RESEARCH ARTICLE

Identification of Genes and MicroRNAs Involved in Ovarian Carcinogenesis

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Abstract

MicroRNAs (miRNAs) play roles in the clinic, both as diagnostic and therapeutic tools. The identification of relevant microRNAs is critically required for ovarian cancer because of the prevalence of late diagnosis and poor treatment options currently. To identify miRNAs involved in the development or progression of ovarian cancer, we analyzed gene expression profiles downloaded from Gene Expression Omnibus. Comparison of expression patterns between carcinomas and the corresponding normal ovarian tissues enabled us to identify 508 genes that were commonly up-regulated and 1331 genes that were down-regulated in the cancer specimens. Function annotation of these genes showed that most of the up-regulated genes were related to cell cycling, and most of the down-regulated genes were associated with the immune response. When these differentially expressed genes were mapped to MiRTarBase, we obtained a total of 18 key miRNAs which may play important regulatory roles in ovarian cancer. Investigation of these genes and microRNAs should help to disclose the molecular mechanisms of ovarian carcinogenesis and facilitate development of new approaches to therapeutic intervention.

Keywords: MicroRNA - ovarian cancer - differentially expressed genes - gene ontology

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Introduction

Ovarian cancer is the second most common gynecologic cancer and the deadliest in terms of absolute figure (Holschneider and Berek 2000). The overall 5-year mortality of ovarian cancer is about 70%, lower than other types of gynecological cancer, although various therapeutic approaches are followed in clinical practice (Colomiere et al., 2009). This is mainly because most cases are not diagnosed until the disease is at an advanced stage (Ono et al., 2000). Therefore, the understanding of molecular mechanisms of ovarian cancer and establish more effective therapies is a critical and urgent issue.

To achieve this goal, identification and characterization of key MicroRNAs that participate in ovarian carcinogenesis are essential steps. MicroRNAs (MiRNAs) are short, non-coding RNAs (~22 nucleotides) that usually play important roles in posttranscriptional regression by binding to partially complementary sites in the 3'-untranslated region (UTR) of their mRNA targets (Lee et al., 1993; Wightman et al., 1993). Changes in the expression of miRNAs during tumorigenesis were discovered in a variety of human malignancies, and they are associated with the prognosis and the progression of cancer in some cases. For instance, the let-7 miRNAs, which are down-regulated in lung cancer, negatively regulated the oncogenes RAS and HMGA2 (Johnson et al., 2005; Lee and Dutta, 2007). In contrast, miR-21 is up-regulated in several tumors and plays an oncogenic role by regulating the expression of the tumor suppressor PTEN in hepatocellular cancer (Meng et al., 2007). In addition, some miRNAs have been implicated in tumor progression by affecting adhesion, migration, and invasion of cancer cells (Ma et al., 2007; Huang et al., 2008). Therefore, the discovery of key miRNAs which are involved in regulating expression of target genes might be important in the development of effective anti-cancer drugs.

Here we report the identification of miRNAs which regulated up- or down-regulated genes in multiple specimens of ovarian carcinoma using cDNA microarray data coupled with computational analysis. In addition, we performed Gene Ontology analysis of the differentially expressed genes to explore the altered biological process in ovarian cancer.

Materials and Methods

Affymetrix microarray data

We downloaded the gene expression profile of GSE18520 from the Gene Expression Omnibus (GEO), which was deposited by Mok and his colleagues (Mok et al., 2009). Total 63 arrays were available for further analysis, including 53 arrays of advanced stage, high-grade primary tumor specimens and 10 arrays of normal ovarian surface epithelium (OSE) brushings. Briefly, the tumor specimens were obtained from previously untreated ovarian cancer patients, who were hospitalized at the Brigham and Women's Hospital between 1990 and

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2000. All patients had advanced stage, high-grade serous ovarian cancer according to the International Federation of Gynecology and Obstetrics standards.

MiRNA- target interactions

The miRNA-target interactions (MTIs) were collected from miRTarBase (Hsu et al., 2011) (Release 2.5) on April 5th, 2012. MiRTarBase has accumulated more than three thousand MTIs, which are collected by manually surveying pertinent literature after data mining of the text systematically to filter research articles related to functional studies of miRNAs. At the time we collected data, the miRTarBase curates 2860 experimentally verified MTIs between 285 miRNAs and 1721 target genes.

Analysis of differentially expressed genes (DEGs)

For GSE18520, we used Affy package in R (v.2.13.0) (Gautier et al., 2004; Team, 2011) to analyze the gene expression. The CEL source files from all conditions were converted into expression estimates and performed background correction and quartile data normalization using RMA (Irizarry et al., 2003) (Robust Multi-array Average) algorithm. Probe sets were mapped to NCBI genes. Delete the probe sets that correspond to multiple genes or no genes. If there are multiple probe sets that correspond to the same gene, the expression values of those probe sets are averaged. As a result, we got a total of 19803 genes from this dataset. We used the two-sample t-test to identify DEGs in ovarian cancer specimens compared to controls. To circumvent the multi-test problem which might induce too much false positive results, the BH method (Benjamini, 1995) was used to adjust the raw p-values into false discovery rate (FDR). The genes with FDR < 0.01 and fold change > 2 were selected as DEGs.

Go Ontology analysis

Gene Ontology (GO) analysis has become a commonly used approach for functional studies of large-scale genomic or transcriptomic data (Ashburner et al., 2000). To better understand the functional relevance of the identified DEGs, we performed GO enrichment analysis using GO-function (Wang et al., 2011). The FDR< 0.01 was defined to be statistically significant.

Identification of miRNA-target interactions related to ovarian cancer

We divided the identified DEGs into two groups: up-regulated genes and down-regulated genes, and used the hypergeometric distribution to identify the significant interactions with $p < 0.05$. The p-value was calculated with the following equation: where N is the number of target genes in all interactions, M is the number of target genes interacted with a particular miRNA, K is the number of differentially expressed target genes and i is the number of overlapping target genes at least.

Results

Differentially expressed genes in ovarian cancer patients and controls

We downloaded publicly available microarray dataset GSE18520 from GEO database and carried out a two-sample t-test to identify the genes specifically differentially expressed in ovarian cancer specimens. Based on the criteria of FDR < 0.01, a total of 1839 genes were considered significantly differentially expressed. Of these 1839 genes, 508 genes were up-regulated (fold change > 2) and 1331 were down-regulated (fold change < -2).

Gene ontology analysis

GO analysis of the dataset showed a total of 20 categories to be altered (Table 1). Some of them were up-regulated, such as mitotic sister chromatid segregation, mitotic prometaphase, DNA packaging and cell cycle.

Table 1. Results of GO Enrichment Analysis (FDR < 0.01)

GO - ID	GO - Term	Gene Count		FDR
		up-	down-	
GO:0000070	mitotic sister chromatid segregation	17	1	5.39E-05
GO:0000236	mitotic prometaphase	21	1	0.006318
GO:0006956	complement activation	0	14	0.006518
GO:0007155	cell adhesion	25	103	6.49E-05
GO:0006323	DNA packaging	23	5	0.002816
GO:0007049	cell cycle	110	68	5.78E-06
GO:0007051	spindle organization	16	3	0.000466
GO:0007067	mitosis	57	12	2.53E-06
GO:0009653	anatomical structure morphogenesis	49	152	0.003148
GO:0008283	cell proliferation	55	127	7.42E-06
GO:0009611	response to wounding	39	101	0.000236
GO:0010033	response to organic substance	44	121	0.006077
GO:0016477	cell migration	19	70	0.003682
GO:0021537	telencephalon development	3	20	0.008201
GO:0030177	positive regulation of Wnt receptor signaling pathway	2	13	0.002535
GO:0035295	tube development	9	41	0.006915
GO:0043627	response to estrogen stimulus	12	19	0.003733
GO:0051301	cell division	59	29	3.35E-10
GO:0060429	epithelium development	24	47	8.11E-05
GO:0065008	regulation of biological quality	77	195	0.006915

Table 2. The Enriched miRNAs of Up-regulated Genes

miRNA	All target genes	up-regulated genes	p-value
hsa-let-7b	145	12	0.0109
hsa-miR-107	15	3	0.0201
hsa-miR-15a	18	4	0.0048
hsa-miR-15b	6	2	0.0216
hsa-miR-192	67	7	0.016
hsa-miR-24	21	3	0.0495
hsa-miR-26a	25	4	0.0161
hsa-miR-373	62	7	0.0106
hsa-miR-424	23	6	2.02E-04
hsa-miR-503	12	4	9.37E-04

Table 3. The Enriched miRNAs of Down-regulated Genes

miRNA	All target genes	down-regulated genes	p-value
hsa-miR-130a	10	4	0.017
hsa-miR-196a	12	4	0.0336
hsa-miR-200a	17	7	0.0012
hsa-miR-200b	13	4	0.0445
hsa-miR-200c	11	4	0.0244
hsa-miR-21	65	12	0.0437
hsa-miR-429	7	4	0.0037
hsa-miR-520h	5	3	0.0108

Some of them were down-regulated, such as complement activation, cell adhesion, response to wounding, and response to organic substance. In fact, most of the up-regulated GO terms were related to cell cycle, and most of the down-regulated GO terms were associated with immune response.

Identification of miRNA-target interactions related to ovarian cancer

MiRNAs can bind to one or more target sites on a gene transcript to negatively regulate protein expression, subsequently controlling many cellular mechanisms (Bartel, 2004; Min and Yoon, 2010). We used the hypergeometric distribution to identify the miRNAs which interact with the identified DEGs in MiRTarBase. At the criteria of p-value < 0.05, a total of 10 miRNAs were enriched in up-regulated genes (Table 2) and 8 miRNAs were enriched in down-regulated genes (Table 3).

Discussion

MiRNAs are post-transcriptional regulators that bind to complementary sequences on target mRNAs, usually resulting in translational repression or target degradation and gene silencing (Kusenda et al., 2006; Bartel, 2009). The identification of miRNAs which are involved in regulating expression of target genes in ovarian cancer might be important in understanding molecular mechanism of this disease. In this study, we analyzed the expression profile of primary ovarian tumor specimens and normal OSE brushings. A total of 1839 genes were identified to be differentially expressed. By mapping these DEGs to miRTarBase database, we obtained a total of 18 key miRNAs which may participate in the initiation of ovarian cancer.

Among the 1839 differentially expressed genes, 508 genes were up-regulated and 1331 were down-regulated. Some of them were consistent with our knowledge for ovarian cancer. Functional annotation of these genes showed that most of the up-regulated genes were related to cell cycle, and most of the down-regulated genes were associated with immune response.

Human malignant tumors are characterized by abnormal proliferation resulting from alterations in cell cycle-regulatory mechanisms (D'Andrilli et al., 2004; Pharoah et al., 2007; Butt et al., 2008). Control and timing of the cell cycle involves checkpoints and regulatory pathways that ensure the fidelity of DNA replication and chromosome segregation (Elledge, 1996). Regulatory mechanism of the cell cycle is a dynamic balance process between cyclins, cyclin-dependent kinases (CDK), and CDK inhibitors. Any differential expression of these molecules would disturb this dynamic balance and result in uncontrolled cell proliferation, eventually lead to carcinogenesis (Nam and Kim, 2008).

The complement system consists of a number of small proteins found in the blood, generally synthesized by the liver, and normally circulating as inactive precursors. This system is a key player in innate immunity, which defends the host against microbes (Carroll, 2004; Morgan et al., 2005; Ricklin and Lambris, 2007). Complement activation can protect the cells by preventing the attack from the membrane attack complex as well as cell lysis mediated by complement. In this study, we found that the GO category of complement activation was down-regulated, suggesting that complement-mediated damage was weakened, thereby tumor cells was allowed the escape from cytolysis and thus promoting carcinogenesis (Murray et al., 2000).

We identified a total of 18 miRNAs in this work. Some of them were also identified to play important roles in ovarian cancer by previous study. For example, miR-21, miR-200, miR-429 and let-7b were differentially expressed in serous ovarian carcinoma compared with normal ovarian tissues (Iorio et al., 2007; Nam et al., 2008), and differentially expression of them were significantly correlated with a poor prognosis. MiR-15a and miR-15b were also aberrantly expressed in human cancer (Zhang et al., 2006). One of them, miR-373, has been described as putative oncogene in testicular germ cell tumors by numbing p53 pathway (Voorhoeve et al., 2006). Besides, miR-130a may associated with chemotherapy resistance (van Jaarsveld et al., 2010). The rest of them, such as miR-107, miR-192, miR-24, miR-26a, and miR-503 may provide valuable alternatives for ovarian cancer mechanism, especially from the miRNA regulation perspective. Further research may pay more emphasis on these miRNAs.

In conclusion, we have demonstrated that cDNA microarray data coupled with computational analysis represent a powerful approach to identify key miRNAs in ovarian cancer. A total of 18 miRNAs which may play important regulatory roles in ovarian cancer were identified. These miRNAs report here may contribute to disclose the molecular mechanism of ovarian carcinogenesis and aid the development of new approaches to therapeutic intervention.

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