Identification of therapeutic target genes with DNA microarray in multiple myeloma cell line treated by IKKβ/NF-κB inhibitor[1]

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ABSTRACT

PURPOSE: To explore the mechanism of resistance to IKKβ inhibitor in multiple myeloma (MM) cells and uncover novel therapeutic targets for MM.

METHODS: We downloaded the microarray data (GSE8476) from GEO (Gene Expression Omnibus) database. The data were derived from the human MM cells lines (L363 cells) treated with IKKβ inhibitor MLN120b (MLN) for eight, 12 and 24 hours. Furthermore, we applied the Search Tool for the Retrieval of Interacting Genes (STRING) and Expression Analysis Systematic Explorer (EASE) database to construct protein-protein interaction networks and identified over-represented pathway among DEGs (differentially expressed genes).

RESULTS: We obtained 108 DGEs in 8h vs. 12h group and 101 ones in 8h vs. 24h group. Most of DGEs were found to be involved in biological regulation. The significant pathways were Ig A pathway and the CAMs pathways. In addition, 24 common DGEs were found in the networks of the two groups such as ICAM 3 and SELL.

CONCLUSION: Intercellular adhesion molecule 3 and SELL may be potential targets in multiple myeloma treatment in the future.

Key words: Multiple Myeloma. Protein Interaction Maps. NF-kappa B.

Introduction

Multiple Myeloma (MM), also named plasma cell myeloma, is a malignant tumor of plasma cells, which is characterized by the accumulation of abnormal plasma cells in the bone marrow resulting in the intervention of normal hematopoiesis[1]. MM can result in lesions, hypercalcemia and the production of an abnormal antibody which contributes to various kidney problems[1]. It is reported that 20.000 new cases are diagnosed with MM in United States annually, among which around 10.710 patients died from MM[2,3]. Besides, there is an increasing trend of MM incidence in China[4]. MM has posed a great threat to public health globally.

Recently, a growing body of studies were conducted to explore the potential mechanism underlying MM development and uncover the effective therapy[5-7]. Nuclear factor kappa pathway has been reported to play a key role in the progression of MM. Nuclear factor kappa-B (NF-kappa B, NF-κB), a protein complex, is associated with the DNA transcription, cellular responses to stimuli and immune response to infection[8-10]. As a critical factor, IKKβ (inhibitor of NF-κB kinase subunit beta) can phosphorylate the IκB (inhibitor kappa-B) proteins that lead to the polyubiquitination and degradation of IκB, and finally activate the NF-κB signaling[11]. The overexpression of NF-κB can lead to the onset of multiple diseases such as MM[12]. Many investigations have focused on the inhibition of NF-κB pathways in order to find a targeting therapy for MM[13,14]. However, the existing mechanism is required depth study.

MLN120b (MLN), served as an IKKβ inhibitor, can suppress the growth of MM cell lines by mediating the NF-κB pathway [15]. In present study, we applied bioinformatics techniques to analyze the genes expression profiles and gene functions in MM cell line treated by IKKβ inhibitor MLN. The purpose of this paper was to provide potential targets for MM treatment.

Methods

Affymetrix microarray and differential expression gene analysis

The gene expression profile dataset GSE8476 was downloaded from GEO (Gene Expression Omnibus) database (http://www.ncbi.nlm.nih.gov/geo/) based on the platform of NCI/Staudt human 15K v13, which was deposited by Annunziata *et al*.[16]. The microarray data were derived from the human L363 cells treated with MLN at different time points (8h, 12h, and 24h). There were a total of 8 samples (including 2 samples collected at 8h, 3 ones at 12h and 3 ones at 24h).

The probe-level data were converted into expression measures and the missing data were imputed based on the K Nearest Neighbors (KNN) method in R[17]. Then the raw data were normalized using the median method[18]. All the samples were assigned into two groups (8h vs. 12h and 8h vs. 24h group) for comparison using the samples of 8h as reference. The differentially expressed genes (DEGs) in the two groups were identified by limma package in R[19]. $p<0.05$ and $|logFC| > 1$ were defined as the cutoff value.

Clusters of orthologous groups of proteins classification

The database of Clusters of Orthologous Groups of proteins (COGs) is a tool for phylogenetic classification of proteins encoded in complete genomes, which currently contains 45350 proteins encoded in 30 complete genomes (http://www. ncbi.nlm. nih. gov/COG) [20].

All the sequences information of proteins in COG database was firstly downloaded. To investigate the DEGs in functional levels, we performed the protein sequence similarity searches via the basic local alignment search tool BLASTX[21]. The similarity degrees between the sequences of DEGs and COGs were evaluated and E values < 1e-05 was defined as threshold value.

Pathway enrichment analysis

Expression Analysis Systematic Explorer (EASE) is a powerful tool for functional analysis of a list of genes[22]. The enriched pathways of DEGs were selected using the Fisher's exact test in EASE and the dysfunctional pathway with $p < 0.05$ was defined as significant.

Construction of protein-protein interaction networks

Search Tool for the Retrieval of Interacting Genes (STRING) database provides comprehensive information of predicted and experimental interactions of proteins[23]. The DEGs from two groups (8h vs. 12h; 8h vs. 24h) were mapped into protein-protein interactions based on STRING database. All the associations of proteins were displayed with a confidence score. We selected significant protein pairs with confidence score > 0.7 and constructed the PPI interaction networks by Cytoscape software[24].

Results

Differentially expressed genes analysis

In order to explore the DEGs in the intervention process of MM, the publicly available microarray dataset GSE8476 was downloaded from GEO. The DEGs with $p < 0.05$ and $|logFC| > 1$ were selected for further analysis. Finally, we obtained 209 DEGs including 108 ones in 8h vs. 12h group and 101 ones in 8h vs. 24h group.

Clusters of orthologous groups of proteins (COGs) classification analysis

To investigate the functional change of genes in the intervention process of MM cell line, we performed COG classification analysis. As shown in Figure 1, the DEGs were enriched in 13 different biological processes including biological regulation, metabolic process, stimulus-response, and cell communications. Most of DGEs were found to be involved in biological regulation. The numbers of DGEs involved in various biological processes were similar between 8h vs. 12h group and 8h vs. 24h group.

PPI networks

We established the PPI networks of DEGs from two groups based on the interactions predicted by the STRING database. The networks of DGEs in 8h vs. 12h group comprised 93 interactions among 64 DEGs, while in 8h vs. 24h group, there were 105 interactions involved with 58 DEGs (Figure 2 A, B). In addition, 24 common DGEs were found in the networks of the two groups such as ICAM 3 and SELL (Figure 2 A, B).

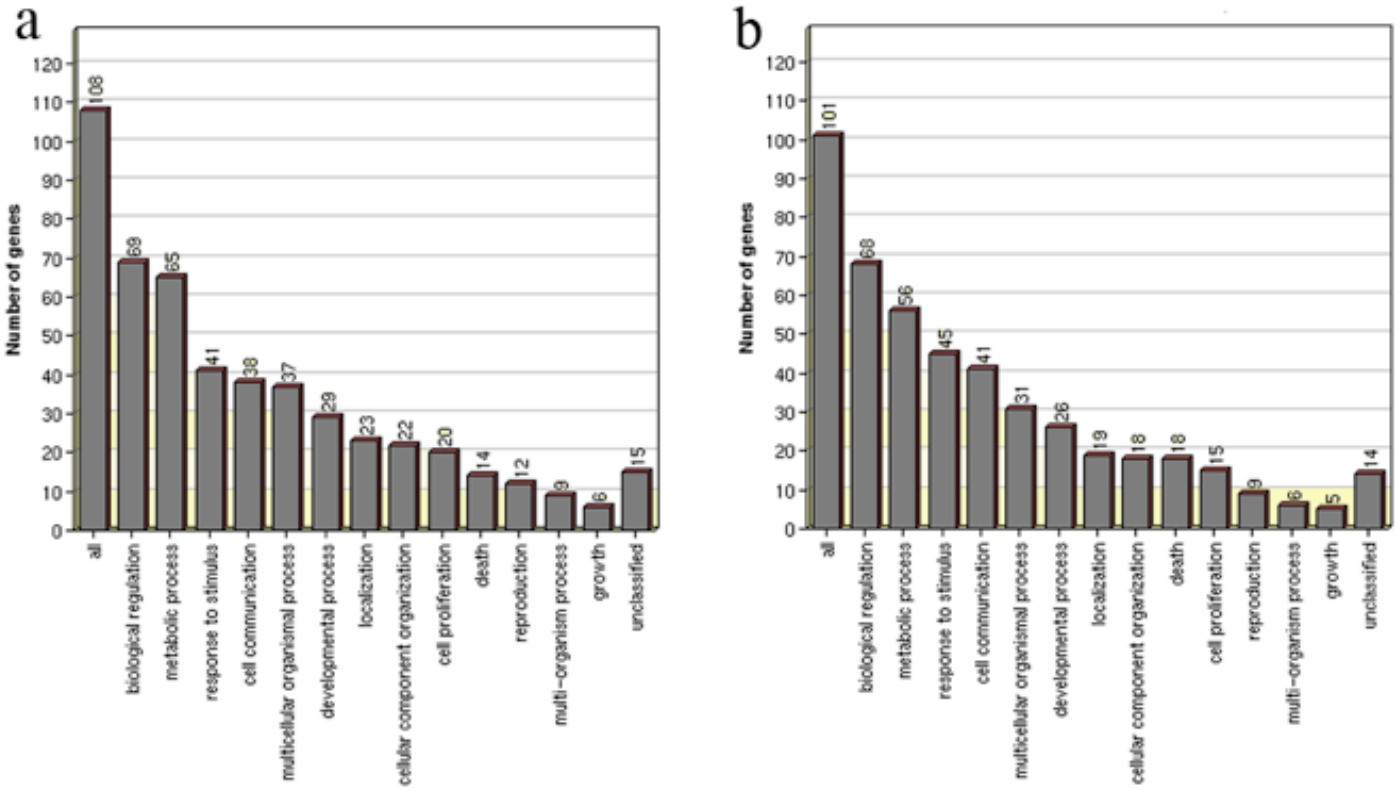

FIGURE 1 - COG classification of DEGs in 8h vs. 12h group (A) and 8h vs. 24h (B). The bar graphs represent the numbers of genes involved in different biological processes.

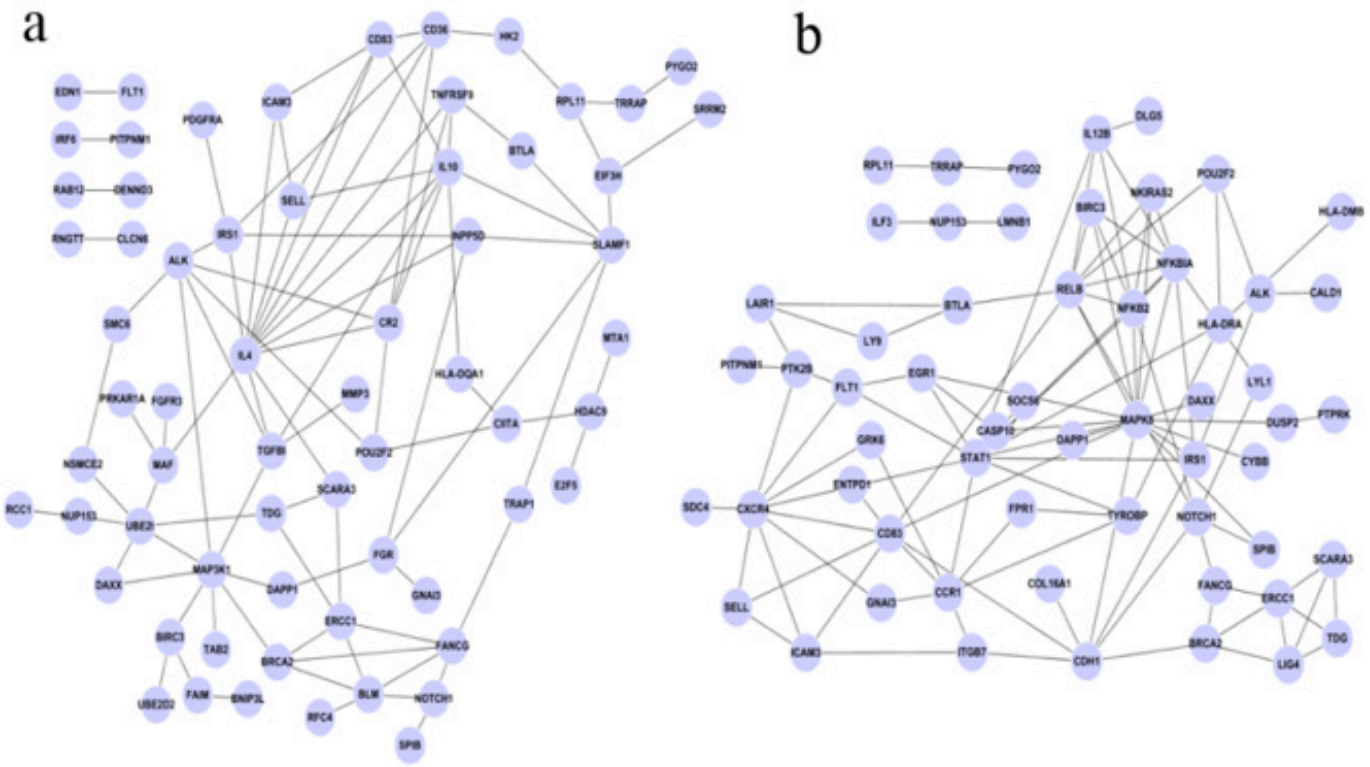

FIGURE 2 - PPI networks of DEGs in 8h vs. 12h group (A) and 8h vs. 24h group (B).

Identification of dysfunctional pathway

The gene-set enrichment analysis was performed by applying EASE. In 8h vs. 12h group, the significant biological pathways enriched by 64 DEGs were intestinal immune network for IgA (Immunoglobulin A) production and autoimmune thyroid diseases. The pathway of immune network for IgA production was defined as the most significant ($p<0.04$) which involved with three DEGs (IL4, Interleukin-4; HLA-DQA1, human leukocyte antigen; IL10, Interleukin-10) (Table 1). In 8h vs. 24h group, the most significant pathway was cell adhesion molecules (CAMs) pathway ($p=2.80$ E-04) and seven DEGs (*ELL*: RNA polymerase II elongation factor; ITGB7: integrin β7; ICAM3: intercellular adhesion molecule 3; CDH1: Cadherin-1; HLA-DMB: HLA class II histocompatibility antigen, DM beta chain; SDC4: syndecan-4; and HLA-DRA: HLA class II histocompatibility antigen, DR alpha chain) were enriched in it. Other dysfunctional pathways in 8h vs. 24h group included immune network for IgA production pathway, cancer involved pathway and toll-like receptor signaling pathway (Table 2).

TABLE 1 - The enriched pathways among DEGs in 8h vs. 12h group.

Term	Pathways	Count	PValue	Genes
hsa04672	Immune network for IgA production	3	0.039731139	IL4, HLA-DQA1, IL10
hsa05320	Autoimmune thyroid disease	3	0.042732582	IL4, HLA-DQA1, IL10

TABLE 2 - The enriched pathways among DEGs in 8h vs. 24h group.

Term	Pathways	Count	P Value	Genes
hsa04514	Cell adhesion molecules (CAMs)	7	2.80E-04	SELL, ITGB7, ICAM3, CDH1, HLA-DMB, SDC4, HLA-DRA
hsa04672	Immune network for IgA production	4	0.004802596	CXCR4, ITGB7, HLA-DMB, HLA-DRA
hsa05200	Pathways in cancer	7	0.025882116	NFKBIA, BRCA2, CDH1, MAPK8, NFKB2, BIRC3, STAT1
hsa04620	Toll-like receptor signaling pathway	4	0.033818056	NFKBIA, MAPK8, IL12B, STAT1

Discussion

Multiple Myeloma (MM), a malignant tumor formed by the plasma cells of the bone marrow, is an incurable disease[25]. The role of NF-κB associated pathways in inhibiting plasma cell apoptosis[26] is verified by many investigators. IKK plays a crucial role in NF-κB pathway, which is composed of two protein subunits (IKKα and IKKβ). Previous reports suggested that IKKα and IKKβ induced the activation of NF-κB, while NF-κB signal transduction pathways were blocked with the absence of IKKα and IKKβ[27].

In our work, we downloaded the gene expression data derived from L363 cells (MM cell line) treated by IKKβ inhibitor MLN120b at different time points to explore the significant DEGs and pathways underlying the mechanism of the resistance of MM cells to MLN. Our results suggested that the most significant pathway was IgA production in immune network in 8h vs. 12h group and cell adhesion molecules (CAMs) pathway in 8h vs. 24h group.

IgA plays an essential role in mucosal immunity. The concentration of IgA (secretory immunoglobulin) is correlated with the progression of oral cancer[28]. Monoclonal IgA was determined to be extremely accumulated in the serum of patients with MM[29]. *IL-4* and *IL-10* were found to be enriched in the pathway of IgA production in immune network. IL-4 and IL-10 are critical regulators in immune responses by activating multiple signaling pathways and affecting the production of IgA[30]. It is reported that IL-4 combined with IL-10 had synergic effect on IgA levels with IgA-deficient (IgAD) patients[31]. The combination of IL-4 and IL-10 have protective effect against lung inflammatory injury[32]. Thus, IgA production is an event in response to MLN and the concentration of IgA may be biomarker in the treatment process of MM.

Cell adhesion molecules (CAMs) mediated the interactions of cell-cell and cell-substratum such as cell adhesion to adjacent cells and to specific extracellular matrix (ECM)[33]. Cell-cell adhesiveness was reduced in the progression of cancer which contributed to tumor invasion, metastasis and destruction of cell morphogenesis[34]. As outlined in previous study, adhesion molecules played crucial roles in several steps of MM progression[35]. CAMs mediated the MM cells migration and adhesion to bone marrow and triggered the activation of interleukin-6. Many genes were found to be involved in the pathway of cell adhesion such as ITGB7, CDH1, SDC4, ICAM 3 and SELL. The activation of ITGB7 (integrin β7) was related with cell adhesion, migration and invasion in multiple myeloma (MM) cells.[36] Silencing of *ITGB7* gene blocked MM cells adhesion to extra-cellular matrix and blocked MM cells migration by inhibiting focal adhesion kinase (FAK). The knockdown of ITGB7 suppressed p65-NF-κB (a member of NF-κB family) activity and affected NF-κB involved immune response[36]. The methylation status of CDH1 and SDC4 promoter was frequently found in the MM progression, which might be potential markers for MM prognosis[37,38].

Besides, ICAM 3 and SELL involved in CAMs pathway were identified to be the common genes in PPI networks of 8h vs. 12h and 8h vs. 24h group. ICAM 3 as an intercellular adhesion molecule was found to be overexpressed in the MM progression and mediated cancer cell adhesion to bone stroma[35]. SELL encoded a cell surface adhesion molecule that belongs to the family of adhesion/homing receptors. The encoded molecule contains multiple domains and plays a key role in lymphocyte-endothelial cell interactions[39]. The single-nucleotide polymorphisms in this gene have been associated with various diseases, such as immunoglobulin A nephropathy. Although reports concerning the role of SELL in MM were rare, ICAM 3 and SELL were critical targets in MM cells intervention by MLN. In conclusion, during the treatment with MLN120b, several pathways were significantly changed in L363 cells, among which the most important ones were Ig A pathway and the CAMs pathways. In our investigation, the comprehensive bioinformatics analysis provided a novel viewpoint to understand the pathophysiology of MM progression.

Conclusion

Intercellular adhesion molecule 3 and SELL may be potential targets in multiple myeloma treatment in the future.

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