve sheath tumor); high grade
- Axial T1-post contrast MRI, thoracic spine: heterogeneously enhancing paraspinal mass (F) with extension into the spinal canal (E).
- Microarray data for block E (B) and F (C) tissue samples. Green arrows indicate abnormalities shared between the specimens, red arrows – abnormalities unique for transformed tumor, purple arrow indicates deletion involving NF2 locus specific for schwannoma.

SNP-CMA Diagnosis: Schwannoma with malignant transformation
Low or high grade tumor?

Case history:
- 69yo female with right thigh medial tumor
- Histology: in differential diagnosis liposarcoma with low grade dedifferentiation and solitary fibrous tumor
- SNP-CMA: Extremely complex genotype with focal amplifications of MDM2 and CDK4

SNP-CMA Diagnosis:
Dedifferentiated Liposarcoma

Sample identity

DNA Microarrays

Advantages over conventional chromosome analysis:
- Identify exact location of chromosome and exact gene content
- Quantitative and objective results
- Evaluates whole genome - high resolution
- Potential to define new syndromes
- Does not require living cells
- Tissue and archival samples can be analyzed

Limitations:
- Cannot detect:
  - Balanced chromosomal rearrangements (still need karyotyping for this)
  - Point mutations in DNA (need PCR or DNA sequencing)
  - Gains or losses in the region of genome, not covered by array (may need whole genome sequencing)
Future of Biomarker for Precision Medicine

Genome Sequencing

- Sequence every gene/whole genome
  - Newborn screening
  - Tumor vs. normal assessment
- Promise of technology
  - Liquid biopsy
- Limitations/Barriers
  - Cost
  - Integration into system
  - Education
  - Evidence base
  - FDA/other regulation
  - Genomics-enabled clinical information systems
  - Data storage
  - Availability of testing

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  - In particular, the microarray group
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