PhD Thesis

Gene expression profiles as a prognostic marker in women with ovarian cancer

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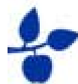

Abstract

Epithelial ovarian cancer (EOC) is the most lethal gynaecologic malignancy with an overall 5 year survival of approximately 30-35%. A more exact prognosis depends on stage, histo-pathological tumour type, grade of differentiation, patient age, size of residual disease after primary operation, and the decline in CA-125 during first line chemotherapy. At the cellular level, the malignant potential of a tumour is presumably reflected by numerous different proteins. Their concentrations are regulated by mRNA for the coding genes, and therefore the copy number of each mRNA reflects the up- and down-regulation of the genes/gene products. Gene expression analysis on a microarray platform is capable of measuring the copy number of thousands of genes in parallel and thereby gives a specific functional signature of the tissue analyzed. It has recently been demonstrated that the gene expression signatures can act as an independent prognostic factors in EOC, but from the few studies conducted so far, it has not been possible to establish a unique profile for prognosis/prediction. The main aim of this study was to extend the knowledge about a prognostic gene expression profile, by conduction of a new microarray study on old EOCs and comparisons with data from the previous published studies. A final number of 43 samples from the MALOVA tissue-bank were used in this study.

To ensure that the expression data we extracted from the tissue/patients we wanted to include in the prognostic study was not biased from previous episodes of thawing, a study concerning repeated thaw-freeze cycles was performed. Eight old EOC samples (collected from1992-1997) from our local bio-bank were used together with 8 samples collected at Odense University Hospital for the present investigation (2004-2005). Three thaw-freeze cycles were performed for each. RNA quality was evaluated in all 3 x 16 samples, while RNA from three different thaw-freeze cycles from 4 old samples underwent microarray analysis.

Furthermore we did not know the degree of heterogeneity of gene expression profiles in EOC. Therefore a second study dealing with this problem was conducted. We used three samples taken with greatest possible mutual distance from 9 different EOCs. Sampling was done at Odense University Hospital from 2004-2005.

In all studies RNA was extracted using the Trizol method and a further clean-up procedure was performed on RNeasy coulombs. RNA quality was evaluated on the on the Agilent 2100 Bioanalyzer. In the first study, evaluating the thaw-freeze parameter, we used the Enzo® labeling kit and the microarray analysis was performed on Affymetrix Human Genome U133A 2.0 arrays with > 14.500 well defined genes. The heterogeneity study and the prognostic study were both performed on Affymetrix GeneChip Human Genome U133 plus 2.0 arrays with >38.500 well defined genes after labelling with the One-Cycle Eukaryotic Target Labeling assay from Affymetrix.

The results from the first study showed that it is possible do at least three divisions of -80 °C frozen tumour tissue at room temperature, without compromising the gene expression signature.

The second study revealed that intra-tumour heterogeneity of gene expression signatures in EOC was very small compared to inter-tumour heterogeneity, and therefore it is sufficient to use only a single macro-dissected sample from each tumour in i.e. prognostic studies.

The prognostic study led to identification of a 15 probe-set (14-gene) signature which can act as an independent prognostic factor in EOC. This signature has the ability to separate short-term survivors with a median survival of 32 months from a group of long-term survivors with a median survival that has not yet been reached after a median follow up time at 76 months (log-rank $P = 3.4 \times 10^{-9}$). The hazard ratio for death in the unfavourable group (short term survivors) was 5.4 ($P = 0.00022$ by univariate Cox analysis). The identified gene list may,

together with other gene set derived from similar patient cohorts, be used in the selection of patients for new experimental treatment. Furthermore it can possibly lead to identification of specific malignant transformed cellular pathways, which may act as targets for new treatment modalities.

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