Validation of a Formalin-Fixed Paraffin Embedded NanoString Assay for Breast Cancer
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Introduction
- Validated breast cancer risk assessment tools do not incorporate molecular factors.
- Goal: Evaluate a 76-gene, microarray-based clinical assay (Malignancy Risk signature, MR) on a custom NanoString platform using formalin-fixed paraffin-embedded (FFPE) tissue.
- MR signature was developed from Affymetrix microarray in fresh frozen (FF) tissue and previously validated to distinguish malignant from benign FF breast and lung lesions.

Aim: Validate the use of the MR NanoString in FFPE breast tissue compared to fresh frozen (FF).

Methods
- Single-institution, IRB-approved, retrospective review of electronic medical records (EMR) and tissue/data banks for female breast cancer patients.
- Included cases:
  - Archived historical, clinical and pathological information
  - Fresh frozen benign breast and malignant tumor specimens available
  - FFPE benign breast and malignant tumor specimens available
  - Automated RNA extraction performed
  - Qubit quantification
  - Agilent TapeStation quality screening
  - Custom NanoString nCounter CodeSet for the MR signature and 18 housekeeping genes
  - Hybridizations performed in randomized FF/FFPE groups
  - NanoString cartridges scanned at 555 FOV

Statistical analysis:
- Background correction by mean+2SD of negative controls
- Normalization by geometric mean of housekeeping genes (18)
- ANOVA between endogenous and housekeeping genes
- Pearson correlation analysis of the 76 gene expression of MR in FFPE and FF cases for validation of NanoString platform

Results
- 137 NanoString Samples Processed
  - 28 cases had all 4 tissue specimens (112 NanoString)
  - 8 cases had at least 1 paired specimen (FF/FFPE tumor or FF/FFPE benign)
- Expression of 18 housekeeping genes had low variation across all samples (CV%: 17 ~23)
- No batch effect noted on ANOVA of housekeeping genes before normalization (p>0.05)
- Normal tissue had poor cellularity and low RNA yield for FF and FFPE
  - FF benign – lowest RNA yields (range 0.05ug-7.13ug; average 0.64ug)
- Pearson correlation (PC) coefficient between FF tumor and FFPE tumor was good at 0.67 (p<0.001) (Figure 1)
- Poor correlation was noted between FF normal and FFPE normal specimens at 0.25 (p = 0.228) (Figure 1)
- Across all 137 specimens, PC coefficient for MR loading coefficient was 0.99 between FF and FFPE (p < 0.001), supporting the validity of the FFPE assay compared to FF (Figure 2)

Figure 1: Correlation of PC1 for 28 paired samples

Figure 2: Correlation of MR loading for FF and FFPE

Conclusions
- Good correlation identified between FFPE and FF specimens on NanoString, validating its use in reproducing the gene signature specifically in FFPE.
- Poor correlation in benign samples is likely due to low cellularity of benign breast tissue and poor quality RNA and is a potential limitation of this approach.

References