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Effects of chemotherapy on global gene expression in non-small cell lung cancer

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Abstract. – INTRODUCTION: Gene expression profiles of peripheral blood monocytes from patients with non-small cell lung cancer (NSCLC) before and after chemotherapy were used to investigate the effect of chemotherapy on gene expression.

MATERIALS AND METHODS: Microarray dataset GSE39345 was downloaded from Gene Expression Omnibus, including 32 NSCLC samples before chemotherapy, 17 NSCLC samples after chemotherapy and 20 healthy samples. Raw data pretreatment and differentially expressed genes (DEGs) analysis between health and NSCLC samples before chemotherapy, health and NSCLC samples after chemotherapy were performed with packages of R. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was done for the common DEGs with WebGestalt to investigate their underlying function.

RESULTS: A total of 904 DEGs were identified in health vs. NSCLC samples before chemotherapy and 2148 in health vs. NSCLC samples after chemotherapy. Further, they were divided into three sets: 584 common DEGs, 320 unique DEGs (health vs NSCLC samples before chemotherapy), and 1564 unique DEGs (health vs NSCLC samples after chemotherapy). Function enrichment analysis showed that these common DEGs were associated with cell cycle and cell differentiation.

CONCLUSIONS: Chemotherapy could not completely reverse the lung cancer development because several cell growth-related genes are still present even after chemotherapy.

Key Words:
Non-small cell lung cancer, Differentially expressed gene, Functional enrichment analysis, Pathway enrichment analysis.

Introduction

Lung cancer is a leading cause of cancer death and its mortality rate has ranked first in both male and female[1]. Based on biological characteristics, clinical treatment and prognosis, lung cancer can be divided into two categories: non-small cell lung cancer (NSCLC) and small cell lung cancer. NSCLC accounts for 80-90% of all lung cancer cases and imposes serious threat to human health[2].

Chemotherapy with cisplatin and gemcitabine has been considered as the first line treatment for NSCLC[3,4]. Nevertheless, due to genetic diversity of tumor cells, the sensitivity to anticancer drugs varies in different patients. In order to improve the efficacy and reduce adverse effect, deep characterization of the molecular mechanisms underlying chemotherapy is rather necessary[5,6].

Microarray technology can detect a series of genes at the same time and, thus, is a useful tool to disclose complicated molecular mechanisms for cancer and cancer patients response to chemotherapy[7]. For example, Lin et al[8] predicted genes including EGF-like domain might be the potential target genes for lung adenocarcinoma development. Dou et al[9] analyzed the gene expression profiles of non-responders and responders to adjuvant chemotherapies for stage III colorectal cancer and found that non-response to adjuvant chemotherapy may be attributed to lower expression of aquaporin-9 (AQP9) gene. Oshita et al[10] conducted a study of cDNA microarray analysis to explore the genes related correlated with the outcome of chemotherapy with paclitaxel (Pac) and irinotecan (CPT) against advanced NSCLC. The results showed that the genes encoding protein phosphatase, IL-1alpha and IgA were independent predictive factors for chemosensitivity.

Though certain achievement has been made, it's still far from enough to guide clinical application and improve curative effect. Therefore, in this study, NSCLC samples before and after chemotherapy were compared with healthy controls to obtain differentially expressed genes

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(DEGs). Functional and pathway enrichment analyses were then conducted to reveal biological functions and pathways associated with chemotherapy, which may provide valuable information for guiding future researches.

Materials and Methods

Microarray Data

Microarray data set GSE39345 was downloaded from Gene Expression Omnibus (GEO), including 32 lung cancer samples before chemotherapy, 17 lung cancer samples after chemotherapy with cisplatin and gemcitabine and 20 healthy samples. Data were collected using GPL6104 Illumina humanRef-8 v2.0 expression beadchip. Probe annotation files were also acquired.

Screening of DEGs

Raw data were converted into recognizable format with package Affy[11,12] of *R*, and then was normalized with median method. Two groups of comparison were carried out: health vs. NSCLC samples before chemotherapy and health vs. NSCLC samples after chemotherapy. Package Limma[13] was chosen for differential analysis. Multiple testing correction was applied with Benjamini-Hochberg method[14]. False discovery rate (FDR) < 0.05 and |logFC(fold change)| > 1 were set as the cut-offs to screen out DEGs.

Comparative Analysis of the two Groups of DEGs

Unique DEGs for each group and common DEGs were selected out. Common DGEs might not be related to chemotherapy while unique DEGs could help to reveal the mechanisms of chemotherapy.

Functional Enrichment Analysis

In order to disclose the biological functions enriched in these DEGs, DAVID (Database for Annotation, Visualization and Integrated Discovery) tool[15,16] was used for functional enrichment analysis. FDR < 0.05 was set as the threshold.

Pathway Enrichment Analysis for Common DEGs

KEGG (Kyoto Encyclopedia of Genes and Genomes) enrichment analysis was performed for common DEGs with WebGestalt (WEB-based Gene SeT AnaLysis Toolkit)[17,18]. FDR < 0.05 was set as the threshold.

Results

Differentially Expressed Genes

After a good performance of data normalization was achieved, DEGs were screened using Limma method. As a result, a total of 904 DEGs were identified in health vs. NSCLC samples before chemotherapy and 2148 in health vs. NSCLC samples after chemotherapy (Figure 1). Further, these DEGs were divided into three sets: (1) 584 common DEGs, which could not be changed after chemotherapy and, thus, other therapeutic measures are needed; (2) 320 unique DEGs in health vs. NSCLC samples before chemotherapy, which had been restored after chemotherapy; (3) 1564 unique DEGs in health vs. NSCLC samples after chemotherapy, which might be associated with side-effects of chemotherapy (Figure 1).

Functional Enrichment Analysis

Functional enrichment analysis was performed for the 3 sets of DEGs and results are shown in Figure 2. As expected, different terms were enriched in the 3 sets of DEGs. The most significant terms for each gene set were "positive regulation of developmental process" (584 common DEGs), "positive regulation of cell differentiation" (320 unique DEGs) and "cell cycle" (1564 unique DEGs).

These results suggested that chemotherapy could affect cell differentiation and cell cycle, which explained its clinical effect in treatment of cancers. Meanwhile, a certain part of DEGs related with development remained unaffected, indicating limited influence of chemotherapy in cellular process.

Pathway Enrichment Analysis Results

Furthermore, KEGG enrichment analysis was also conducted for common DEGs with We-

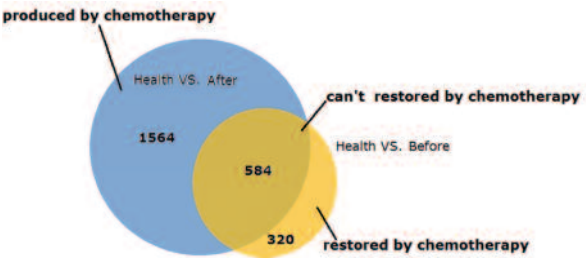

Figure 1. Venn diagram for differentially expressed genes between health vs. non-small cell lung cancer samples after chemotherapy and health vs. non-small cell lung cancer samples before chemotherapy.

Table I. Five significantly over-represented pathways in common differentially expressed genes.

Term	Count	*p* value
hsa04060: Cytokine-cytokine receptor interaction	15	0.002337718
hsa04350: TGF-beta signaling pathway	7	0.013948038
hsa04520: Adherens junction	6	0.030446774
hsa05200: Pathways in cancer	14	0.034954137
hsa04510: Focal adhesion	10	0.039104582

bGestalt and results (Table I). A total of 5 terms were disclosed, including "cytokine-cytokine receptor interaction", "TGF (Transforming Growth Factor)-beta signaling pathway", "adherens junction", "pathways in cancer" and "focal adhesion". Among them, "Cytokine-cytokine receptor interaction" was the most significant one, containing 15 DEGs (Figure 3) that belonged to several cytokine families like CXC family, CC family, IL family and TNF family.

Discussion

In present study, a comparative analysis was carried out with gene expression data for NSCLC before chemotherapy, NSCLC after chemotherapy with cisplatin and gemcitabine and healthy control. Two groups of DEGs were obtained for health vs. NSCLC samples before chemotherapy and health *vs*. NSCLC samples after chemotherapy, which were further divided into 3 sets: unique DEGs in health vs. NSCLC samples before chemotherapy and health *vs*. NSCLC samples after chemotherapy, as well as common DEGs. Those unique DEGs might be associated with chemotherapy. Functional enrichment analysis indicated that those genes were enriched in cell cycle and cell differentiation.

Cell cycle control is closely linked with cancer chemotherapy[19]. Pohl et al[20] report that expression levels of p21, p53 and cyclin D3 are significantly increased after preoperative chemotherapy in breast carcinomas. Mohamed et al[21] analyze cell cycle-related proteins in mediastinal lymph nodes of patients with N2-NSCLC and find that p53 and p21 expressions are significantly related to the response to platinum-based chemotherapy. We obtained 55 DEGs related to cell cycle. Cyclin D1 (CCND1) forms a complex with and functions as a regulatory subunit of CDK4 or CDK6, whose activity is required for cell cycle G1/S transition. Mutations, amplification and overexpression of this gene, which alters cell cycle progression, are observed frequently in a variety of tumors and may contribute to tumorigenesis. Ishiguro et al[22] report that CCND1 amplification predicts sensitivity to chemotherapy and chemoradiotherapy in head and neck squamous cell carcinoma. Feng et al[23] suggest that CCND1 can serve as a predictive biomarker of neoadjuvant chemotherapy in patients with locally advanced head and neck squamous cell carcinoma. Gautschi et al[24] find that CCND1 A870G gene polymorphism modulates smoking-induced lung cancer risk and response to platinum-based chemotherapy in NSCLC patients. CCND2 also presents a prognostic value in newly diagnosed multiple myeloma treated with high-dose chemotherapy and tandem autologous stem cell transplantations[25].

Induction of cell differentiation is a strategy to treat cancers[26,27]. Brambilla et al[28] report a cytotoxic chemotherapy induces cell differentiation in small-cell lung carcinoma. A total of 13 unique DEGs were found in health vs. NSCLC samples before chemotherapy in our study. Colony stimulating factor 1 (CSF1) is a cytokine that controls the production, differentiation, and function of macrophages. It can predict the risk of recurrence and metastasis in breast cancer[29]. It is involved in macrophage recruitment and, thus, related to regulation of response to chemotherapy[30]. Peroxisome proliferator-activated receptor gamma (PPARG) activation by specific agonists leads to growth inhibition, apoptosis and differentiation of tumor[31]. Therefore, PPARG agonists are often combined with cisplatin chemotherapy to treat cancers[32].

Common DEGs were also investigated to have an insight into the basic regulatory mechanisms of NSCLC. Functional enrichment analysis showed that "positive regulation of developmental process" was most significantly over-represented, containing 22 genes. Besides, many metabolic pathways were also revealed, certifying those common DEGs were involved in rather fundamental process and thus remained unaffected by chemotherapy. Pathway enrichment analysis pointed out that "cytokine-cytokine receptor inter-

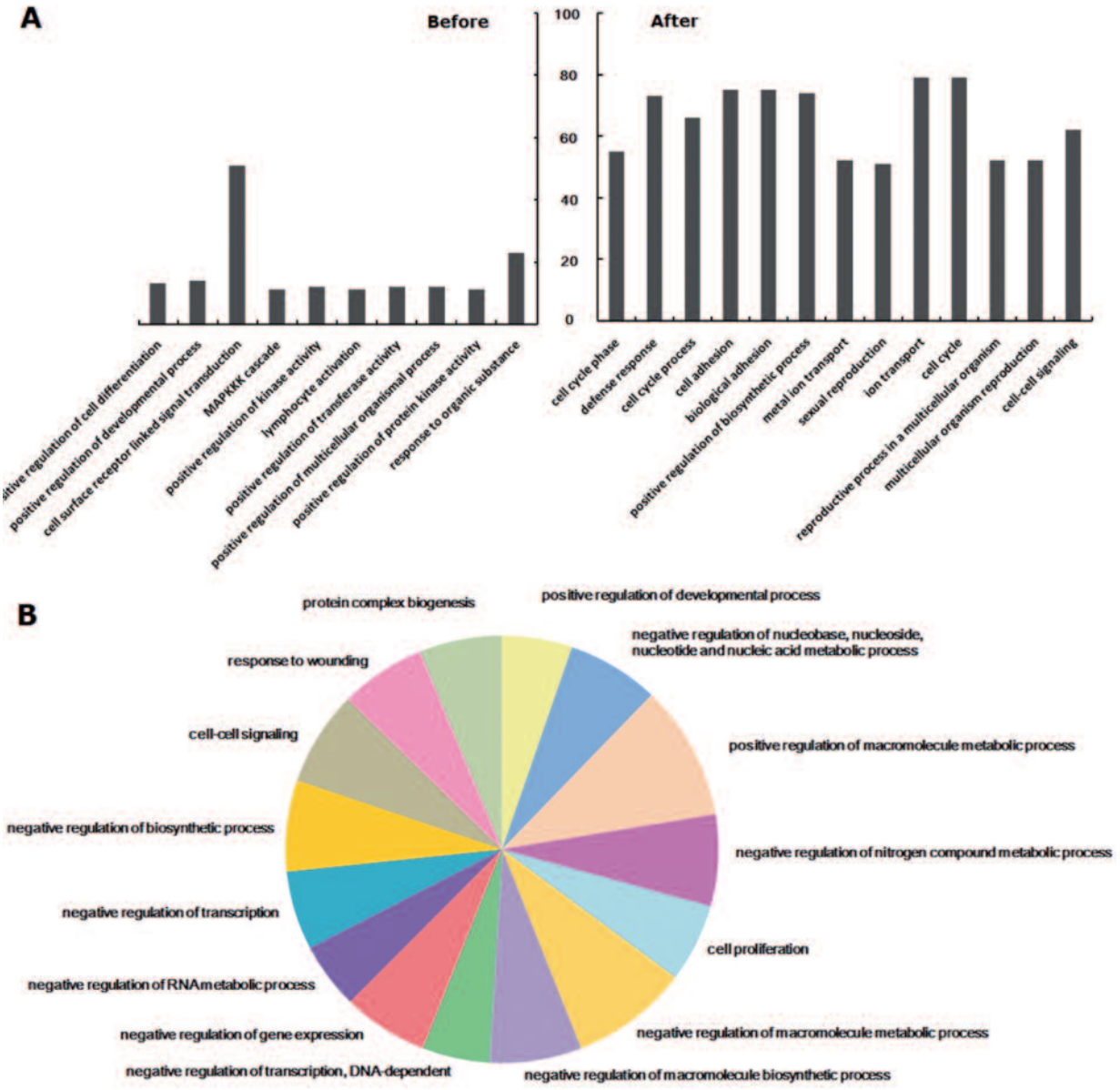

Figure 2. Functional terms significantly over-represented in the 3 sets of differentially expressed genes (DEGs). ***A,*** Unique DEGs in non-small cell lung cancer samples before chemotherapy vs. health (left) and unique DEGs in non-small cell lung cancer samples after chemotherapy vs. health (right). ***B,*** Common DEGs.

action" was the most significant one. Several families of cytokines were included, such as CXC family, CC family, IL family and TNF family. Chemokines and cytokines mediate the link between innate immunity, inflammation, and cancer[33-35]. Immunotherapy just exploits the functions of cytokines to treat cancers. Chemokine (C-C motif) ligand 3 (CCL3) is found to be involved in hepatocellular carcinoma[36], oral squamous cell carcinoma[37] and so on. Zhu et al report[38] the production and upregulation of chemokine (C-X-C motif) ligand 6 (CXCL6) by IL-1[2] and hypoxia in small cell lung cancer. Our findings could offer a good guidance for future researches.

Conclusions

Overall, a range of DEGs associated with chemotherapy with cisplatin and gemcitabine

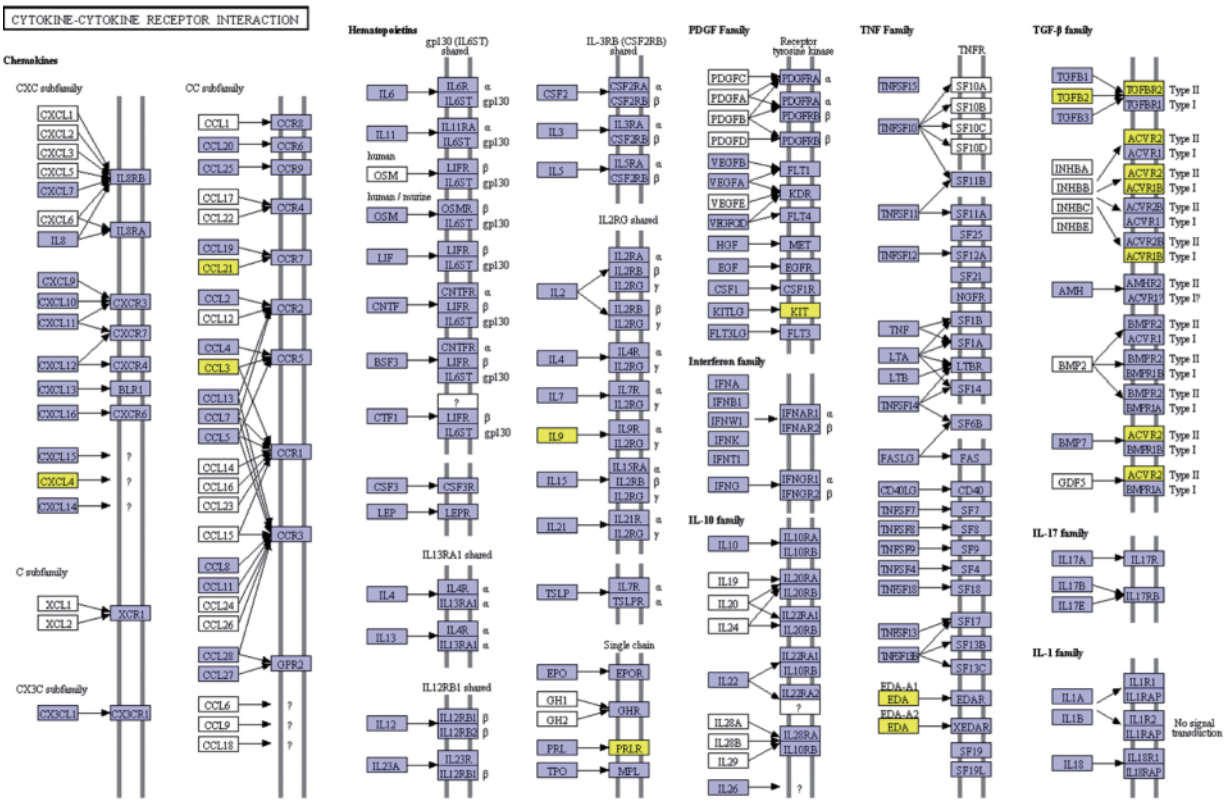

Figure 3. Cytokine-cytokine receptor interaction pathway. Yellow boxes indicate differentially expressed genes identified in this study.

were identified in our study, especially those associated with cell cycle and cell differentiation. They could be utilized to improve the effectiveness of chemotherapy and even design new treatments. In addition, many cytokines were also discovered as common DEGs, which might be exploited to develop targeted therapies.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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