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Integrated analysis of gene expression profile and genetic variations associated with ovarian cancer

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Abstract. – OBJECTIVE: Ovarian cancer is the most lethal gynecologic cancer worldwide, since most patients are diagnosed at an advanced stage. To improve the early diagnosis and treatment of ovarian cancer, we performed a integrated analysis of transcription profile and genetic variations to study on the molecular pathogenesis in ovarian cancer.

METHODS: mRNA expression profiles of ovarian cancer and normal controls downloaded from ArrayExpress database were applied to identify differentially expressed genes (DEGs). The chromosomal distributions of these DEGs were established using DAVID. Then, DNASeq data from the Cancer Genome Atlas (TCGA) were extracted to analyze gene mutational information including the number of mutations (mut), the number of mutational genes (mutG) and chromosomal distributions of mutations. Statistical method was offered to carrying on correlation analysis of gene mutations and differential expression.

RESULTS: A total of 1732 DEGs were identified, and the chromosomal distributions of 97 genes were unknown. These DEGs were most significantly distributed on chromosome 4 with *p* value = 1.34E-7. Chromosome 1 enriched the most DEGs (11.56%). Statistical algorithm showed that DEGs presented significantly positive correlation with mut (*p* = 0.000009) and mutG (*p* = 0.00001). In 48.7% DEGs, gene mutations were found.

CONCLUSIONS: We conducted scientific analysis on integration of DEGs in expression profiles and genetic mutations in ovarian cancer, displayed the correlation of differential expression and genetic variations. The result indicated that expression profiles were significantly correlated to genotype.

Key Words:
Ovarian cancer, Differentially expressed genes, Single nucleotide polymorphism, Genetic mutations.

Introduction

Ovarian cancer is the second most common gynecologic cancer, however, with mortality at the top[1] because of a typically late detection and laggard therapeutic strategies[2]. It was estimated that nearly 21,980 new cases would be diagnosed and 14,270 would die from ovarian cancer in the United States alone in 2014[1]. Based on symptoms and effective screening programs, other gynecologic cancers such as cervical cancer and endometrial cancer could usually be diagnosed in the early stage[3]. Unfortunately, the vast majority of ovarian cancer patients present with an advanced stage because of the lack of specific symptoms and reliable biomarkers[3], and the 5-year survival is less than 30%[4]. Clearly, ovarian cancer presents a challenge which is making correct detection as early as possible. A better understanding of the molecular pathogenesis associated with ovarian cancer is greatly needed, so that available biomarkers and drug targets which are helpful to early diagnosis should be identified.

Like other cancers, hereditary factors are suspected to cause ovarian cancer. Several evidences show that genetic events play an important role in some ovarian cancer women[5-9]. It was reported[7,8,10,11] that ovarian cancer could be induced by mutations in specific genes, such as breast cancer susceptibility gene (BRCA), including BRCA1 and BRCA2, which were susceptive to breast and ovarian cancer. Harmful mutations in either of these two genes conferred a woman lifetime risk of ovarian cancer from 15% to 40%[7]. Lakhani et al[12] also demonstrated the connection between BRCA and ovarian cancer that BRCA gene accounted for 5%-13% of

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ovarian cancers and Ashkenazi Jewish women were proven to suffer ovarian cancer at an earlier age with a higher risk[13]. A integrated analysis of 489 high-grade serous adenocarcinomas demonstrated that TP53 was mutated in almost all cases (96%), and other 9 genes including NF1, BRCA1, BRCA2, RB1 and CDK12 were commonly mutated[14]. KRAS-variant was considered as a new genetic marker of cancer risk for ovarian cancer[15].

Next generation DNA sequencing technology (DNASeq) which greatly influences clinical researches is rapidly developed in recent years. Compared to microarray data, DNASeq can be used to generate genome-wide genetic data with less signal noises[16]. DNASeq technology has been applied for unprecedented discovery in various types of cancer, including breast cancer[17], acute myeloid leukemia[18] and non-small cell lung cancer[19]. Bioinformatics approach provides new perspectives to study the pathogenesis and therapy of cancer. ArrayExpress Archive is a public database of microarray gene expression data, which is designed to hold data from microarray platforms[20]. The Cancer Genome Atlas (TCGA) database (http://cancergenome.nih.gov/) is a integrative and multidisciplinary project where different diseases are categorized using a variety of genome-wide platforms[21], included DNASeq data. Previous studies have offered amount of preliminary data on gene expression profiles in ArrayExpress Archive and DNASeq data such as single nucleotide polymorphism (SNP) of ovarian cancer in TCGA[22-25]. Although these studies have provided some useful insights, systematically integrative analysis comparing gene differential expressions and genetic variations is greatly needed, which offers potential for identifying novel and specific biomarkers for the early detection of cancer.

Many scientists believe that high-grade serous ovarian cancer, the most common type of ovarian cancer, is a fallopian tube malignancy disguising as an ovarian one[26-30]. Now, scientists have presented the model of this cancer that proves the hypothesis powerfully and facilitates the development of better treatments for cancer[30,31]. Women with strong genetic risk for ovarian cancer may consider the use of prophylactic salpingo-oophorectomy, since they also have an increased risk of fallopian tube cancer[32].

In the present study, to examine the role of genetic factors in ovarian cancer development, we attempted to correlate genetic alterations with the gene expression profile. An integrated approach was applied to identify statistically significant genes associated with ovarian and fallopian tube cancer using microarray data and patient matched data from ArrayExpress and TCGA. We first identified the differentially expressed genes (DEGs) between tumor samples and normal controls based on the expression level, then validated the chromosomal distributions of these DEGs using DAVID (http://david.abcc.ncifcrf.gov/). The genetic variations were downloaded from TCGA database to proceed correlation analysis between DEGs and genetic mutations. This study revealed the relevance of genetic mutations and differential expression, which provided information for understanding the molecular pathogenesis of ovarian and fallopian tube cancer.

Methods

Data Collection and Identification of DEGs

The microarray expression profiles of fallopian tube and ovarian cancer and normal control were downloaded from ArrayExpress (http://www.ebi.ac.uk/arrayexpress/), a publicly available repository, with access number of E-GEOD-10971[23], E-GEOD-14001[24], E-GEOD-18520[25] and E-GEOD-27651[33]. For each gene expression dataset, the original expression information from all conditions were preprocessed into expression estimates by RMA method in Bioconductor. Each probe was mapped to one gene, where the probes were discarded if they could not match any genes. The value averaged over probes was selected if the gene had multiple probes. RankProd package is a powerful meta analysis tool to detect DEGs by integrating multiple microarray data[34], which is developed from the rank product method[35]. We provided the RankProd package to identify the DEGs between patients and control by combining these multiple experiments. The up-and down-regulated DEGs were identified by assimilating a set of gene-specific *t* tests. Genes with a percentage of false-positives (pfp) < 0.01 and $|\log_2 FC| > 2$ were considered as DEGs between patients and controls.

Chromosomal Distributions of DEGs

In order to ascertain the chromosomal distributions of the DEGs identified by RankProd, we performed chromosomes enrichment analysis using Functional Annotation Chart module in

DAVID (http://david.abcc.ncifcrf.gov/). The significant enrichments were identified by EASE score with the correction of false discovery rate (FDR). The threshold of EASE score was less than 0.05.

Analysis of DNASeq Data

TCGA database (http://cancergenome.nih.gov/) is a central bank which provides multiple experimental data of more than 20 different types of human cancer, including DNA changes of ovarian cancer. To study the connection and difference between DEGs identified by microarray analysis and gene variations in TCGA, DNASeq data of ovarian cancer were downloaded and analyzed. In this study, level 2 data were used to identify somatic mutations, including single-nucleotide polymorphism (SNP), base deletion and insertion. Whole-genome sequencing was done with the Illumina HiSeq sequencer. Reads were aligned to the reference human genome build hg19. Somatic mutations obtained by WUSM mutation calling model were selected for our study. A total of 9 valid batches with 460 samples (230 tumors and 230 normals) were extracted. Then the genetic variational information was obtained for further analysis.

Correlation Analysis of DEGs and Genetic Variations

By analysis of the DNASeq data, we could gain the number of mutations (mut), the number of mutational genes (mutG), the ratio of total mutations to total genes (Amut/G) the percent of mut in total gene (Pmut), the number of genes in chromosomes (Gene) and chromosomal distributions of genetic mutations. Spearman correlation test[36] was used to analyze the correlation between the DEGs and mutational information with p value < 0.01 considered as significant correlation.

Results

Identification of DEGs

A total of 1732 genes that were consistently differentially expressed in tubal and ovarian cancer across studies were identified. Among the 1732 DEGs, 580 genes were up-regulated and 1152 genes were down-regulated.

Chromosomal Distributions of DEGs

By the functional annotation chart analysis in DAVID, the chromosomal distributions of a total of 1664 DEGs were identified (Table I), excepted for the other 68 DEGs which were unknown

Table I. Chromosomal distributions of differentially expressed genes in expression profiles.

Chromosome	Count	%	*p* value	Genes
1	189	11.6	0.001852*	S100A4, STIL, RNASEL, PTGS2…
2	121	7.4	0.087593	LYPD1, LTBP1, ZAK, DYNC2LI1…
3	98	6.0	0.058346	NCBP2, RARRES1, PLXNA1, RBM6…
4	110	6.7	1.34E-07*	FAM200B, GNPDA2, SGMS2, PDLIM5…
5	81	5.0	0.157247	MEF2C, FGF18, SNCAIP, IL6ST…
6	104	6.4	0.596139	TUBB2A, TTK, ANKRD6, DSE…
7	71	4.3	0.98725	STK31, CLDN4, CLDN3, EZH2…
8	74	4.5	0.06419	DLC1, CTHRC1, TUSC3, PNMA2…
9	68	4.2	0.568477	CTNNAL1, C9ORF72, ALAD, CDC14B…
10	68	4.2	0.268881	ZCCHC24, BTRC, PWWP2B, PRKG1…
11	77	4.7	0.962824	MPZL2, TSPAN4, IL18, E2F8…
12	97	5.9	0.014105*	SYT1, LMO3, C12ORF73, NELL2 …
13	39	2.4	0.151873	CAB39L, WASF3, DZIP1, CLDN10…
14	56	3.4	0.924428	SGPP1, ATL1, FLJ39632, JAG2…
15	64	3.9	0.079959	C15ORF48, AQP9, PRC1, CRABP1…
16	35	2.1	0.999989	BCLAF1, HSD17B2, C16ORF72, PHKB…
17	74	4.5	0.870948	HLF, CLDN7, COX11, PRR11…
18	37	2.3	0.04189*	ZNF516, VAPA, SYT4, TYMS…
19	54	3.3	0.999992	SLC44A2, RFXANK, LSR, CCNE1…
20	36	2.2	0.793087	TMX4, CTCFL, AURKA, NECAB3…
21	20	1.2	0.655431	ADARB1, SYNJ1, CHODL, CYYR1…
22	24	1.5	0.998606	TRIOBP, PRAME, SELM, SLC5A1…
X	67	4.1	0.910104	ZMAT1, KDM6A, NAP1L3, FGF13…

*p value < 0.05.

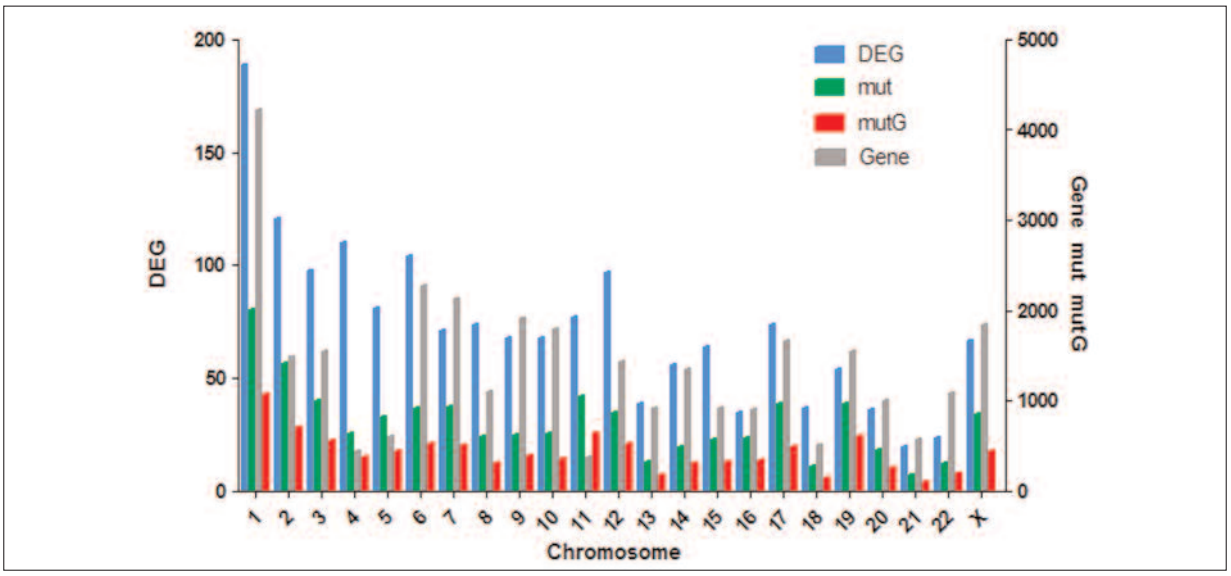

Figure 1. Chromosomal distributions of the DEGs in expression profiles and genetic mutational information. (DEGs: differentially expressed genes; mut: genetic mutations; mutG: mutational genes; Gene: genes in chromosomes).

genes. These genes were significantly distributed on four chromosomes including chromosome 1, 4, 12 and 18. Thereinto, chromosome 4 had the most significant distribution with p value = 1.34E-7, followed by chromosome 1 (p = 1.85E-3) and chromosome 12 (p = 0.014). In terms of count, chromosome 1 enriched the most DEGs (11.6%), followed by chromosome 2 (7.4%) and chromosome 4 (6.7%).

Correlation Analysis of DEGs and Genetic Variations

The TCGA ovarian cancer data set consisted of 230 tumors and 230 normals with genetic variation data were obtained. The DEGs and genetic mutational information of chromosomes including mut, mutG and Gene were shown in Figure 1.

Across DEGs in expression profiles and DNASeq analysis, we obtained the correlation of DEGs and logFC, mut, Pmut, Gene, mutG and Amut/G using spearman correlation test (Table II). The analysis found that the number of DEGs had significantly positive correlation with mut (p = 0.000009) and mutG (p = 0.00001), that is, the more the mut and mutG, the more the DEGs, but had no significant correlation with logFC (p = 0.401), Pmut (p = 0.112), Gene (p = 0.111) and Amut/G (p = 0.112).

Genes which were both DEGs in expression profiles and mutational genes, and their chromosomal distributions were identified by screening DEGs and mutational genes in our study (Figure 2). It was shown that there were 811 genes which were both DEGs and mutational genes, and genetic mutations were found in an average of 48.7% of DEGs. That showed that the differential expressions of genes might originate from genetic variations, or the differential expressions of genes could influence the genetic structure.

Table II. The correlation of differentially expressed genes in expression profiles and genetic variations by Spearman correlation test.

		logFC	mut	Pmut	Gene	mutG	Amut/G
DEGs	Correlation coefficient	0.184	0.7844	-0.340	0.341	0.778	0.340
	p value	0.401	0.000009	0.112	0.111	0.00001	0.112

mut: the number of genetic mutations; Pmut: the percent of mut in total gene; Gene: the number of genes in chromosomes; mutG: the number of mutational genes; Amut/G: the ratio of total mutations to total genes in chromosomes, i.e. the average of genetic mutations in each gene.

Discussion

The lack of effective early detection and therapy makes ovarian cancer patients present high mortality and poor prognosis. Molecular mechanism of ovarian cancer becomes a burning question. To identify molecular basis involved in ovarian cancer, the present study attempted to investigate the association between gene expression profiles and genetic variations on genome-wide in ovarian cancer.

As described in previous study, genetic variations and differential expressions of transcription profiles had been investigated widely[37-40]. Evidence from epidemiology suggested that genetic factors might play an essential role in the development of ovarian cancer[39,41]. Predispositions to certain cancers have been linked to an ever-increasing number of mutations[42]. To date, multiple mutations in different genes have been associated with the development of ovarian cancer. Activating mutations in fibroblast growth factor (FGF) had been associated with an increased risk of ovarian cancer[41]. Genetic variation in insulin-like growth factor 2 (IGF2) also played an important role in influencing risk of ovarian cancer[38,43]. More recently, in older to build a complete catalog of cancer genes, Lawrence et al[44] identified almost all known cancer genes as well as 33 novel mutated genes by analyzing nearly 5,000 patient samples from 21 tumor types, and found that about 20 percent cancer genes existed high-frequency mutations and most genes were mutated within intermediated frequencies.

Previous researches[45-47] showed that there were enormous amount of DEGs between tumor patients and normal people. Identifying DEGs of cancers by gene expression profiles had been popularized recently[48-52]. Two genes (DOC-1 and DOC-2) were identified as DEGs using a DNA-fingerprinting approach with Northern analysis confirmed[39]. Hough et al[53] exhibited global gene expression profile from various ovarian cell lines and tissues using a serial analysis of gene expression, and gained a total of 444 up-regulated genes. A study on ovarian metastasis[51] showed that fifty-six genes demonstrated differential expression between ovarian and metastasis samples by microarray analysis of global gene expression patterns.

Recently, differential expression has been related to genetic variations[37,54]. Jazaeri et al[54] indicated that gene mutations would lead to the change of molecular phenotypes by comparing gene expression in ovarian cancer associated with mutations in BRCA1 and BRCA2. A study about pseudohypoxic pheochromocytomas and paragangliomas associated with SDHB, SDHD, and VHL mutations showed that the gene expression profiles depended on tumor location as well as underlying mutation[42]. In another research of Lawrence[55], somatic mutation frequency in cancers was found strongly correlated with gene expression level by whole-genome and whole-ex-

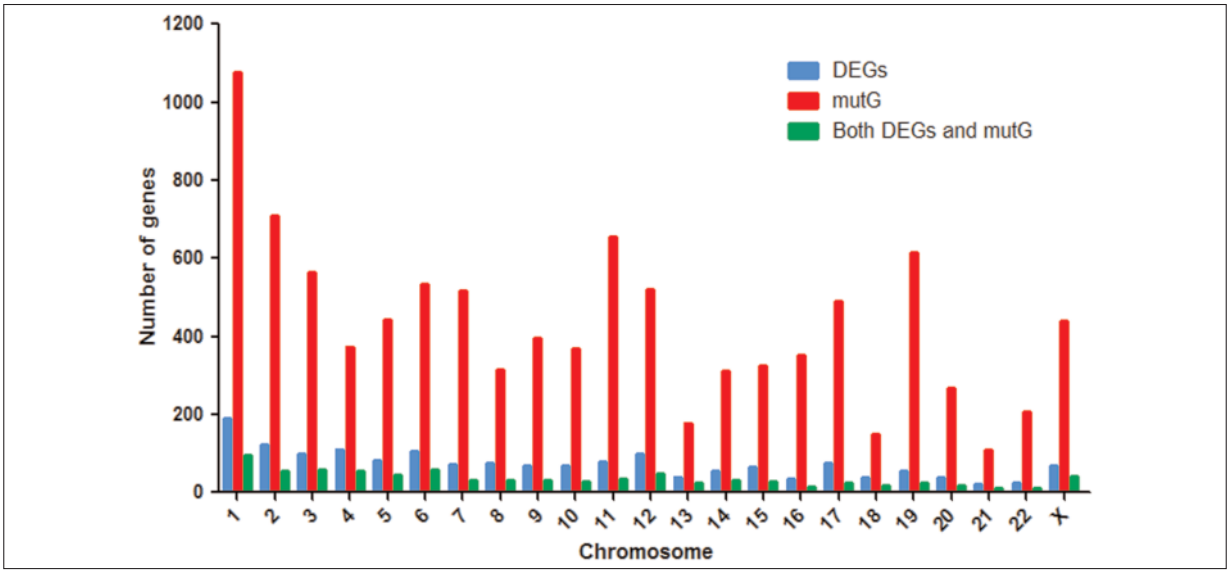

Figure 2. Genes which were both DEGs in expression profiles and mutational genes, and their chromosomal distributions. (DEGs: differentially expressed genes; mutG: mutational genes).

ome data analysis. Consistent with previous study, our finding also illustrated the correlation between expression profile and genetic variations in genome-wide scale.

Conclusions

In the present study, gene expression profiles of ovarian cancer were compared with normal controls to identify new biomarkers for disease early diagnosis. Association of specific genes may lead to identification of new diagnostic markers and potential therapeutic targets. Various genetic mutations in chromosomes and DEGs in expression profiles resulted in ovarian and fallopian tube cancer were identified. We showed that DEGs of expression profiles significantly positive correlated with genetic variations including mut and mutG. In about half of the DEGs, gene mutations were found. It is possible that changes of expression profiles might originate from genetic variations, or differential expressions could impact the genetic variations. In a word, the expression profiles and the genetic changes have significant correlation.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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