Delineating Genomic Alterations in Cancer Using a Novel CGH+SNP Array

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1960:
“The acquired genetic instability and associated selection process, most readily recognized cytogenetically, results in advanced human malignancies being highly individual karyotypically and biologically. Hence, each patient's cancer may require individual specific therapy”
Cancer-Associated Genomic Aberrations

- Cancer diagnosis/prognosis
- Disease classification
- Risk stratification
- Treatment selection
Methods of Detecting Cancer-Associated Genomic Aberrations

<table>
<thead>
<tr>
<th></th>
<th>Cytogenetics</th>
<th>Microarray</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resolution</td>
<td>10 – 20 Mb</td>
<td>1 – 10 Kb</td>
</tr>
<tr>
<td>Sample type</td>
<td>Live sample</td>
<td>PB, BM, FNB, FF, FFPE, etc.</td>
</tr>
<tr>
<td>Aberrations</td>
<td>Del/Dup, Translocation</td>
<td>Define the origin, size, and content of Del/Dup/Amp</td>
</tr>
<tr>
<td>UPD/LOH</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Cytogenetics:

4-6 DM

High-level focal amplification on Chr1q32.1; Copy number 4, 5, 6
Methods of Detecting Cancer-Associated Genomic Aberrations

Chromosome Microarray Analysis

CGH-based arrays       SNP-based arrays
## DNA Microarrays

<table>
<thead>
<tr>
<th></th>
<th>aCGH</th>
<th>SNP-based arrays</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reference</strong></td>
<td>Intra-experimental</td>
<td>Ex-experimental</td>
</tr>
<tr>
<td><strong>Probes</strong></td>
<td>BAC/PAC, oligos</td>
<td>oligos</td>
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<tr>
<td><strong>SNP genotyping</strong></td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>SNP desert coverage</strong></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><strong>UPD detection</strong></td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

A platform that offers both copy number and genotype information.
Probe Coverage of Different CCMC Designs

- 4x44K, 2x105K, 8x60K, 4x180K platforms
- Same coverage for targeted regions
- Different coverage for backbone regions
- CCMCv2: 4x180K with 60K SNPs
BCM 400K CGH+SNP Cancer Array

- 2,300 cancer genes or cancer-related genes
- 235 cancer associated-miRNAs
- Average of 6 probes per exon.
- Average resolutions <1 Kb (large exons) to <10 Kb (cancer genomic regions) in targeted regions, and
- ~12 Kb in backbone regions.
Mechanism of CGH+SNP Arrays

- Signal measures copies of uncut allele
- Raw SNP data: black curve
- Colored Gaussians: fits to distributions

AluI cuts: AGCT
Cytogenetically Normal AML
Cytogenetically Normal AML
CN-AML: Deletion of 5q
The fragmentation of a chromosome and its subsequent highly imperfect reassembly
Genomic Aberrations in Solid Tumors

MYCN amplification in neuroblastoma in which no metaphases could be obtained
Origin of DM in Hepatoblastoma

High-level focal amplification on Chr1q32.1; Copy number 4, 5, 6

4 – 6 copies
Identification of Small Copy Number Alterations (CNAs)

FISH: nuc ish(EGR1x1)[96/100], (ETV6, AML1)x2 [67/100], (ETV6x3, AML1x2)[30/100], (ETV6x5, AML1x2) [3/100], (CBFBx1)[94/100], (D20S108x2)[100]

Multiple clonal abnormalities

12p13.2 mosaic dup. 0.79 Mb
Unbalanced “Balance Translocation”

47,XXX,t(3;12;3)(p21.3;p13.3;q26.2),-7,+mar.arr r(7)(p11.2q31)
SNP Probes Help CNV Calls and Provide Important Allelic Information

CLL Sample
2 deletions on p-arm of Chr8
Amplification to 4 copies on q-arm
SNP shows copy number of 0, 2, 4
SNP Probes Help CNV Calls and Provide Important Allelic Information

Amplification to 4 copies on 6p

SNPs that were homozygous show 0 or 4 copies;
SNPs that were heterozygous show 1 or 3 copies
Clinical Significance of CNV + SNP Array

Childhood ALL:
High hyperdiploid ALL - Good prognosis
Hypodiploid ALL – Poor prognosis
Duplication of hypodiploid ALL – Poor prognosis
Clinical Significance of CNV + SNP Array

Loss of a copy of chr. 19 followed by duplicate the remaining copy
Clinical Significance of using CNV + SNP Array

Duplication of two homologous chr.18
Clinical Significance of CNV + SNP Array
Normal Disomy Chromosome 5
Trisomy Chromosome 6
Tetrasomy Chromosome 10
CGH+SNP Array on FFPE Samples

Glioblastoma Sample

Whole Genome View
CGH+SNP Array on FFPE Samples

glioblastoma

Chromosome 1, Two copies

Chromosome 3, del/dup/amp

Deletion

amp ~8 copies

Dup 3-4 copies
CGH+SNP Array on FFPE Samples

glioblastoma

Chromosome 7, del/amp

Chromosome 3, del
Detection of Low-level Mosaic Aberrations

Chromosome 1 Gain in Breast Cancer
Skin Metastatic Ca of Unknown Origin

- Metastatic cancer of unknown origin
- Two pieces of core skin biopsy received
- 6 ml blood sample received
CGH + SNP Array on Solid Tumor
Paired Tumor/Normal Samples
CGH + SNP Array: amp. of chr. 1q
CGH + SNP Array: gain of chr. 5
CGH + SNP Array: del chr. 8q

~20.5 Mb
CGH + SNP Array: 16p+ and 16q-
CGH + SNP Array: dup chr. 2q

dup2q36.1-36.3; ~3.5 Mb
CGH + SNP Array: del of chr. 3p

del3p12; ~1.45 Mb; CADM2
CGH + SNP Array: Germline Deletion

del8p22;
~70 Kb;
MSR1:
Germline CNV
– Prostate cancer
## NGS MUTATION PANEL

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Reference</th>
<th>Variant</th>
<th>VarFreq</th>
<th>cDNA change</th>
<th>Amino Acid change</th>
<th>Exon</th>
<th>Confirmed</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIK3CA</td>
<td>A</td>
<td>T</td>
<td>12.08</td>
<td>c.3140A&gt;T</td>
<td>p.H1047L</td>
<td>20</td>
<td>Low level mutant peak by Sanger sequencing</td>
</tr>
<tr>
<td>PDGFRA</td>
<td>C</td>
<td>T</td>
<td>49.74</td>
<td>c.2472C&gt;T</td>
<td>p.V824V</td>
<td>18</td>
<td>dbSNP rs2228230 synonymous - in both tumor and blood - germline polymorphism</td>
</tr>
<tr>
<td>KIT</td>
<td>A</td>
<td>C</td>
<td>51.25</td>
<td>c.1621A&gt;C</td>
<td>p.M541L</td>
<td>10</td>
<td>db SNP rs3822214 - in both tumor and blood - germline polymorphism</td>
</tr>
</tbody>
</table>
PIK3CA c.3140A>T(p.H1047L)
Sanger Sequencing

3140A>T

Tumor

3140A

Blood
Significance of Genomic Profiling

- Genomic CNV profile suggested breast cancer
- Genomic CNV profile associated with good prognosis
- NGS identified a PIK3CA mutation
- Diagnostic and therapeutic significances
Summary – Cancer CMA

• Confirm, clarify, and further characterize cytogenetic and FISH results
• Identify many submicroscopic genomic aberrations
• CGH+SNP arrays advantages:
  • LOH
  • Low-level mosaicism
  • Important allelic information
  • Ploidy status
Acknowledgements

http://www.bcm.edu/cancergeneticslab/
Thank You! Questions?