Comparative whole-genome microarray analysis of two estrogen-responsive breast cancer cell lines

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Motivation

Estrogens promote the growth of female secondary sexual characteristics, such as breasts, and play a fundamental role in the development and maintenance of the reproductive cycle. Being steroid hormones, estrogens control cellular key functions by diffusing through the cell membrane to interact with estrogen receptors. The consequences of these interactions are changes in the expression of genes [1-2]. In pathologies, such as human hormone-responsive breast tumors, specific gene expression patterns are observed [3]. Through an innovative cRNA microarray technique, the Illumina genome-wide expression analysis, we monitored the expression of more than 46.000 genes in two human breast cancer cell lines (ZR-75-1 and MCF-7) and compared the gene expression profiles so obtained with those relative to the Universal Human Reference RNA [4], representing a pool of RNAs from 10 different human cell lines derived from brain, breast, B- and T-lymphocytes, uterine cervix, liver, fat tissue, macrophages, skin and testis. Out of the data we produced, we searched for gene expression signatures that may characterize the hormone-responsive breast cancer phenotype.

Methods

Microarray analyses were carried out in replicates: 4 hybridizations each for the two cell lines under study and 7 for the reference RNA. The Illumina Sentrix Human-6 v2 BeadChips platform was used, which contains six arrays on a single BeadChip, each with over 46,000 probes derived from human genes in the National Center for Biotechnology Information (NCBI) Reference Sequence (RefSeq) and UniGene databases.

The data was pre-processed and the expressed genes lists extracted using the BeadStudio software.

Results

At first we addressed the choice of the best normalization strategy to apply for chip-to-chip comparisons and, on the other hand, to capture as much significant genes as possible while reducing the number of false positives. To achieve this, we compared our results to a gold standard using different normalization methods implemented in our microarray analysis software tools. After this comparison, we found that a global average normalization method for the raw data was the most suitable to our aims. The ratio between the average signal value of expressed genes and the average value of the reference genes was calculated and this allowed us to define a list of genes which are over- or under-expressed in either or both cell lines under study, compared to the Universal Human Reference RNA. Furthermore, we were able to deduce a list of genes with very high or very low differential expression in either ZR-75-1 (e.g. RDC1), MCF-7 (e.g. SPANXB2) or both cell lines (e.g. FBP1), suggesting chromosomal imbalances and gene silencing events underlying the hormone-responsive phenotype.

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Availability: http://crisceb.unina2.it/geneexpression/

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