(Intra-Tumoral) Genomic Heterogeneity of Breast Cancer

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No Financial Disclosures
**Intra-Tumoral Heterogeneity (ITH)**

“The coexistence, within an individual cancer, of multiple subclonal populations of cancer cells that differ in their genomic, epigenomic, transcriptional, morphological or behavioral features”\(^1\)

[1] The European Society for Medical Oncology (ESMO) Precision Medicine Glossary, Annals of Oncology, 2017
Approaches for Studying Intra-Tumoral Heterogeneity (ITH)

Next Generation Sequencing properties suited to studying ITH

- **Quantitative** (1 read = 1 molecule of DNA)
  - Fine resolution
  - Highly specific

Outline

- Key clinical questions that studies of genomic intra-tumoral heterogeneity (ITH) are attempting to address:

  1. Can we confirm or refute the clonal relationship between tumors in the same patient?

  2. Can we identify the subclonal origins of aggressive disease in the primary tumor?

  3. What is the difference between a primary tumor and subsequent relapse?
Every Cancer Genome is Unique

- Each breast cancer contains hundreds to thousands of somatic mutations
- The complement of genomic mutations is specific to a given cancer
- All cells from any given cancer are **clonally related** and as such share a large number of somatic mutations

Contralateral tumors vs Distant metastasis?

- **Contralateral tumors** are more common amongst BRCA1/2 carriers, ILC and younger individuals and are routinely treated as new primary tumors.

- Two recent studies used NGS and showed across a total of 35 individuals 10-12% of contralateral breast cancers are distant metastases\(^1,2\)

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Relapse vs Second Primary Tumor

- 13 year history of ‘relapsing’ disease
- At least 3 different grade 3, triple negative primary breast cancers during her lifetime
- Would this knowledge alter your treatment recommendation?
Question 1. Can we confirm or refute the clonal relationship between tumors in the same patient?

• YES we can!

Important on an individual patient basis and for interpreting a wide range of clinical trials outcomes - 3.2% - 1.8%
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Spatially Distinct Sub-clonal Driver Mutations are Common & Diverse

- 12 patients’ primary tumors at primary surgery
- Genomic sequencing of 98 samples
- Tumor size = 1.3-4 cm
- Local clonal expansions in 10/12 cases and contained driver alterations in 4/12 cases: **PIK3CA, BRCA2, TP53** mutations
  **MYC** amplification

Alternative Driver Alterations in Heterogeneously Amplified HER2+ Cancers

Heterogeneous HER2 amplification in around 6% of cancers, has been associated with:

- **Worse survival**
- **Poor response** to trastuzumab (metastatic setting)

- Analysis of HER2 negative and positive compartments of 12 heterogeneously HER2+ tumors using aCGH and NGS approaches

- HER2 negative compartments contain some known drivers but none recurrent

- **Evidence that alternative alterations may compensate for lack of HER2 amplification** (eg HER2 p.I767M mutation & over expression / amplification of BRF2 and DSN1)

Ng et al. *Genome Biol*. 2015
Therapy Unmasks Resistant Subclones

- 22 cases, sampled pre and post 4 months of AI
- Substantial remodeling of the cancer genome
- In one case an aggressive ER negative tumor was effectively unmasked after the application of endocrine therapy that resulted in a good response in the initially sampled ER positive tumor.

Subclonal Origins of Aggressive Clinical Features

- Multi-region whole genome sequencing of 10 primary breast cancers sampled across clinically important timepoints
- Phylogenetic tree reconstruction

Molecular time

Chemotherapy Resistant Subclones
Subclones Seeding Metastasis
Invasive Subclones Arising from DCIS

Question 2: Can we identify the subclonal origins of aggressive disease in the primary tumor?

• YES, but many questions remain unanswered...

• We do not know if there is anything genomically special about the subclone of origin or if it ‘won’ by chance

• We do not know if treatment resistant subclones reflect subsequent metastases more closely

• There are likely to be important none genomic features of ‘successful’ subclones
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Paired Primary and Metastasis Samples Define Patterns of Genomic Evolution

Aim of the study
To understand patterns of cancer progression in 3 scenarios
1. Distant Metastasis
2. Loco-regional
3. Synchronous axillary

Method
For 17 patients we performed whole genome sequencing of samples from:
– Primary tumor
– Germline
– Metastasis/ relapse deposit form one or more of the above 3 scenarios

We used previously described methods to identify the major classes of mutations: substitutions, indels & structural variants
Evolution in the Primary Tumor

- Each colour change reflects the emergence of a new genomically distinct subclone.
- MRCA = Most recent common ancestor (the most recent cell from which all cancer cells in this cancer are directly descended).
- A cancer is a dynamic entity.

Key question: Where in primary tumor evolution does the metastasis seeding cell leave?

LR Yates et al. Cancer Cell, 2017
The Metastatic Clone Diverges Late in Primary Tumor Evolution

1. Dissemination at 87% in primary tumor molecular time on average
2. Similar for all relapse/metastasis types
1. Distant metastases and local relapses have changed significantly by the time that they are diagnosed
   • With, on average 63% (range = 24-244%) additional mutations compared to the primary tumor
2. Synchronous lymph nodes are very similar to the primary tumor

LR Yates et al. Cancer Cell, 2017
The Driver Landscape Across Metastasis

• Within a given cancer most cancer genes are usually clonal
• But, in distant metastasis samples we usually find 1 or 2 additional cancer genes
• What to ‘action’ and when is not yet clear

LR Yates et al. *Cancer Cell*, 2017
Selective Pressures Re-Shape the Metastatic Landscape

- Patient with an activating PIK3CA mutation treated with a PI(3)Ka inhibitor BYL719

  After clinical response disease progressed and after death the patient had an autopsy (14 metastatic regions sampled)

- Convergent evolution with homozygous loss of PTEN in all progressive lesions

Summary

1. We can use genomics to confirm the clonal relationship between tumor deposits
   • New primary breast cancer vs distant metastasis or local relapse
   • Distant metastasis vs second primary tumor

2. The subclones responsible for the aggressive features of a cancer can often be identified in a primary tumor
   • But these are genomically diverse
   • Treatment resistant subclones MAY be informative BUT whether these reflect the subclones that are destined/liable to seed relapse is unclear

3. Relapsed cancers contain most of the mutations seen in the primary tumor but typically a substantial burden of additional mutations
   • Whether metastasis specific driver mutations or those shared with the primary tumor are more important is still unclear
Acknowledgements and Thanks!

The Cancer Genome Project