Breast Cancer Heterogeneity and Metastases

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Disclosures

• We receive unrestricted educational grants from Pfizer, Novartis and Takeda
Defining the term “heterogeneity”

• INTER tumoural heterogeneity; variation between patients in the composition and behaviour of breast cancers. I will discuss this in the afternoon session

• INTRA tumoural heterogeneity; clonal variation within a single patient’s tumour – one patient, potentially several diseases. This will be the subject of my talk this morning.
CLONAL EVOLUTION AND CANCER
(colours represent cancer cells bearing distinct mutations)

- **Early tumour**
  - Stable clonal mix, Little/no selection

- **Mid-stage tumour**
  - Selection of some number of common pre-existing clones

- **Late stage tumour**
  - Dominance of a rare initial clone
  - De novo mutation

Some possible outcomes
What are the clinical/biological implications of intra tumoural heterogeneity?

• If different tumour cell populations exist bearing different somatic aberrations, potentially the different populations will behave differently in response to therapy.

• Current indications for targeted therapy do not generally explicitly account for somatic heterogeneity (HER2 is a recent exception). Current clinically used biomarkers do not generally report or take account of somatic mutational heterogeneity.

• Most drug development is done against cell line models or sometimes xenografts with incomplete knowledge of the genomic background and with no idea of the extent of mutational heterogeneity.
HER2 clonal heterogeneity, noted in between 5-30% of primary cancers


Primary->metastasis, divergence in IHC

Over 30 papers examining the rate of divergence in IHC status between primary and mets. Most show between 10 and 20% divergence but the quality/size of studies is limited. Strong level 1 evidence not available yet. Eg:

• Receptor conversion in 233 metastases (Breast Cancer Research 2010, 12:R75); rates between 5.2 to 32.6%, PR- to PR+ most prevalent; +ve -> -ve >> -ve -> +ve

• BRITS study (prospective in 209 subjects, Thompson et al. Breast Cancer Research 2010, 12:R92), altered receptor status in mets resulted in a management change in 17% of patients
Metastases – to re-biopsy or not?


- **UK - NICE guidelines (CG81, 2009; [http://guidance.nice.org.uk/CG81/NICEGuidance/pdf/English](http://guidance.nice.org.uk/CG81/NICEGuidance/pdf/English)** advise against biopsy and retesting of ER and HER2 recurrent disease
Next generation – whole genome sequencing of primary and metastatic tumours, recent insights
Somatic and germline variation in the genome/epigenome underpins tumour development – however it occurs at every scale.

Eg. BRCA2, p53, BRAF, etc

**Single base error/mutation**

...ATTTGATCCGGG...

\[ \downarrow \]

...ATTTGAACCGGG...

Until very recently it has been impractical to comprehensively establish genome variation at all scales.
Principles of next-gen sequencing

1. Flowcell

- anchor single DNA molecules to solid surface
- copy each molecule in situ by PCR to amplify template

2. Repeat cycle ~50-100 times

- universal adapter
- 3' - CATAAAGCGTGTC...
- 5' - universal primer
- template addition of chain terminator
- add 4 colour-labelled reversible terminators, polymerase, universal primer
- remove unincorporated nucleotides
- detect with laser

3. Reverse termination (chemically or enzymatically)

4. Repeat cycle 1..100 times

~2.5 billion sequence reads at 100bp x 2

~500 Gb of sequence or 172x haploid coverage or 5.5 genomes

Aparicio and Huntsman 2010 J. Pathol

10 days
Measuring the frequency of alleles in a heterogeneous DNA population

Mixture of cells containing different mutations

Library construction and sequencing separates single DNA templates

Reference sequence

ATGCCGCG
ATGCCGCG
ATGCCGCG
ATGCCGCG
ATGCCGCG
ATGCCGCG
ATGCCGCG
ATGCCGCG
ATGCGCG
ATGCGCG
ATGCGCG
ATGCGCG
ATGCGCG
ATGCGCG
ATGCGCG
ATGCGCG
ATGCGCG

align each sequence

Heterozygous germline SNV
present in every cell -> expected frequency 50%

Heterozygous somatic SNV
present in every malignant cell -> expected frequency 50% x cellularity
eg in 80% cellular tumour a frequency of 0.5x0.8 = 0.4

Sub-dominant somatic SNV
present in only a proportion of malignant cells -> less than 0.5 x cellularity

Count of nucleotides at each position gives the frequency of the alleles in the sequenced population of DNA

Count at surrounding positions gives hypothesised mean for binomial exact test

Aparicio, Huntsman; J.Pathol. 2010
Detecting clonal evolution in a breast cancer – How many diseases in one patient?

Malignant cells +/- mutations

Pre-malignant state

Primary

Metastasis

Lymphocytes
Supporting normal cells
Mutations

Method: Measure the somatic allele frequency in diploid or copy number invariant regions of the genome.
Genome sequence of an estrogen sensitive lobular breast cancer

HOW DID THE PRIMARY AND METASTASIS GENOMES DIFFER?

9 YEAR INTERVAL

primary

SURGERY, RADIOTHERAPY
TAMOXIFEN. NO CHEMO

EXMESTANE

metastasis

E-CAD
LOW / NEGATIVE

Shah et al, Nature 2009
EXAMPLE - TWO RANDOM GERMLINE SNVs
Predicted frequency ~ 0.5 (50%)

<table>
<thead>
<tr>
<th>Position</th>
<th>RATIO in primary tumour</th>
<th>RATIO in metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>chr14:22142501</td>
<td>0.48</td>
<td>0.48</td>
</tr>
<tr>
<td>chr12:54801169</td>
<td>0.34</td>
<td>0.48</td>
</tr>
</tbody>
</table>

Shah et al, Nature 2009
Subclonality revealed over a 9 years by somatic mutation allele frequency

<table>
<thead>
<tr>
<th>MUTATION CLASS</th>
<th>PRIMARY</th>
<th>METASTASIS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>“dominant”</strong> $f &gt; 0.2$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MUTATIONS PRESENT IN EVERY</td>
<td>chr19:9314428</td>
<td>0.51</td>
</tr>
<tr>
<td>CELL OF PRIMARY AND MET</td>
<td>chr2:169497197</td>
<td>0.33</td>
</tr>
<tr>
<td><strong>“sub-dominant”</strong> $f &lt;0.2, &gt; 0$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MUTATIONS PRESENT ONLY IN</td>
<td>chr11:113185109</td>
<td>0.01</td>
</tr>
<tr>
<td>A SUBSET OF PRIMARY</td>
<td>chr17:10248420</td>
<td>0.14</td>
</tr>
<tr>
<td>Undetectable in the primary.</td>
<td>chr1:44650831</td>
<td>0.00</td>
</tr>
<tr>
<td>Origin unknown</td>
<td>chr12:52063157</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Shah et al, Nature 2009
SOMATIC CODING MUTATION FREQUENCY
EVOLUTION OVER 9 YEARS

5 somatic mutations were present at dominant frequencies in the primary: PALB2, HAUS3, MORC1, ABCG11, SNX4

6 somatic mutations were present at low frequencies (1-14%) i.e. SUBDOMINANT

The rest (19 of 32) somatic coding non-synonymous mutations, were not detectable in the primary tumour cell population
Basal TNBC may have stronger clonal dominance than non-basal TNBC.
Mutational evolution underpinning the progression of breast cancers

Ding et al, Nature 2010 (Wash U) – basal (triple negative) breast cancer primary and brain metastasis 8 months later, compared with a xenograft of the primary tumour. Sequence of a trio – primary tumour, metastatic tumour and xenografted tumour.

Evolution of somatic allele frequency suggests that tumour xenografting selects for a mutational landscape more similar to the metastatic tumour than the primary.
Future – single cell genomes

Single cell/nucleus analysis is needed to understand which mutations co-occur in the same clone. This will be relevant in the context of defining the biology of individual clones. (eg Navin et al, Nature 2011)

Sequencing libraries and mutation analysis on hundreds of single nuclei in parallel

Collaborators, Carl Hansen, Marco Marra
Current questions

• What is the relationship between the genomes of clones and the tumour initiating cells identified by xenotransplantation assays?
• Are circulating tumour cells multi-clonal or oligoclonal?
• What is the relationship between clonal complexity and outcome?
• Will knowledge of clone genomes help with targeting?
Summary points

• Intra-tumoural heterogeneity is common among breast cancers and leads to “multiple diseases in one patient”.

• One manifestation - divergence between primary and mets in phenotype - is well established but guidelines for re-biopsy are still non uniform.

• Next generation sequencing is providing important insights into the evolution of breast cancer and may provide actionable information in the near future.