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Bioinformatics analysis reveals potential candidate drugs for psychological stress in ovarian cancer

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Abstract. – BACKGROUND: Women with ovarian cancer may be at increased risk for psychological distress around the time of diagnosis relative to patients diagnosed with other cancers, because of the seriousness of the disease. However, the molecular mechanism of this effect is far from clear.

AIM: We sought to investigate the influence of psychological status in regulating gene expression among women with primary ovarian cancer and to identify the small molecules which exhibit similar effects with different psychological status.

MATERIALS AND METHODS: DNA microarray analyses of 10 ovarian carcinomas (GSE9116, downloaded from GEO) identified 916 human transcripts that were differentially expressed in tumors from patients with high depression relative to grade-and stage-matched tumors from low depression patients, and pathways related to immune system were dysfunctional.

RESULTS: Our results suggest that psychosocial stress is related to impaired immunity in ovarian cancer patients. Besides, we identified a group of small molecules which can be exploited as adjuvant drug to improve therapeutic effect for ovarian cancer, such as MS-275 and adiphenine.

CONCLUSIONS: Our findings may be useful for the development of management strategies for psychological distress, and we suggest that there is a need for improvement in the quality of life of cancer outpatients being treated with chemotherapy.

Key Words:
Ovarian cancer, Psychological stress, Small molecules.

Introduction

Ovarian cancer is the second most common gynecologic cancer and the deadliest in terms of absolute figure[1]. The survival rate of ovarian cancer still remains lower than other types of gynecological cancer, with an overall 5-year mortality of 70%, although considerable progress achieved in the management of gynecological cancers[2]. Because of the seriousness of the disease, women with ovarian cancer may be at increased risk for psychological distress around the time of diagnosis relative to patients diagnosed with other cancers[3-4]. The psychological distress worse over the course of cancer treatment, persist after cancer therapy, significantly impair quality of life, and decrease adherence to cancer therapy and survival. However, no treatments have been established for depression in ovarian cancer patients. As surviving cancer becomes increasingly common, there is an urgent need to develop an empirical basis to provide effective, evidence-based treatments to this population.

Though psychological status has long been suspected to influence cancer onset and disease progression, the molecular mechanisms of these effects are just beginning to be investigated and not univocal[5]. Depression may influence cancer biology via a direct effect of the neuroendocrine system on the functional activity of tumor cells[6]. The neuroendocrine system has been shown to regulate several biological processes involved in cancer progression, such as tissue invasion[7-8], angiogenesis[6,9], cell motility[10] and programmed cell death[11-12].

In this present study, we used the gene expression profile of GSE9116 to explore the influence of psychological status in regulating gene expression in primary ovarian cancer, and to seek the small molecules which exhibit similar effect with low expression. The availability and integration of high-throughput gene expression data and the computational bioinformatics analysis may shed new lights on therapeutic studies of ovarian cancer.

Materials and Methods

Affymetrix Microarray Data

We downloaded the gene expression profile of GSE9116 from a public functional genomics data repository GEO (http://www.ncbi.nlm.nih.gov/geo/)

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which is based on the Affymetrix Human Genome U133A Array. Total 10 chips were available for further analysis, including 5 low depression specimens and 5 high depression specimens. Tumor tissue and psychosocial data were collected from 10 patients undergoing primary surgical resection of ovarian carcinoma. Patient samples were selected for analysis based on high vs. low biobehavioral risk profiles defined by depressive symptoms and social support. Individual tumor tissues from high- vs. low-risk patients were matched based on stage, grade, and histological subtype prior to genome-wide expression analysis[13].

Small Molecules Data

The connectivity map (CMap) can be used to find connections among small molecules sharing a mechanism of action, chemicals and physiological processes, and diseases and drugs[14]. It is the first installment of a reference collection of gene-expression profiles from cultured human cells treated with bioactive small molecules, together with pattern-matching software to mine these data. The CMap dataset comprises genomic profiling data from 6100 treatment-control pairs (instances) involving 1309 bioactive molecules (perturbagens). The output consisted of a group of chemical perturbagens with a connectivity score ranging from +1 and −1. The score represented the correlation between the query signature profile and the gene profile of a treatment-control pair (instance). A high positive connectivity score indicated that the corresponding perturbagen induced the expression of the query signature, whereas a high negative connectivity score indicated reversal of expression of the query signature by the perturbagen. A zero or "null" connectivity score indicated that no effect upon expression of the query signature was recorded. We downloaded all the profile data for further analysis.

Pathway Data

KEGG (Kyoto Encyclopedia of Genes and Genomes) is a collection of online databases dealing with genomes, enzymatic pathways, and biological chemicals[15]. The PATHWAY database records networks of molecular interactions in the cells, and variants of them specific to particular organisms (http://www.genome.jp/kegg/).

Differentially Expressed Genes Analysis

For the GSE9116 dataset, the classical t-test method was used to identify differentially expressed genes (DEGs). We used the limma[16] package in R[17] to preprocess the data of profile GSE9116. The raw expression datasets from all conditions were processed into expression estimates using robust multiarray averaging (RMA) method[18] with the default setting implemented in Bioconductor, and then constructed the linear model. The p value less than 0.05 were chosen as the cutoff criterion.

Pathway Enrichment Analysis

DAVID (The **D**atabase for **A**nnotation, **V**isualization and **I**ntegrated **D**iscovery)[19], a high-throughput and integrated data-mining environment, which checks for an enrichment of genes with specific GO, KEGG, and SwissProt terms. We used the DAVID to identify over-represented pathways using the genes in our result. We selected the pathways with p-value less than 0.05 and count larger than 2.

Results

Microarray Data Analysis

Publicly available microarray dataset GSE9116 was obtained from GEO. We used the classical t-test method to identify the DEGs between low depression specimens and high depression specimens. Expression of 916 genes with p value less than 0.05 were identified differed across high- and low-risk groups.

Pathway Enrichment Analysis

To functionally classify these 916 significant genes, we used the online biological classification tool DAVID and observed significant enrichment of these genes in multiple pathways. In order to focus on the most significantly relevant pathways, the p value less than 0.05 and the count larger than 2 were chosen as the thresholds. The most significant enrichment was the pathway of acute myeloid leukemia with p-value = 0.004315. The other significant pathways included mitogen activated protein kinase (MAPK) signaling pathway (p value = 0.012661), phosphatidylinositol signaling system (p value = 0.020644), pathways in cancer (p value = 0.024412) and so on (Table I).

Identification of Candidate Small Molecules

In order to identify candidate small molecules capable to cause similar effect with low depression and high depression, we performed compu-

Table I. The enriched pathways.

Term	Description	Count	*p* value
hsa05221	Acute myeloid leukemia	10	0.004315
hsa04010	MAPK signaling pathway	25	0.012661
hsa04070	Phosphatidylinositol signaling system	10	0.020644
hsa05220	Chronic myeloid leukemia	10	0.022357
hsa05200	Pathways in cancer	28	0.024412
hsa04142	Lysosome	13	0.028497
hsa04910	Insulin signaling pathway	14	0.036425

tational bioinformatics analysis of the derived gene signature using the Connectivity Map dataset. Enrichment scores and *p* values were computed for 1309 bioactive small molecules. A search against 6100 treatment-control pairs (instances) representing 1309 bioactive small molecules identified large amount small molecules which may exhibit similar effect to low depression and high depression. The top 20 significant small molecules were listed in Table II. In Table II, the small molecules of adiphenine (enrichment score = –0.947) and MS-275 (enrichment score = –0.963) were associated with highly significant negative scores and the small molecule of 1, 4-chrysenequinone was associated with highly significant positive score (enrichment score = 0.951).

Table II. Most significative known genetic variants and their effect in pharmacotherapy.

Cmap name	Enrichment score	*p* value
Adiphenine	-0.947	0
Genistein	-0.513	0.00008
Ginkgolide A	0.892	0.00014
Nadolol	-0.856	0.00074
Eucatropine	0.706	0.00173
Diphenhydramine	-0.751	0.00176
MS-275	-0.963	0.00304
Estradiol	-0.289	0.00324
8-azaguanine	0.797	0.00328
Iloprost	-0.88	0.00343
Gliclazide	0.795	0.00346
Famprofazone	0.671	0.00352
1,4-chrysenequinone	0.951	0.00447
Cefalexin	0.714	0.00457
Rimexolone	0.777	0.00464
Ethotoin	0.652	0.00528
Desipramine	-0.774	0.00533
Viomycin	-0.771	0.00561
Phthalylsulfathiazole	0.7	0.00585
Trimethobenzamide	-0.688	0.00661
Prestwick-1082	-0.845	0.00743

Discussion

The severe emotional distress accompanying a diagnosis of cancer and its initial treatment has been documented extensively[20-21]. However, the molecular mechanism of effect that psychological status has an effect on cancer onset and disease progression is far from clear. In this work, we sought to investigate the influence of psychological status in regulating gene expression among women with primary ovarian cancer and to identify the small molecules which exhibit similar effects with different psychological status. Results show that gene expression profiles in primary human tumor tissues are altered in association with patient-level biobehavioral risk factors, and bioinformatics analyses confirmed that pathways related to cellular immune system were changed. Furthermore, we identified large amount of small molecules which can provide new ideas for the therapeutic studies in ovarian cancer.

In the result of microarray data analysis, up to 916 genes were identified expressed differentially in tumors from patients with low depression relative to grade- and stage-matched tumors from high depression patients. This result indicates that the gene expression profiles changed significantly along with the patients' psychological status. Our result is in line with previous studies[13,22-23]. Further investigation of these DEGs may aid in exploring the molecular mechanism of effect that psychological status has an effect on cancer onset and disease progression.

Pathway enrichment analysis identified several pathways related to immune system were dysfunctional, such as pathway of acute myeloid leukemia, pathway of chronic myeloid leukemia and pathway of lysosome. The effects of psychological stress on various parts of immunological function and the association with cancer have been investigated in many prospective studies[24-27].

At the cellular level, high depressed patients had an overall leucocytosis, mild reduction in absolute NK-cell counts, reduced mitogen-stimulated lymphocyte proliferation and neutrophil phagocytosis, and moderate decreases in T-cell and natural killer-cell (NK-cell) functions and so on[27-30]. At the molecular level, serum and plasma concentrations of basal cortisol, specific antibodies against herpes simplex virus type 1 and Epstein Barr virus, and acute-phase proteins were higher in high depressed patients than in healthy controls[28-31].

Data in Table II shows that the small molecules of MS-275 (enrichment score = –0.963) and adiphenine (enrichment score = –0.947) were associated with highly significant negative scores, which suggest that these small molecules is capable to cause similar effect with low depression, that is, they can be used as adjuvant drug to improve therapeutic effect for ovarian cancer. MS-275 has garnered some attention in recent years because of its ability to inhibit histone deacetylase (HDACs) in the brain[32]. Covington et al demonstrated that MS-275 infusion reverses the effects of chronic defeat stress on global patterns of gene expression in the nucleus accumbens, and suggested that stress-regulated genes whose expression is normalized selectively by MS-275 may provide promising targets for the future development of novel antidepressant treatments[33]. MS-275 shows promise in the search for drugs that might be useful for treating depression[34]. We are not aware of any reports of the evaluation or use of adiphenine as systemic therapies for depression. Although this compound is widely used in clinical practice of local anesthetics, there is a lack of knowledge as to their effects on psychological stress of human ovarian cancer. Given the widespread use of this agent for anesthetics, it is unlikely that this compound will be exploited as therapeutics by itself, but may have promise for use in combination therapy.

The small molecule of 1, 4-chrysenequinone (enrichment score = 0.951) was associated with highly significant positive score, which suggest that this small molecules are capable to cause similar effect with high depression. This small molecule can be used to simulate the effect of high depression in the research of ovarian cancer.

Overall, we have demonstrated that psychosocial stress is related to impaired immunity in ovarian cancer patients. Pathways related to immune system were dysfunctional, such as pathway of acute myeloid leukemia, pathway of chronic myeloid leukemia and pathway of lysosome. Besides, we identified a group of small molecules which can be exploited as adjuvant drug to improve therapeutic effect for ovarian cancer, such as MS-275 and adiphenine. Although it may be premature to suggest that these drugs might be ready for psychiatric clinical trials, it is clearly a direction that warrants additional consideration.

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