

Analysis of novel gene targets in breast cancer in younger women (<35 years)

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The aim of this study was to use real-time PCR to validate novel gene expression changes identified by Affymetrix Human Genome UI33A GeneChip® microarray in sporadic breast cancers in young women. The array study compared two breast tumours with normal breast organoids isolated from reduction mammoplasty and the non-tumourigenic breast cell line HBL-100. Nine novel target genes (NCOA3, AKAP1, RBBP4, C-EBP α , Granulin, DDB2, RARRES3, APRIL and TGF- β I) were selected by SAM analysis of the primary array data, and which have not been identified previously in other array investigations.

mRNA was extracted from five breast cancer cell lines and 25 tumour tissues (13 cases >35 years, 12 cases < 35 years in age) using Tri-reagent. RNA (1 μ g for cell lines and fresh tissue, and 3 μ g for FFPE tissues) was converted to cDNA using reverse transcriptase. Target gene expression was investigated using both manual RT-PCR and quantitative real time RT-PCR.

Most mRNA changes identified in the microarray were confirmed in the cancer cell lines, with good corroboration between the data from manual RT-PCR and real time experiments. Of the nine targets investigated in breast cancer cases, TGF- β I showed differential expression between younger and older cases, with elevated levels of mRNA expression in breast cancers <35 years. Whereas, AKAP1, RARRES3 and DDB2 showed a reduced level of expression in younger cases compared to older cases and the normal breast tissue. These novel gene expression changes identified will be followed up using protein studies and where appropriate the functional significance of these novel gene changes will be investigated.