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Original Article

Identification of genes associated with apoptosis-sensitive acute lymphoblastic leukemia responsive to ionising radiation by bioinformatics analyses

Ying Sun, Meng Huo, Chunyan Zhang, Rengui Wang, Tingguo Wen

Radiation Center, Beijing Shijitan Hospital of Capital Medical University, Beijing, China

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Abstract: Objective: This study was aimed to characterize radiosensitivity for apoptosis sensitive acute lymphoblastic leukemia (ALL) by identifying differential genes using bioinformatics analysis. Materials and Methods: The microarray data of GSE13280 were downloaded from the Gene Expression Omnibus database. The differentially expressed genes (DEGs) between apoptosis-sensitive B-precursor ALL tumors responsive to ionising radiation (IR) and not responsive to IR were identified. Then, biological process (BP) and pathway enrichment analyses of DEGs were performed, and protein-protein interaction (PPI) network was constructed. Results: Total 59 up-regulated and 48 down-regulated DEGs were selected in IR samples. Besides, 109 PPI relationships were obtained from the 107 DEGs. The up-regulated DEGs, such as BCL2-associated X protein (*BAX*) and Fas cell surface death receptor (*FAS*), were mainly enriched in the BP terms related to apoptosis and p53 signaling pathway. The down-regulated DEGs including cyclin D3 (*CCND3*) were mainly enriched in the BP terms related to cell proliferation. Additionally, FBJ Murine Osteosarcoma Viral Oncogene Homolog and CD40 molecule, TNF receptor superfamily member 5 (*CD40*) were found to be hub genes in the PPI network. Conclusions: DEGs including *BAX*, *FAS*, *CCND3* and *CD40* may be associated with the radiosensitivity of ALL.

Keywords: Acute lymphoblastic leukemia, differentially expressed genes, functional enrichment analysis, protein-protein interaction network

Introduction

Acute lymphoblastic leukemia (ALL) is the most common pediatric malignancy with a peak incidence at age 2 to 5 years, and remains the leading cause of cancer-related death in children and adolescents [1]. It is characterized by the overproduction and accumulation of cancerous, immature lymphoblasts [2]. Despite considerable progress in cure rate over the past 10 to 15 years, 20% of children with B-precursor ALL still experience disease progression with current treatment [3].

Generally, tumor is caused by the damage of DNA which leads to uncontrolled cell proliferation [4]. Study has found that in animals and humans, ALL is associated with exposure to radiation and chemical radiation which is considered to cause damage to cellular DNA [5]. Presently, ionising radiation (IR) is an established DNA-damaging agents in ALL treatment [6]. Weston *et al*. [7] have reported that some ALL exhibits defective induction of apoptosis following IR. Kruyt [8] and Gong *et al*. [9] have reported that in B-precursor ALL cell lines, IR can activate the tumor necrosis factor-related apoptosis-inducing ligand signaling pathway, which has been found to cooperate synergistically with the cytotoxic effect of radiation. Despite progresses achieved in exploring the pathophysiology of IR-related ALL, there remains a lack of understanding of the molecular basis behind this disease.

Marston *et al*. [10] addressed the mechanisms behind *in vitro* cellular responses to IR-induced DNA double strand breaks in 74 paediatric ALL patients. They found an apoptosis-sensitive response in 64% of patients and an apoptosis-

resistant response in the remaining 36% of leukaemias. Importantly, they deposited all microarray data of these patients in Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) under accession number GSE13280. Data above suggest that most of ALL are apoptosis-sensitive. Therefore, we downloaded the gene expression microarray data GSE13280 associated with apoptosis-sensitive response to explored the roles of IR in apoptosis-sensitive ALL. In the present study, we identified the differentially expressed genes (DEGs) between apoptosis-sensitive B-precursor ALL tumors responsive to IR and not responsive to IR. Then functional enrichment analysis and protein-protein interaction (PPI) network construction for the DEGs were performed. We aimed to characterize radiosensitivity for apoptosis-sensitive ALL by identifying differential genes.

Materials and methods

Affymetrix microarray data

The exon array data of GSE13280 [10] were downloaded from GEO database. Twenty-two B-precursor ALL tumors including 11 apoptosis-sensitive responsive to 5 Gy IR (cobalt Co^{60}) for 8 h and 11 apoptosis-sensitive not responsive to IR were analyzed based on the platform of [HG-U133A] Affymetrix Human Genome U133A Array (Affymetrix Inc., Santa Clara, California, USA).

Data preprocessing and differential expression analysis

The original array data were preprocessed with background correction, quartile data normalization and probe summarization by robust multi array average (RMA) [11], then they were converted into expression measures by the algorithm in R affy [12] package. The paired t-test based on the limma package [13] in R was used to identify the DEGs between the two groups of samples. The genes with and adjusted p-value < 0.05 and $|\log_2 FC| > 1$ were regarded as DEGs.

PPI relationship prediction

The Search Tool for the Retrieval of Interacting Genes (STRING) database (http://string-db.org/) [14] is a precomputed global resource which has been designed to evaluate the PPI information. In this paper, the STRING online tool was applied to predict the PPI relationships of DEGs and only those experimentally validated interactions with a combined score > 0.4 was selected as significant.

Functional enrichment analysis

The Database for Annotation, Visualization and Integrated Discovery (DAVID) [15] is a comprehensive set of functional annotation tool and has been developed for relating the functional terms with gene lists by clustering algorithm. In this study, the DEGs involved in the PPI relationships were performed functional enrichment analysis, including Gene ontology (GO) biological process (BP) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) [16] pathways analyses, using the DAVID online tool.

PPI network construction

Based on the obtained PPI relationships, the PPI network was constructed using the Cytoscape software [17] which was used for visualization and analysis of biological networks. From the previous obtained biological network we found that most of the PPI networks obeyed the scale-free attribution. Thus, the connectivity degree was analyzed by statistics to obtain the important nodes (hub proteins) [18] in the PPI network.

Results

Identification of DEGs

For the dataset of GSE13280, 107 DEGs in samples responsive to IR were selected, including 59 up-regulated DEGs and 48 down-regulated DEGs.

PPI relationship prediction and functional enrichment analysis

In total, 109 PPI relationships were obtained from the 107 DEGs. The DEGs that were involved in the PPI relationships were enriched in several BP terms and pathways. Specifically, the up-regulated DEGs, such as BCL2-associated X protein (*BAX*) and Fas cell surface death receptor (*FAS*), were mainly enriched in the BP terms related to cell death and pathways of p53 signaling pathway and Apoptosis (**Table 1**). The down-regulated DEGs including

Table 1. Functional enrichment analysis for the up-regulated differentially expressed genes (DEGs)

Category	Term	Description	Count	*P*-value
BP	GO:0042981	Regulation of apoptosis	16	6.18E-08
BP	GO:0043067	Regulation of programmed cell death	16	7.04E-08
BP	GO:0010941	Regulation of cell death	16	7.39E-08
BP	GO:0006915	Apoptosis	13	8.65E-07
BP	GO:0012501	Programmed cell death	13	1.01E-06
BP	GO:0008219	Cell death	13	5.51E-06
BP	GO:0016265	Death	13	5.91E-06
BP	GO:0042127	Regulation of cell proliferation	13	1.38E-05
BP	GO:0007242	Intracellular signaling cascade	13	1.15E-03
BP	GO:0006955	Immune response	12	2.25E-05
KEGG	hsa04115	p53 signaling pathway	9	2.30E-09
KEGG	hsa04060	Cytokine-cytokine receptor interaction	10	9.00E-06
KEGG	hsa04210	Apoptosis	5	1.37E-03
KEGG	hsa04621	NOD-like receptor signaling pathway	3	4.81E-02

BP: Biological process; KEGG: Kyoto Encyclopedia of Genes and Genomes.

Table 2. Functional enrichment analysis for the down-regulated differentially expressed genes (DEGs)

Category	Term	Description	Count	*P*-value
BP	GO:0008283	Cell proliferation	7	1.25E-03
BP	GO:0006955	Immune response	6	4.25E-02
BP	GO:0007610	Behavior	5	4.16E-02
BP	GO:0002684	Positive regulation of immune system process	4	2.89E-02
BP	GO:0022409	Positive regulation of cell-cell adhesion	2	3.05E-02
BP	GO:0045730	Respiratory burst	2	3.59E-02
BP	GO:0051412	Response to corticosterone stimulus	2	4.40E-02
KEGG	hsa04662	B cell receptor signaling pathway	4	1.50E-03

BP: Biological process; KEGG: Kyoto Encyclopedia of Genes and Genomes.

cyclin D3 (*CCND3*), CD79a molecule, immunoglobulin-associated alpha (*CD79A*) and CD79B were mainly enriched in the BP terms related to cell proliferation and immune (**Table 2**).

PPI network construction

Based on the STRING database, the PPI network was constructed (**Figure 1**). Fourteen nodes were selected as hub genes (degree ≥ 5), such as FBJ murine osteosarcoma viral oncogene homolog (*FOS*, degree = 17), CD40 molecule, TNF receptor superfamily member 5 (*CD40*, degree = 12), and growth arrest and DNA-damage-inducible, alpha (degree = 10).

Discussion

In the present study, a total of 59 up-regulated and 48 down-regulated DEGs were identified between IR and control samples through gene expression profile of GSE13280. The up-regulated DEGs, such as *BAX* and *FAS*, were mainly enriched in the BP terms related to apoptosis and p53 signaling pathway. The down-regulated DEGs were mainly enriched in the BP terms related to cell proliferation and immune response. Additionally, *FOS* and *CD40* were found to be hub gene in the PPI network. The results suggested that IR might affect the progression of ALL by regulating these genes or pathway.

Apoptosis is an active process that can be induced through signal transduction by DNA-damaging agents including IR. The regulation of apoptosis is delicately balanced by signaling pathways between apoptosis-promoting factors such as p53 [19]. In the present study, BP terms related to apoptosis and p53 signaling

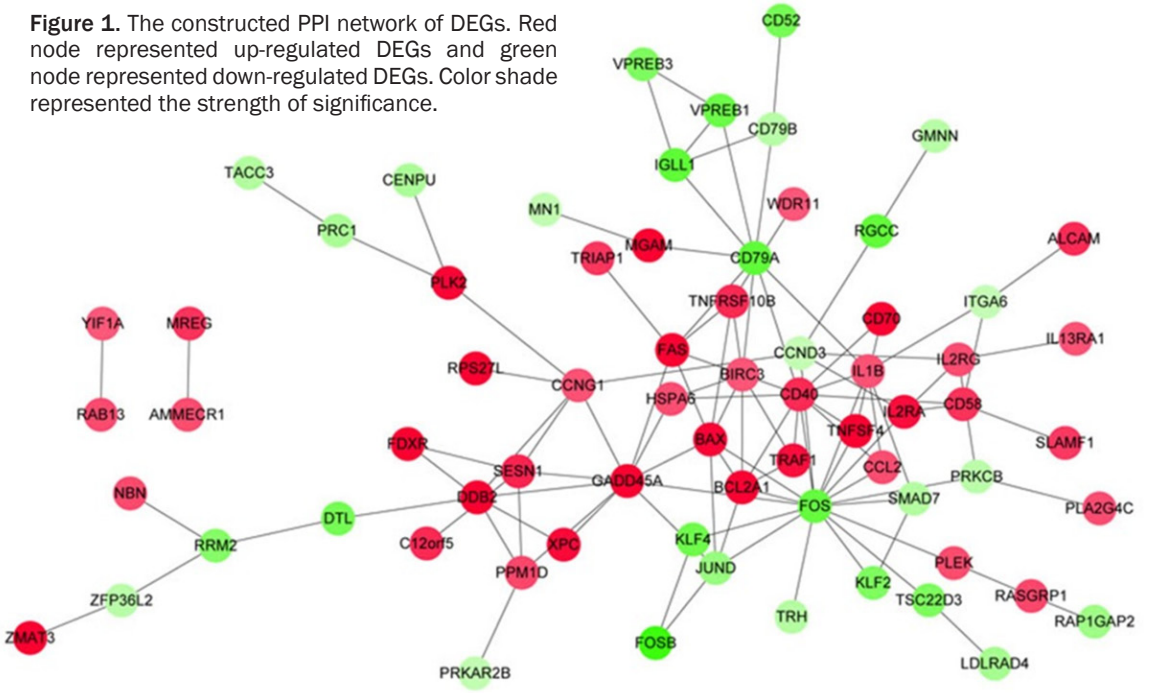

Figure 1. The constructed PPI network of DEGs. Red node represented up-regulated DEGs and green node represented down-regulated DEGs. Color shade represented the strength of significance.

pathway were found enriched by some up-regulated DEGs, such as *BAX* and *FAS*. *BAX* encoding protein belongs to the BCL2 protein family which regulates and contributes to programmed cell death or apoptosis [20]. Bax has the ability to form heterodimers with the other BCL2 protein family members and acts as a positive regulator of apoptosis [21]. Additionally, *BAX* has been found to be expressed in a variety of acute myelogenous leukemia and ALL cell line [21]. For the other DEGs *FAS*, its encoding protein is a member of the TNF-receptor superfamily which has been shown to play an important role in the physiological regulation of apoptosis, and has been implicated in the pathogenesis of various malignancies [22]. Lenardo *et al*. [23] revealed that the apoptosis of B- and T-lymphocyte was initiated by the binding of the FAS ligand to FAS. Taken together, IR may promote apoptosis through up-regulating the DEGs such as *BAX* and *FAS*.

In addition, the down-regulated DEGs were mainly enriched in BP terms related to cell proliferation. In other words, the up-regulated DEGs in samples not responsive to IR were mainly associated with functions of cell proliferation, such as*CCND3*. CCND3 belongs to the highly conserved cyclin family, which is a key regulator of the progression from G1- to S-phase of the cell cycle [24, 25]. Doglioni *et al*. [26] have suggested that *CCND3* expression is associated with cell proliferation in lymphoid tissues. Filipits *et al*. [27] also reported that high CCND3 expression was an prognostic factor associated with poor clinical outcome in patients with diffuse B-cell lymphoma. In our study, *CCND3* was up-regulated in the ALL tumors B-precursor not responsive to IR, which was in accordance with the findings above. Therefore, the down-regulation of *CCND3* in the IR samples may be due to the influence of IR.

Furthermore, the down-regulated DEGs were mainly enriched in GO terms associated with immune response as well. Study has suggested that host genetic variation within immune response genes may contribute to risk of childhood ALL [28]. For instance, in our study, *CD79A* and *CD79B*were found down-regulated in term of immune response. The two genes play multiple and diverse roles in B cell development and function [29]. Lai *et al.* [30] indicated that CD79A was a reliable marker for ALL of B cell lineage. Additionally, *CD79B* methylation has been found in leukemic cells and has been suggested to be a critical determinant of lineage specification in ALL [31]. Taken together, immune response may play important roles in the development of ALL when tumor cells of

ALL are not responsive to IR. In the PPI network, *CD40* was a up-regulated hub gene with a higher degree. CD40 belongs to the tumor necrosis factor receptor superfamily and is expressed on a wide variety of cell types [32]. This receptor has been found to be essential in regulating a broad variety of immune and inflammatory responses [33]. Additionally, CD40 has been found to have a widespread expression on tumor cells, including lymphomas, myeloma and some carcinomas [34]. Importantly, some evidence suggests that CD40-CD154 interactions may play a role in the control of B cell haematopoiesis. In the present study, *CD40* was up-regulated in the IR group, which might indicate that *CD40* was a gene response to IR in ALL.

In conclusion, our data provide a comprehensive bioinformatics analysis of DEGs and functions which may be responsive to IR. DEGs including *BAX*, *FAS*, *CCND3* and *CD40* in ALL may be involved in the radiosensitivity of ALL. However, further genetic studies with larger sample size are still needed to confirm our observation.

Disclosure of conflict of interest

None.

Address correspondence to: Rengui Wang and Tingguo Wen, Radiation Center, Beijing Shijitan Hospital of Capital Medical University, Beijing 100038, China. E-mail: renguiwang@aliyun.com (RGW); wtg195911@hotmail.com (TGW)

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