Gene Expression Profiling of Serous Ovarian Cancers

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Abstract

Introduction: Serous carcinoma, the most common type of ovarian cancer, has a high mortality rate, due in part to the extraovarian spread of disease that is usually present at diagnosis. Standard treatment involves debulking surgery followed by combined platinum/taxane chemotherapy. Optimal debulking is not always possible, and in recent years neoadjuvant chemotherapy has been used to decrease tumor bulk prior to surgery. The effects of chemotherapy on tumor cells *in vivo* is poorly understood, and currently we are not able to predict which patients are most likely to benefit from this approach.

Objective: This study aims to profile gene expression patterns associated with serous ovarian carcinomas exposed to chemotherapy before (neoadjuvant) and after (adjuvant) surgery.

Design: RNA from 44 serous ovarian cancers were analyzed using Affymetrix U133 plus 2.0 Gene Chips (23 Adjuvant and 21 Neoadjuvant treated tumors). A combination of supervised and unsupervised data analyses followed (ArrayAssist, Stratagene). A parallel study used SAM (Significance Analysis of Microarrays, Stanford) and the data from that approach was compared to this method.

Results: Significance analysis using ArrayAssist (Stratagene) identified 82 significant genes able to help distinguish between adjuvant and neoadjuvant treated tumors (p value of 0.01). 32 of these genes are over expressed and 50 are under expressed in the neoadjuvant samples compared to the adjuvant samples. Hierarchical clustering of the 82 significant genes indicates that this gene set is not a perfect discriminator as 5 adjuvant samples clustered with the 21 neoadjuvant samples. Analysis of the 82 genes with PathwayExpress (Wayne State) indicated that these genes do not belong to any specific biological pathway. The most highly represented molecular function was ATP binding, with 23% of the significant genes showing this activity. When subcellular localization was analysed, most of the genes were widely distributed, however 15% were determined to be localized to the nucleus.

Conclusions: While there is indication of altered gene expression in tumors which have been treated by neoadjuvant therapy compared to standard debulking followed by chemotherapy, the results are as of yet inconclusive. It appears that neoadjuvant treated tumors can largely be distinguished from the majority of adjuvant treated tumors however it appears that there may be several subgroupings of adjuvant treated tumors, one of which behaves more like neoadjuvant treated samples.. Further work, including the analysis of additional clinical and outcome data in warranted.

Introduction

Serous carcinomas are the most common type of malignant ovarian tumor in female adults and are generically referred to as ovarian cancer. Serous tumors occur most often in women who are between 40 and 60 years of age. Both high- and low-grade tumors can be identified. About 50% of these tumors are malignant, 33% are benign and 17% are of borderline malignancy. Surface epithelial neoplasms are classified into subtypes based on the type of epithelial differentiation that is present in the tumor. The subtypes include serous, mucinous, endometrioid, clear cell and transitional cell. The subtypes derive their names from the tissue that they most closely resemble.

Serous carcinoma has a high mortality rate, due in part to the extraovarian spread of disease that is usually present at diagnosis. Standard treatment involves debulking surgery followed by combined platinum/taxane chemotherapy. Optimal debulking is not possible in some patients, and in recent years neoadjuvant chemotherapy has been used to decrease tumor bulk prior to surgery. However, the effects of chemotherapy on tumor cells *in vivo* is poorly understood, and currently we are not able to predict which patients are most likely to benefit from this approach.

Design

Snap-frozen tissues (n=44) were selected from the Toronto Ovarian Tissue Bank using the following criteria: serous type, stage III-IV, completion of 1st line chemotherapy, sample tumor content >70%. Quantity and quality of Trizol extracted RNA for each sample was assessed on the Nanodrop ND-1000 spectrophotometer and Agilent 2100 BioAnalyzer, respectively. All RNA samples to be studied were labelled and hybridized following standard protocols from Affymetrix.

Results

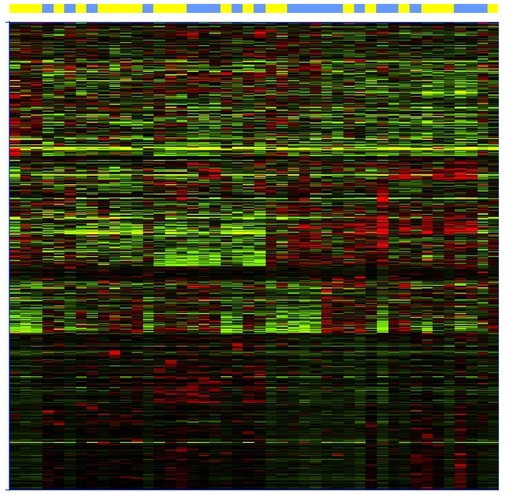

Fig 1. Hierarchical clustering on GC-RMA normalized data (unsupervised analysis). The entire dataset was entered into the clustering algorithm to see if a natural seperation would be detected on the basis of treatment. As can be seen adjuvant (yellow) and neoadjuvant (blue) treated samples do not separate.

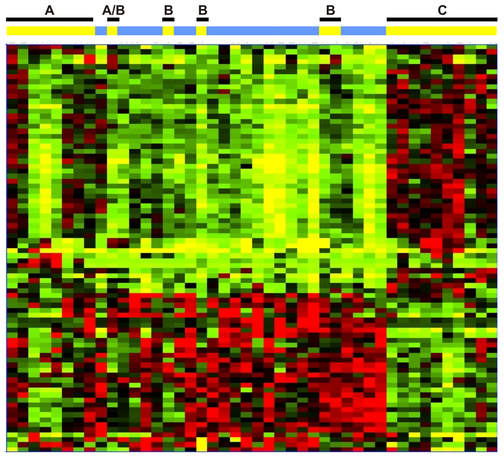

Fig 2. Hierarchical clustering of the 82 significant genes identified as part of a supervised analysis (two class unpaired, variance inflation, ArrayAssist). The neoadjuvant treated tumors (blue) cluster together. There appears to be three subgroups (A-C) of adjuvant (yellow) treated tumors including one subtype that clusters most closely with the neoadjuvant treated tumors (B).

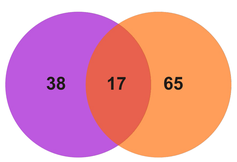

Fig 3. Significance analysis by SAM (Stanford) identified 55 genes the distinguished between adjuvant and neoadjuvant treated tumors. Comparison back to the 82 identified via ArrayAssist reveals 17 genes which are in common.

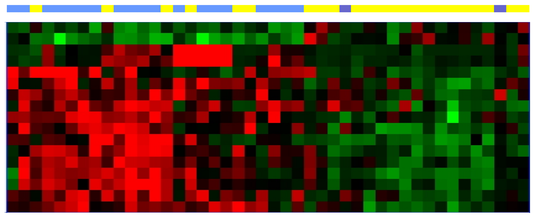

Fig 4. Hierarchical clustering of the 17 significant genes found in common between the two analysis methods. This reduced set of genes does not separate the two treatment types, neoadjuvant treated tumors (blue) and adjuvant treated tumors (yellow) as effectively as the larger 82 gene set.

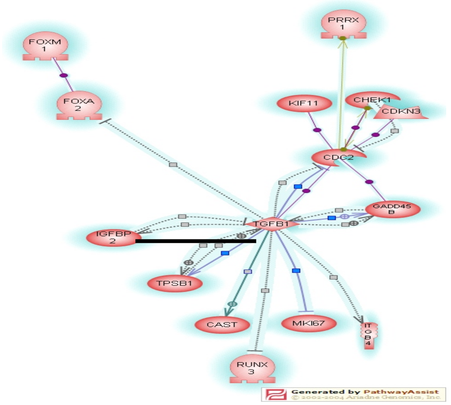

Fig 5. A direct interaction pathway was built using the list of 82 significant genes as input (PathwayAssist, Stratagene). Blue lines represent expression controls, grey lines represent regulation, purple lines represent binding and green lines represent protein modification. TGF-ß is at the centre of the identified complex.

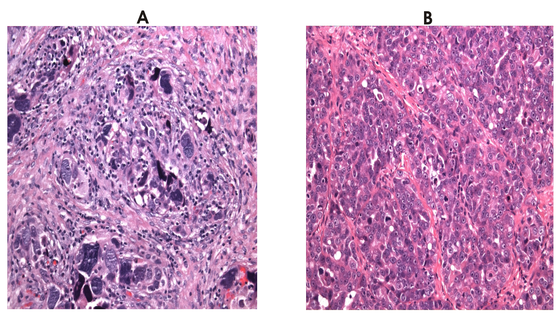

Fig 6. Haematoxilin and eosin stained neo adjuvant tumor tissues. Cytotoxic effects are shown in (A) and no chemo effects for (B).

Discussion and Conclusions

Ovarian cancer has been studied by other groups using microarrays. Many of these studies have compared gene expression between normal ovarian epithelial cells and ovarian cancers.

In this study, we used expression arrays to identify gene expression patterns that reflect response to chemotherapy. All individuals received chemotherapy post surgery for both adjuvant and neoadjuvant groups. Chemotherapy consisted of Platinum and Taxol treatment soon after surgery and were repeated once a month for 6-8 cycles if all went well.

Hierarchical clustering (unsupervised analysis) of all of the genes on the array (Fig 1) showed mixed clusters with no clear distinction between the two treatment groups. This was not unexpected as the vast majority of genes represented on the array are expected to not be involved in either response to chemotherapy or in the cancerous condition. Biological variance between patients as a result of ethnic background, age, diet et cetera would confound any potential relevant discrimination between the treatment groups. A subsequent supervised significance analysis using ArrayAssist (modified T-test using variance inflation) identified 82 significant genes at a p value of 0.01. Of these, 32 are over expressed and 50 are under expressed in neoadjuvant compared to adjuvant treated tumors. Hierarchical clustering of the 82 significant genes (Fig 2) indicated that there was better, but still incomplete partitioning of the two treatment groups. It appears that the neoadjuvant treated samples can be grouped together, but not fully separated from a potential subgroup of adjuvant treated tumors. Two other potential subgroups (one containing ten individuals and another containing eight). The reason for this stratification may become apparent in follow up studies including additional clinical data (see below). Further analysis of the data using Pathway Express (Wayne State) did not reveal any specific biological pathway, however, 23% of proteins are involved in ATP binding and 15% are localized to the nucleus. PathwayAssist (Strategene) indicates that there may be a role of the TGFß pathway (Fig 5). PathwayAssist has also indicated that RB tumor suppressor and ATM signaling are two pathways that are most similar to the direct interaction pathway built from the significant list.

This data set was also analyzed with SAM and it found 55 significant genes at a false discovery rate (FDR) of 4.09%. These 55 genes again are also unable to fully separate the two classes of treatments providing similar results to the 82 found via ArrayAssist. In order to determine if a subset of these genes may actually be more effective (through reduction of noise) we looked at the genes which were found in common between the two analysis methods. 17 genes were found to overlap (Fig 3) and again clustering does not show an ability to fully distinguish between treatments (Fig 4.)

Future Work

Indicators of response to chemotherapy including morphology and serial serum CA125 values will be assessed, and outcome parameters including disease-free survival and overall survival will be included in further analysis. Selected significant genes will be validated via QRT-PCR. *In situ* hybridization and immunohistochemistry will be applied to tissue microarrays of a larger series of both adjuvant and neoadjuvant samples for additional validation.

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Toronto Ovarian Cancer Research Network

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