African Journal of Pharmacy and Pharmacology Vol. 6(28), pp. 2119-2122, 29 July, 2012
Available online at http://www.academicjournals.org/AJPP
DOI: 10.5897/AJPP12.272
ISSN 1996-0816 ©2012 Academic Journals

Full Length Research Paper

Mouse ovarian-related gene expression profiles change with intraperitoneal injection of cisplatin

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Accepted 28 May, 2012

Cisplatin (DDP), an important anti-cancer drug, can affect normal ovarian function. However, the underlying pathophysiological mechanism is unclear. Thus, to analyze the mechanism of its ovary function, modulation effect was evaluated by noting the effect of its injection on ovary related gene expression in mice. Total mRNA of mice ovary tissue after administration of cisplatin and the normal control group was extracted by TRIzol method. After reverse transcription and fluorescent labeling by Cy3, Cy5, we obtained two groups of mouse ovarian cDNA probes and then hybridized with the cDNA gene expression profiles microarray hybridization. The cDNA microarray was scanned using the fluorescent signals and showed differences between two groups with the help of statistical analysis program. By analyzing the ovarian tissue gene expressions after the administration of cisplatin, 109 differentially expressed genes were identified, among which were 19 up-regulated expression genes and 90 down-regulated expression genes. These genes may participate in the occurrence and development of ovary dysfunction induced by cisplatin and provides diagnosis evidence for ovary dysfunction induced by chemotherapy.

Key words: Ovary, cisplatin, gene expression pattern, nucleotide sequence analysis, microarray hybridization.

INTRODUCTION

Premature Ovarian Failure (POF) is a syndrome clinically defined by failure of the ovary before the age of 40 (McGee and Hsueh, 2000). It is characterized by primary or secondary amenorrhea, hypoestrogenism and elevated gonadotropin serum levels (deMoraes-Ruehsen and Jones, 1967; Fonseca et al., 2012). This syndrome is very heterogeneous with a multi-causal pathogenesis and any of the following: chromosomal, enzymatic, iatrogenic, autoimmune or infectious aberration may be the cause of the disease (Hoek et al., 1997), although in most cases the etiology of POF is still unclear. Hence, it is necessary to improve our understanding of the molecular mechanisms responsible for premature ovarian failure development and p rogression, and in turn develop novel strategies for the early detection, prevention, and treatment of POF.

In recent years, the incidence of carcinoma has shown an increasing tendency (Jiao et al., 2012), and there are more and more patients suffering from ovarian deficiency and infertility caused by chemotherapy and an anti-cancer drug, which becomes one of the important etiological factors of POF (Goswami and Conway, 2005; Krishna et al., 2010). A previous study showed that cisplatin (DDP), an important anti-cancer drug, could cause serious damage to ovarian tissue, resulting in ovarian deficiency and infertility (Dube et al., 1998). However, the underlying pathophysiological mechanism of ovarian deficiency and infertility caused by chemotherapy and DDP is unclear.

Thus, in this study, the animal model of POF induced by chemotherapy damage was established by using ICR mice injected with DDP. Using normal ovary and damaged ovary induced by DDP as models for cDNA microarray analysis, we detected gene expression

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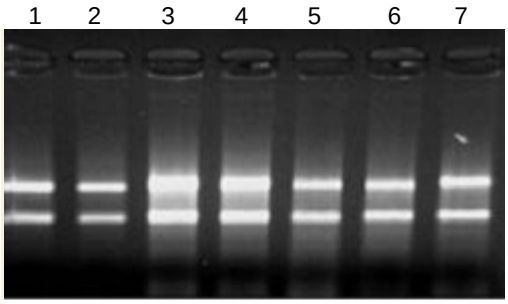

Figure 1. The RNA isolated from ovary of rats. Lanes 1 to 4, RNA from damaged ovary by DDP; Lanes 5 to 7, RNA from normal ovary tissue.

change of ovary induced by DDP, which would be of help in explaining the molecular mechanisms of ovary dysfunction induced by chemotherapy.

MATERIALS AND METHODS

Experimental rats and sample preparation

Forty female ICR rats (28 to 30 g) were studied after 1 weeks of observation. ICR rats were randomly divided into control group (n = 20) and experimental group (n = 20) after one week. The animal model of POF was established by administration of cisplatin (1 g/L, 3.0 mg/kg) to the experimental group. Ovaries were collected from rats after feeding of the drug for one week.

Isolation of high-quality RNA

Total RNA was isolated from fresh ovarian tissue using TRIzol reagent (Invitrogen, USA) according to the manufacturer's protocol and as described in the online supplement. In brief, the tissue was ground with mortar and pestle cooled by liquid nitrogen of the ground tissue, then 100 mg was incubated with 1 ml TRIzol for 5 min at room temperature (RT). Cell debris was removed by centrifugation (12,000×g at 4°C for 10 min) and 0.4 ml chloroform was added. After vortexing, the mixture was incubated for 5 min at RT. The phases were separated by centrifugation (12,000×g at 4°C for 15 min) and the aqueous phase was transferred to a new tube. Furthermore, 0.6× volume of isopropyl alcohol and a 0.1× volume of 3 M sodium acetate were added to this aqueous phase and incubated for 10 min at 4°C. The precipitated RNA was pelleted by centrifugation (12,000×g at 4°C for 15 min) and after the removal of the supernatant, the RNA was washed twice with 70% ethanol. Subsequently after drying, the RNA was re-suspended in 30 µL DEPC-treated water. Then the RNA was purified using QIAGEN RNeasy Kit (QIAGEN, German). The quality and quantity of the RNA was verified by the presence of two discrete electropherogram peaks corresponding to the 28S and 18S rRNA at a ratio approaching 2:1.

cDNA microarray

Isolation and validation of high-quality mRNA from ovary of both normal and cisplatin treated mice was carried out and transcribed to cDNAs, then cDNAs were labeled with the directly incorporated fluorescently dUTP (cy-5 or cy-3) to prepare the hybridization probes. High density Oligo gene chip of mice, including 16143 Oligo DNA genes came from the National Engineering Center for Biochip at Shanghai (China). The mixed probes were hybridized to the cDNA microarray. The result was scanned by the laser scanner and processed by software for image analysis, standardization, the ratio of value analysis, cluster analysis.

RESULTS

Isolation of high-quality RNA for gene chip analysis

Total RNA isolated from fresh ovary tissue was subjected to gene chip analysis. As shown in Figure 1, the electropherogram ladder of RNA was clear and bright and the presence of two discrete electropherogram peaks corresponding to the 28S and 18S rRNA at a ratio approached 2:1, which demonstrated that the quality and quantity of the RNA was fit to be used for gene chip analysis.

Results of cDNA microarray

We were interested in identifying functionally related genes that distinguished the ovary damage by DDP with normal ovary from the control subjects. It was observed that the permutation P values were < 0.05 for the ovary damage by DDP versus the control subjects. There were 109 differentially expressed transcripts genes in the ovary damaged by DDP compared with the control subjects, 19 genes were upregulated, while 90 were downregulated in the ovary damage by DDP. The gene list is available for review on the National Center for Biotechnology Information's Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/query). Some important genes are related to synthesis of DNA, energy metabolism crossing membrane, activation of cellular nuclear factor which have an inhibiting effect on functional expression of ovarian cells, and then result in imbalance of net regulation of some genes (Table 1).

DISCUSSION

cDNA microarray technology is a burgeoning molecular biology technology in recent years, which can provide access to enormous genetic data sets with opportunities to discover novel disease mechanisms. This approach has been applied to diseases of unknown cause to create new hypotheses relating to disease pathogenesis (Calvano et al., 2005; Ergun et al., 2007) and is shown to have prognostic (Korkola et al., 2007) and diagnostic applications (Takahashi et al., 2005; Aldred et al., 2004). However, a study of gene expression change of ovary by chemotherapy damage has not been reported so far, thus, cDNA microarray was utilized in the present study to detect corresponding gene transcripts on ovary damage by DDP. It was found that 19 genes were up-regulated and 90 were down-regulated in the ovary damage by DDP. These results are helpful in explaining the poison effect of ovary by anti-cancer drug and chemotherapy. The inorganic drugs of the metal complexes have been considered as new anticancer drugs since the introduction of cisplatin as an anticancer drug (Chai et al., 2000). Side effects of DDP are significantly increased along with the increases of the administration dosage and drug use time, which were

Table 1. Differently expressed partial genes of premature ovarian failure of the mouse induced by DDP.

Accession	Ratio	Chromosome	Position	Name	Gene card
BC018161	2.125	10	C2	Solute carrier family 25 (mitochondrial carrier, phosphate carrier), member 3	Slc25a3
NM_008517	2.103	10	C3	Leukotriene A4 hydrolase	Lta4h
NM_008806	2.057	5	F	Phosphodiesterase 6B, cGMP, rod receptor, beta polypeptide	Pde6b
AK014593	2.069	-	-	Not Present	
AF037205	2.437	3	D	Ring finger protein 13	Rnf13
AF267660	2.423	11	D	Pyruvate dehydrogenase kinase, isoenzyme 2	Pdk2
NM_013647	2.539	7	A3	Ribosomal protein S16	Rps16
AJ251508	2.113	7	D3	Zinc finger, AN1-type domain 6	Zfand6
NM_010238	2.191	17	D	Bromo domain containing 2	Brd2
AK010677	2.062	1	H2.2	ATPase, Na+/K+ transporting, beta 1 polypeptide	Atp1b1
AK003423	2.393	1	C5	Solute carrier family 16 (mono-carboxylic acid transporters), member 14	Slc16a14
BC021588	2.23	2	F1	RIKEN cDNA 2310003F16 gene	2310003F16Rik
NM_021314	3.071	7	F4	Transforming, acidic coiled-coil containing protein 2	Tacc2
NM_011823	3.708	X	X A1.3	G protein-coupled receptor 34	Gpr34
NM_021487	4.528	X	X F1	Potassium voltage-gated channel, Isk-related family, member 1-like	Kcne1l
NM_008889	2.09	19	A	Protein phosphatase 1, regulatory (inhibitor) subunit 14B	Ppp1r14b
NM_008683	2.161	14	C3	Neural precursor cell expressed, developmentally down-regulated gene 8	Nedd8
AK004325	2.112	14	D1	RIKEN cDNA 1110060D06 gene	1110060D06Rik
NM_025586	2.584	9	A3	Ribosomal protein L15	Rpl15
NM_011439	3.512	1	E4	SRY-box containing gene 13	Sox13
NM_053090	2.051	5	-	Down-regulated by Ctnnb1, a	Drctnnb1a
BC003432	2.074	9	B	Electron transferring flavoprotein, alpha polypeptide	Etfa
AK007641	5.988	18	E1	Sec11-like 3 (S. cerevisiae)	Sec11l3
NM_008536	2.933	3	D	Transmembrane 4 superfamily member 1	Tm4sf1
AK017889	2.222	6	C	CD8 antigen, alpha chain	Cd8a

consistent with other anti-cancer drug (Harrison and Davis, 2001; Cheng et al., 1998). These side effects would lead to premature ovarian failure (POF) and POF could cause some gene expression change, therefore, gene change of ovary damage by DDP might clarify pathophysiological mechanisms of POF.

POF is a highly heterogeneous condition and can be associated to autoimmune disorders (Hoek et al., 1997), ovarian surgery, iatrogenic causes such as chemoradiotherapy or to systemic diseases like galactosaemia (Waggoner et al., 1990), but the details of the etiology of POF is still unknown so far (Fonseca et al., 2012). Genetic causes are also involved in the genesis of POF (Beck-Peccoz and Persani, 2006; Conway et al., 1996; Goswami and Conway, 2005; Suzumori et al., 2007; Woad et al., 2006). X monosomy, X rearrangements and mutations of follicle-stimulating hormone (FSH) b-subunit gene and FSH and luteinizing hormones (LH) receptors genes, as well as other autosomal genes, are known to be responsible for POF (Christine-Maitre et al., 1998). Rosenbaum et al. (2000) found that IL-8 plays important role in POF and low level expression and high level expression of IL-8 in ovary could cause ovary damage. The present study shows that IL-8 expression is significantly down-regulated in ovary damage by DPP in comparison to the normal ovary tissue by cDNA microarray. This might be the direct reason for the irregular ovulation and its cessation after chemoradiotherapy. In addition, over-expression tumor necrosis factor receptor could inhibit secretion of progesterone and estrogen of the granulosa cell (Azcoitia et al., 2005), which was consistent with our result that tumor necrosis factor expression was increased by DDP induced. These results demonstrated that gene changes are involved in POF. However, how these related genes affect POF was unclear; hence clarification of this aspect would require further studies.

In the present study, cDNA microarray analysis was used to determine functionally related genes that may be expressed at different levels between ovary damage by DDP and controls using pooled samples. The results obtained indicated that 19 genes were markedly up-regulated, while 90 genes were down-regulated in the ovary damage by DDP. These data help to reveal complex gene regulation network role in POF caused by chemotherapy and clarify the pathophysiological mechanisms of POF which provide a new idea for ovarian disease diagnosis and treatment.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial support provided by the National Natural Science Foundation of China (no. 81173443), Scientific Research Fund of Liaoning Provincial Education Department, Liaoning Province Science and Technology Program (no. 2009225010-47) and Liaoning BaiQianWan Talents Program (no. 2009921040).

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