DNA Microarray profiling of BRCA1 and BRCA2 deficient human tumors and cell-lines using a breast specific platform to identify a biomarker of DNA repair deficiency

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Introduction

There is increased focus within breast cancer research, on identification of reliable biomarkers to aid in accurate classification of the disease, predicting its progression and patients' response to both available therapies and those in development. DNA microarrays are a powerful tool for global analysis of gene transcript expression and they have in recent years become one of the key elements of biological research. We have characterised the transcriptome of breast cancer and used this information to create a unique disease focused microarray that is designed to work with formalin fixed paraffin embedded (FFPE) tissue.

Gene expression profiling was carried out on a number of ERα-positive and ERα-negative cell-line models in which BRCA1 and BRCA2 was silenced using siRNA technology. A cohort of BRCA1 and BRCA2 mutant tumors and matched wild-type controls were also profiled. The differentially expressed genes from the cell-lines were used for comparison with, and functional enrichment of, the gene expression profiles of the tumor samples. We found that the differentially expressed genes common to both the cell-line and tumor data were primarily concerned with cell-cycle control and DNA-damage response. Unsupervised analysis using this list of common genes identified a subset of sporadic tumor samples that were classified amongst the BRCA1 and BRCA2 mutant tumors. Further analysis of the genes characteristic of these tumours and the BRCA1 mutant tumours show a strong correlation in abrogation of DNA response pathways in both cohorts. This would suggest that the sporadic tumors are defective in DNA-repair response, and are therefore candidates for targeted therapy such as PARP-1 inhibitors.

Methods

(1) siRNA transfection
Three validated siRNAs to both BRCA1 and BRCA2 were obtained from Qiagen. siRNAs were transfected into cells using Lipofectamine RNAiMAX Transfection reagent from Invitrogen. Total RNA was harvested 72hrs following transfection.

(2) qPCR analysis
qPCR analysis was performed on the Roche Lightcycler using primers and the Quantitect OneStep RT-PCR kit from Qiagen.

(3) Gene expression profiling
Total RNA from cell-lines was amplified using the NuGEN Ovation® RNA Amplification System. Total RNA from FFPE tumor tissue was amplified using the NuGEN WT-Ovation™ FFPE System. The amplified product was hybridised to the Almac Breast Cancer DSA™ research tool.

(4) Analysis
Feature selection analysis – background, group distance, fold change and permutational t-test filtering (FDR and MTC) were applied.
Unsupervised analysis – agglomerative cluster analysis was performed using Euclidian distance metric and average linkage method.
Functional analysis – performed using GeneGo Metacore knowledgebase.

Cell-line and tumor gene-expression analysis

The expression of BRCA1 and BRCA2 was downregulated using three separate siRNAs to each gene in MCF-7s, T47Ds, Hs578Ts and MDA-231s followed by microarray analysis using the Breast Cancer DSA™ research tool. BRCA1 and BRCA2 mRNA expression was downregulated by an average of 90% and 82% respectively in all 4 cell-lines as determined by qPCR (Figure 1).

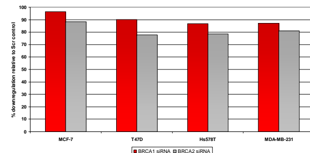

Figure 1
Percentage downregulation of expression relative to Scr control of BRCA1 and BRCA2 in MCF-7s, T47Ds, Hs578Ts and MDA-231s

In parallel, a cohort of 146 macrodissected BRCA1 mutant, BRCA2 mutant and matched sporadic breast cancer FFPE tissue samples were also profiled using the Breast Cancer DSA™ research tool. A list of differentially expressed transcripts was derived for BRCA1 and BRCA2 loss in the cell-lines (Figure 2A and 2B respectively). Likewise, a list of differentially expressed transcripts was derived from the comparison of BRCA1and BRCA2 mutant tumors to matched sporadic tumors (Figure 2C and 2D respectively).

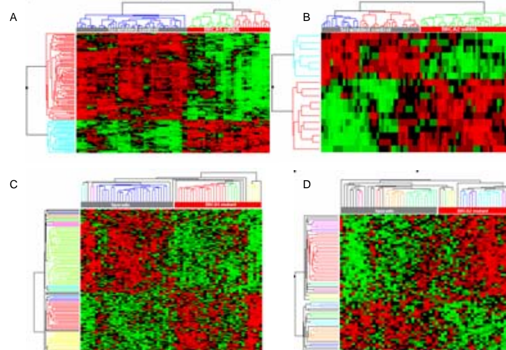

Figure 2
Clustergram representation of sample distribution using differentially expressed genes between BRCA1 siRNA and Scr control (A), BRCA2 siRNA and Scr control in cell-lines (B), BRCA1 mutant and matched sporadic tumors (C) and BRCA2 mutant and matched sporadic tumors (D)

Functional analysis of common transcripts

The transcripts that were differentially expressed in both the cell-line and tumor models for BRCA1 and BRCA2 loss were identified. Functional analysis was performed to characterise the main pathways and processes that are modulated by these transcripts (Figure 3). BRCA1 loss was found to abrogate pathways concerned with the mitotic checkpoint, DNA damage response and metabolism. BRCA2 loss was found to modulate pathways responsible for DNA replication, apoptosis and also DNA damage response.

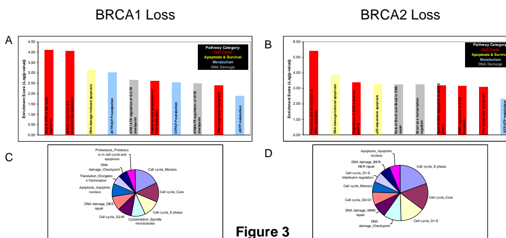

Figure 3
Functional analysis of the transcripts common to cell-lines and tumors: A and B – pathways abrogated following loss of BRCA1 and BRCA2 respectively; C and D – cellular processes abrogated following loss of BRCA1 and BRCA2 respectively

Unsupervised analysis of tumors

In order to assess how the common differentially expressed transcripts can distinguish BRCA mutant tumor samples from their matched sporadic tumor samples, we applied unsupervised 2D clustering to the tumor set. The tumors clustered broadly into 4 subgroups – 2 sporadic groups, a BRCA2 mutant group and a BRCA1 mutant group (Figure 4). Of note, one of the sporadic groups clustered in between the BRCA2 and BRCA1 mutant tumor groups.

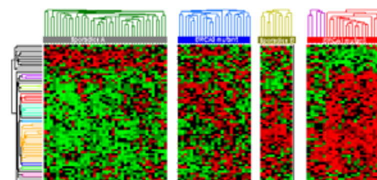

Figure 4
Clustergram representation of tumor sample distribution following unsupervised analysis using transcripts common to cell-line and tumor samples

Functional analysis of tumor subgroups

The clustering of a subset of sporadic breast tumors with the BRCA mutant tumors would suggest that they share some commonality at a transcriptional level. BRCA1 and BRCA2 mutant tumors are known to be defective in DNA double-strand break repair due abnormal homologous recombination. As such, we hypothesised that the sporadic tumors clustering amongst the BRCA mutant tumours (Sporadic B) may also display an enhanced abrogation of pathways associated with DNA-damage response compared to the larger sporadic group (Sporadic A).

Functional analysis was performed on the genes most upregulated in the Sporadic A, Sporadic B and BRCA1 mutant subgroups for comparison. It was found that pathways governing development and estrogen receptor signalling were enriched within the Sporadic A subgroup (Figure 5A). In comparison, of the top pathways affected in the Sporadic B and BRCA1 mutant subgroups, 6 were common. As expected, these are primarily concerned with DNA-damage response as well as G2 and M phase cell-cycle control (Figure 5B).

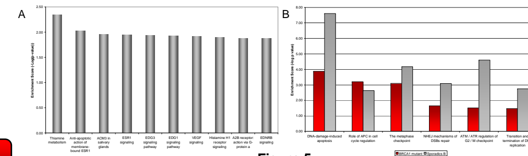

Figure 5
Pathway analysis of the Sporadic A (A), Sporadic B and BRCA1 mutant subgroups (B)

Conclusion

This study demonstrates the power of using gene expression profiling of cell-line models to refine the analysis of expression data from FFPE archived human tumor samples. This approach has identified a set of transcripts that could be used to identify an abnormal DNA-damage response within BRCA1/2 mutant tumours as well as a subset of sporadic tumors. This approach has the potential to identify cancer patients that benefit from conventional DNA damaging chemotherapeutic agents or from novel DNA repair targeted agents such as PARP-1 inhibitors.

Work is currently on-going to refine and validate the genes that characterise the Sporadic B, BRCA1 and BRCA2 mutant subgroups with the intention of developing a predictive test.

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