

TUMOR GENE EXPRESSION AND PROGNOSIS IN BREAST CANCER: MULTI-GENE RT-PCR ASSAY OF PARAFFIN-EMBEDDED TISSUE

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Purpose: To identify candidate genes that predict prognosis of early breast cancer using a new assay for quantifying gene expression in fixed paraffin-embedded (FPE) tumor tissue. **Methods:** Patients with invasive breast cancer diagnosed from 1990 to 1997 were identified in the cancer registry and stratified into 4 groups by tumor size and nodal status. RNA was extracted from three 10 micron sections of the FPE tumor tissue obtained at the initial diagnosis, and expression was quantified for 7 reference genes and 185 cancer-related genes using RT-PCR. **Results:** Of the 146 patients with invasive breast cancer studied, 1 was a male. The mean age was 63 yr; 119 had negative nodes and 27 had positive nodes. Median follow-up was 8.8 yrs. As of Aug. 2002, 105 patients were alive without breast cancer recurrence. In univariate Cox survival analyses, a number of genes were associated with disease-free survival (DFS) (20 genes $p < 0.01$; 43 genes $p < 0.05$). Higher expression was associated with shorter DFS ($p < 0.01$) for FOXM1 (HNF-3), STK15 (BTAK), PRAME, Ki-67, NME1 (NM23), YB-1 (NSEP), Transferrin Receptor, Carbonic Anhydrase IX, Survivin, RPS6KB1, and c-Src. Higher expression was associated with longer DFS ($p < 0.01$) for Bcl2, CEGP1, GSTM1, PR, Bbc3 (Bcl2 binding component 3), GATA3, and GSTM3. Multivariate analysis indicates that inclusion of multiple genes has greater predictive power than use of single genes. The results are largely concordant with reported prognostic and biological activities of the genes, and provide additional new insights to clusters of genes with prognostic value. This RT-PCR assay has also been used to study a higher risk group of breast cancer patients (Cobleigh et al, Abstract, ASCO 2003). Notably, 8 of the 16 genes associated with DFS ($p < 0.05$) in that study are represented in the genes identified here. **Conclusion:** RT-PCR assay of paraffin-embedded tissue can be used to efficiently evaluate and validate large numbers of genes associated with breast cancer recurrence and response, and is a promising technology for new standard multi-gene clinical assays.