

Multiple GSTM gene family members are recurrence risk markers in breast cancer

Michael Kiefer, Kenneth Hoyt, James Hackett, Michael Walker and Joffre Baker Genomic Health Inc., Redwood City, CA

The human GSTM (GST μ) gene family consists of five different closely related isotypes, GSTM1-GSTM5. GSTM proteins conjugate glutathione to various electrophilic small molecules, facilitating clearance of the electrophiles from cells. Evidence exists that several metabolites of estrogen, including estrogen semi-quinones and estrogen quinones (catechol estrogens), are toxic and mutagenic (Cavalieri *et al.*, *Proc Natl Acad Sci* 94:10937-42, 1997). The activity of one or more GSTM enzymes may limit mutational damage caused by these estrogen metabolites. Accordingly, cancer cells expressing elevated levels of GSTM enzymes might be expected to exhibit better differentiation and reduced heterogeneity. We have reported five independent clinical studies in which GSTM gene expression was examined by quantitative RT-PCR in formalin-fixed, paraffin embedded primary breast cancer tissues. GSTM expression correlated strongly with favorable clinical outcome in each of these studies (Esteban *et al.*, *Prog Proc Am Soc Clin Oncol* 22:850 abstract, 2003; Cobleigh *et al.*, *Clin Cancer Res (in press)*; Paik *et al.*, *Breast Cancer Res Treat* 82:A16 abstract, 2003; Habel *et al.*, *Breast Cancer Res Treat* 88:3019 abstract, 2004; Paik *et al.*, *N Engl J Med* 351:2817-26, 2004). In these studies the probe used could not discriminate between GSTM1 and several other GSTM family members as a result of the strong sequence similarity of the GSTM genes, amplicon size limitations and the stringent sequence criteria for probe-primer design, leaving the possibility that several of the GSTM genes may be favorable markers. To address this, we have designed gene-specific probe/primer sets for GSTM1-5 by utilizing noncoding sequences as the source of probe/primer sets. We have now examined the levels of these GSTM RNA transcripts in 125 breast cancer tumor specimens that have linked clinical records. Statistical analysis reveals a modest range of correlated expression between the gene family members (Pearson R ranging between 0.15-0.47) in breast tumor tissue. Importantly, in this study, increased expression of all five GSTM genes correlated with increased disease-free survival when analyzed in a univariate Cox Proportional Hazards (PH) Model (HR and p values ranging between 0.57-0.79 and 0.0003-0.0493, respectively). Furthermore, a multivariate stepwise Cox PH analysis indicated that GSTM2 and GSTM3 contribute independent recurrence risk information. In summary, we have used noncoding gene sequence to design RT-PCR assays that discriminate between the closely related members of the GSTM gene family, and have studied the relationship between expression of these genes and breast cancer recurrence risk. The results indicate that all five GSTM genes are correlated with the likelihood of breast cancer recurrence and suggest that certain GSTM family members contribute independent prognostic information.