Genomic Characterization of Cancer-Adjacent Breast: Evidence of field effects and expression subtypes

Melissa Troester, PhD, MPH
What Predicts Breast Cancer Recurrence?

- Recurrence rates are higher for breast conserving therapy.
- Local recurrence commonly occurs in the lumpectomy bed.
- Local recurrence rates are higher among basal-like breast cancers.

Field carcinogenic events

- Slaughter *et al.* (1953) observed abnormal tissue surrounding oral squamous cell carcinoma
  - Field cancerization explains the development of multiple primaries and local recurrences.
How does cancer-adjacent tissue respond to tumor?

- Response to wounding
- Stress response
- Immune response
- Angiogenesis
- Extracellular matrix
- Chemotaxis

Troester et al. (2009) Clin Cancer Res
Double Normal Breast Committee

Chair: Melissa Troester, UNC

RNA and DNA from 40 triplets:

- Blood <- normal breast -> tumor

40+ tumor-normal pairs

- normal -> tumor

Exome Seq
D. Koboldt, L. Ding, WashU

Copy Number Alterations
A. Cherniack, Broad

Methylation
H. Shen, S. Mahurkar, P. Laird, USC

microRNASeq
G. Robertson, BCCA

RNAseq, microarray
M. Troester, K. Hoadley, M. D’Arcy, UNC
Q1. Detectable field effects?
Q2. Detectable tumor cells?

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Tumor-like copy number alterations

Chromosome 8

Chromosome 17

Focal peak in chromosome 10 is also seen in normal.

Courtesy of Andy Cherniack
Tumor-like copy number alterations

7% with ‘field effect’ OR tumor contamination
10 cases (25%) had strong evidence of field effect (many mutations with VAF >=2% in the adjacent normal).

- 1,760 mutations from 41 cases
- $R^2=0.288$
Tumor-like mutations

7% with ‘field effect’ OR tumor contamination
25% with ‘field effect’ OR tumor contamination
21 Tumors

HM27

21 Adjacent Normals

PathAveEpi (Epi): 0 - 15

PathAveStr (Str): 0 - 100

Expression signature (Exp)

Active

Inactive

1000 probes with highest positive and negative tumor-normal differences

Courtesy of Swapna Mahurkar
1000 probes with highest positive and negative tumor-normal differences

Expression signature (Exp)

PathAveStroma (Str): 0 - 100
PathAveEpi (Epi): 0 - 15

Courtesy of Swapna Mahurkar
Tumor-like methylation patterns

7% with ‘field effect’ OR tumor contamination
25% with ‘field effect’ OR tumor contamination
7-10% with ‘field effect’ OR tumor contamination
Q1. Detectable field effects?

Q2. Detectable tumor cells?
DNA data types: Comparison & Validation

A ‘positive control’ – all three DNA platforms detected the sample with tumor contamination
Histopathologic Assessment

Melissa Troester, UNC
Rupninder Sandhu, UNC
Andy Beck, Harvard
Nicole Johnson, Harvard
Kim Allison, U of Wash

SCORING:
Pathology (tumor, benign)
Immune infiltrations
Percent Composition:
e.g. 30% Stroma
63% Adipose
7% Epithelium
Methylation Reflecting Composition

• Epithelial Content on HM450 platform (qvalue<0.05).
  – 13000 probes were positively correlated
  – 12500 probes were negatively correlated

• Stromal Content on HM450 platform (qvalue<0.05):
  – 5700 probes were positively correlated
  – 2300 probes were negatively correlated

• Correlation composition and DNA methylation on 27k was weak. This needs further investigation.
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Q1. Detectable field effects?
   Normal vs. blood
   Normal vs. tumor

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Q2. Detectable tumor cells?

Q3. Other heterogeneity?

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Two Subtypes of Cancer-Adjacent Tissue

Active

Inactive

increased
- cell movement
- inflammation
- fibrosis
- chemotaxis

decreased
- cell adhesion
- differentiation
- cell-cell contact

Roman-Perez et al. (2012) Breast Cancer Res
Cancer-Adjacent Subtype vs. Tumor Subtype

Active microenvironment occurs in all tumor subtypes

Roman-Perez et al. (2012) *Breast Cancer Res*
Active Microenvironment Predicts Survival

**Graphs:**

- **ER positive**
  - Active (orange) vs. Inactive (gray)
  - Hazard Ratio (HR): 2.5, p = 0.062

- **Hormone Treated**
  - Active (orange) vs. Inactive (gray)
  - Hazard Ratio (HR): 2.6, p = 0.045

*Roman-Perez et al. (2012) Breast Cancer Res*
mRNA and microRNA subtypes

- RNA expression clusters
  - Two main clusters by microRNA-seq
  - Two main clusters by RNA-seq

- RNA and miRNA concordance

- Tumor characteristics (ER status, intrinsic subtype, etc.) not strongly associated with main clusters

- ‘Probable contamination’ samples not readily detected.

Courtesy of Gordon Robertson
RNA Expression Subtype vs. Composition

![Box plots showing the comparison of RNA expression subtypes for different tissue compositions.](image-url)
Conclusions & Future Directions

• DNA shows field effects/tumor contamination
  RNA identifies expression subtypes

• Distinguishing field effects vs. tumor cells
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