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Biopsy Cavities in Breast Cancer Specimens: Their Impact on Quantitative RT-PCR Gene Expression Profiles and Recurrence Risk Assessment

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Background: Tissue heterogeneity may influence profiles of gene expression. Manual microdissection (MMD) to remove biopsy cavities (BxC) is routinely performed in processing of specimens for the Oncotype DX assay. The objective of this study was to characterize by quantitative RT-PCR the impact of BxC on gene expression and the Recurrence Score (RS).

Methods: 48 (15 well, 18 moderate, and 15 poorly differentiated) invasive breast carcinomas were evaluated for differences in gene expression between whole sections (WS; which contained BxC) and enriched tumor (ET; BxC were excluded by MMD). An additional 27 invasive carcinomas were MMD to compare BxC profiles with ET and WS. Standardized quantitative RT-PCR analysis for the 21 genes was performed; reference normalized gene expression measurements ranged from 0 to 15, each 1-unit reflects an ~2-fold change in RNA. Correlation analyses used Pearson's R, concordance analysis by Lin's sample concordance and paired t-tests characterized differences.

Results: In the 48 cases there were statistically significant differences in gene expression between ET and WS for 6 cancer-related genes: BAG1 (ET-WS: 0.13 units, $p=0.0025$), CD68 (ET-WS: -0.64 units, $p<0.0001$), ER (ET-WS: 0.29 units, $p=0.0012$), GSTM1 (ET-WS: 0.18 units $p=0.0025$), STK15 (ET-WS: -0.18 units, $p=0.0041$) and STMY3 (ET-WS: 0.62 units, $p<0.0001$). Expression of CD68 was higher and ER was lower in WS containing BxC. The correlation (0.95) and concordance (0.92) were generally high between WS and ET for RS overall. Among moderately differentially tumors, there was a statistically significant mean increase in RS for WS of 3.3 units ($p = 0.0012$) while among poorly differentiated tumors, there was a trend toward a statistically significant decrease in RS for WS of 2.2 units ($p=0.0569$). RNA was obtained to analyze the BxC in 26 of the 27 additional cases. The RS was higher in the BxC than in the ET in 22 of the 26 cases. The average difference between BxC and ET was very large, 13.4 ± 2.1 RS units (mean \pm SE, $p<0.05$).

Conclusions: The inclusion of BxC in breast cancer specimens is associated with significant changes in the expression of individual genes and can impact the RS. Removal of BxC by MMD from breast cancer specimens assessed for gene expression levels is warranted.