Gastroenterology and Hepatology From Bed to Bench.

ORIGINAL ARTICLE

Evaluation of cellular response to *Clostridium difficile* toxin-A: a network analysis

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ABSTRACT

Aim: The current study aimed to determine crucial genes targeted by toxin-A through network analysis.
Background: *Clostridium difficile* (*C difficile*) produces toxin-A and toxin-B and is known as a risk factor for hospital infection, especially after broad spectrum antibiotic therapy. Bioinformatics findings have led to the introduction of a set of genes and biological terms that are targeted by toxin-B in colon epithelia.
Methods: The significant differentially expressed genes (DEGs) of human intestinal Caco-2 cells treated by toxin-A versus control were retrieved from gene expression omnibus (GEO). The queried DEGs were analyzed using by protein-protein interaction (PPI) network analysis through STRING database and Cytoscape software v.3.7.2.
Results: Among 157 significant DEGs, JUN, VEGFA, CDKN1A, ATF3, SNAI1, DUSP1, HSPB1, MCL1, KLF4, FOSL1, HSPA1A, and SQSTM1 were determined as hubs and JUN, DUSP1, DUSP5, EZR, MAP1LC3B, and SQSTM1 were highlighted as bottlenecks.
Conclusion: JUN, DUSP1, and SQSTM1 are possible drug targets to prevent and treat *C difficile* infection.

Keywords: *Clostridium difficile*, Infection, Drug target, Network analysis, Treatment.

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Introduction

Clostridium difficile (*C difficile*) is a common hospital infection that is tied to broad spectrum antibiotic therapy. *C difficile* reportedly produces two toxins, toxin-A and toxin-B (1). Toxin-A and toxin-B inactivate the small GTP-binding proteins, cytosolic Rac, Rho, and Cdc42. Following *C difficile* toxin functions, the affected cells become round and die. While toxin-B targets broad cell types, toxin-A causes effects mainly inside the intestinal epithelium cells (2). Investigations have revealed that antibiotic exposure, hospitalization, and older age are significant patient-related risk factors for *C difficile* infection (3). Sandra Maab et al. evaluated the response of *C difficile* to several antibiotics through proteomics. This study presents proteomic signatures of bacteria in response to metronidazole, vancomycin, and fidaxomicin (4). Sandra Janezic et al. published their comparative genomic findings related to the epidemiology of the

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disease, understanding of the evolution of *C difficile*, and infection control (5). Bioinformatics is another method that is used to detect various aspects of *C difficile* infection. Integrated bioinformatics analyses revealed that a set of genes and biological terms are dysregulated by the toxin-B bacterium; these results can improve our understanding of the molecular mechanism underlying infection of the colon epithelia (6). Various kinds of network analysis, such as social network analysis and network meta-analysis, are applied to study different aspects of *C difficile* infection (7, 8). The well-known PPI network analysis is a useful method that has attracted the attention of researchers in medicine and related fields. The molecular mechanism of many diseases can be studied through PPI network analysis. Safari-Alighiarloo et al. reported on the application of PPI network analysis on evaluating complex diseases (9). PPI network analysis provides valuable information about limited numbers of genes known as central genes that are involved in the initiation and development of diseases (10). Because of the importance of intestinal infection, DEGs related to the response of human intestinal Caco-2 cells to *C difficile* toxin-A retrieved from the GEO database were evaluated by PPI network analysis in the current study to detect the core genes affected by toxin A. The findings can be considered as drug targets or prevention agents.

Methods

In this study, GSE100541 was selected for analysis from GEO datasets (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=gse100541). Gene expression profiles of human intestinal Caco-2 cells incubated with *clostridium difficile* (*C difficile*) toxins are presented in this GSE. The gene expression profiles of three sample cells incubated with toxin A (Tox A) versus those of cells in the presence of Dulbecco's modified eagle medium (DMEM) as control samples were analyzed by the GEO2R program. The relationship between fold change and p-value was evaluated by volcano plot analysis. Samples were evaluated by density plot analysis to explore gene expression patterns. In total, 250 top DEGs (considering fold change) were downloaded to be analyzed. The characterized significant DEGs were identified among the top DEGs considering a p-value <0.01 and fold change >1.5.

The characterized significant DEGs were found in the STRING database (which contains the related proteins) by "protein query," and the recognized DEGs were interacted by Cytoscape software v.3.7.2 (11). Because of the poor number of interactions between the nodes of the main connected components, 50 first neighbor genes from STRING were added to the recognized significant DEGs, and the network was reconstructed. The network was analyzed using the "Network Analyzer" application of Cytoscape, and the top 10% of queried nodes based on degree value were identified as hub nodes. The top 5% of queried nodes based on betweenness centrality were introduced as bottlenecks. The common hubs and bottlenecks were identified as hub-bottlenecks.

The relationship between hub and bottleneck nodes was investigated from STRING database (https://string-db.org/). Connections between the interacted hub-bottlenecks identified through co-expression, experimentation, and text mining were determined and discussed.

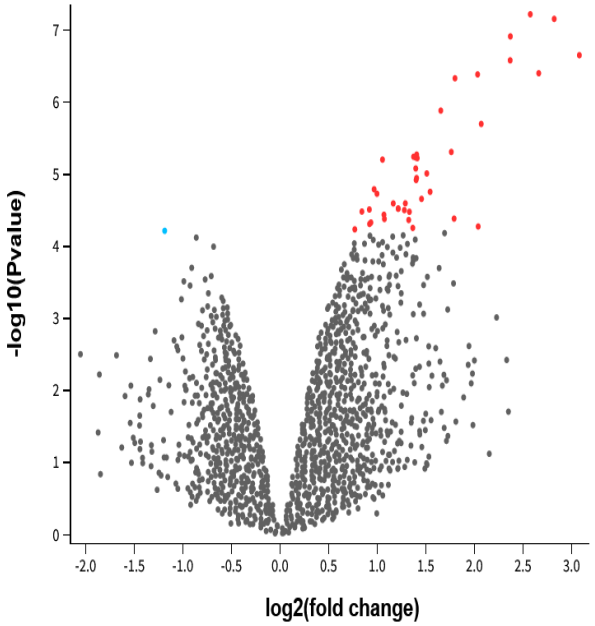

Figure 1. Volcano plot presentation of gene expression profiles of human intestinal Caco-2 cells incubated with *C difficile* tox A versus controls.

Results

Volcano plot analysis (see Figure 1) indicated that a considerable number of DEGs are statistically

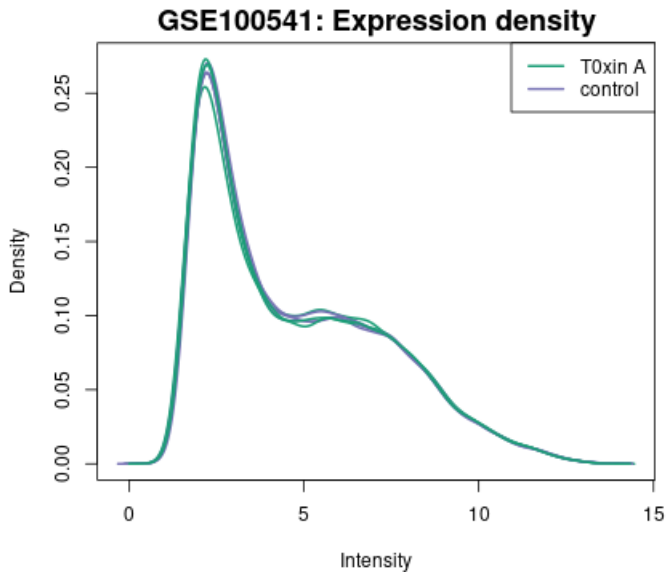

Figure 2. Density plot presentation of gene expression pattern of human intestinal Caco-2 cells incubated with *C difficile* tox A versus controls.

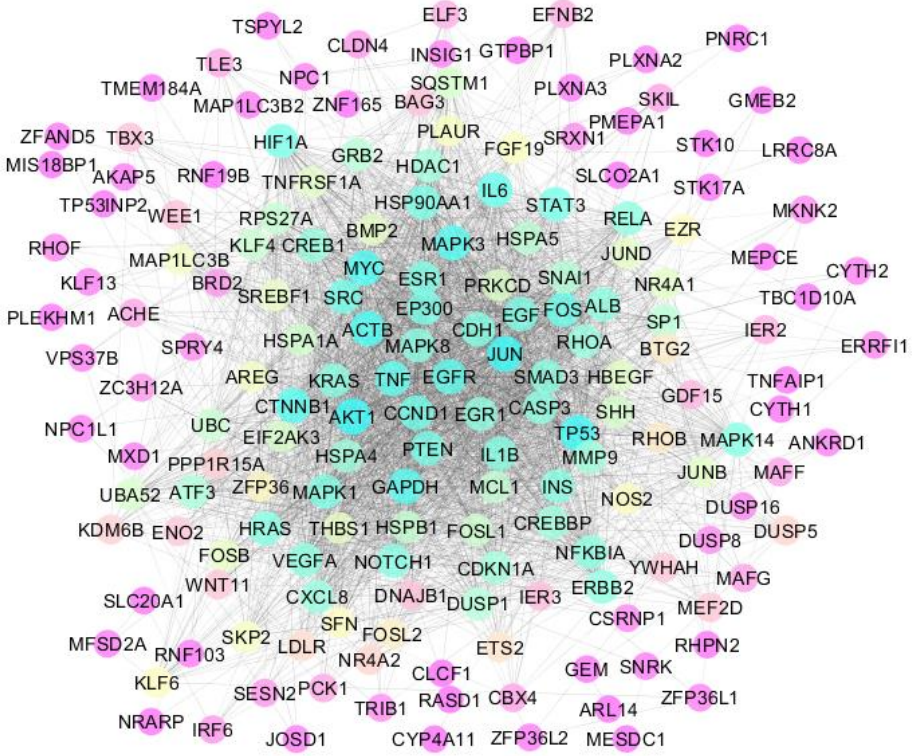

Figure 3. Main connected component of PPI network related to gene expression profiles of human intestinal Caco-2 cells incubated with C difficile tox A versus controls. Bigger sizes and the blue color of nodes refer to a higher value of degree.

significant. Density plots of the studied gene expression profiles are presented in Figure 2. As depicted in Figure 2, the gene expression patterns of samples are similar and comparable. A total of 157 characterized significant DEGs from among 192 queried individuals were selected for further analysis.

Among the 157 queried DEGs, 152 individuals were recognized by STRING database. The constructed network including a main connected component of 97 nodes, and 319 edges were formed (data not shown).

Adding 50 first neighbors allowed the creation of a network with a main connected component comprising

173 (123 queried DEGs + 50 added first neighbors) nodes and 2773 connections (see Figure 3).

JUN, VEGFA, CDKN1A, ATF3, SNAI1, DUSP1, HSPB1, MCL1, KLF4, FOSL1, HSPA1A, and SQSTM1 were determined as hubs, and JUN, DUSP1, DUSP5, EZR, MAP1LC3B, and SQSTM1 were highlighted as bottlenecks. It was appeared that Jun, DUSP1, and SQSTM1 are hub-bottlenecks (see Table 1). The connections between hub-bottleneck nodes, including relationships determined by co-expression, experimentation, and text mining, are presented in Figure 4.

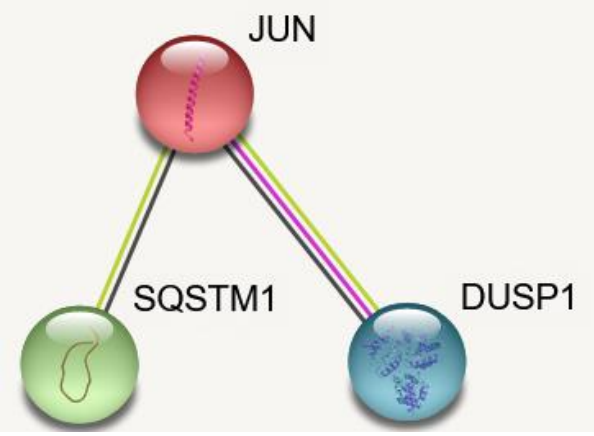

Figure 4. Connections between three hub-bottlenecks. Black, purple, and lemon colors refer to co-expression, experimentally determined, and text mining-identified, respectively.

Discussion

As depicted in Figure 1, the expression of a considerable number of genes is significantly changed. Gene expression patterns shown in Figure 2 indicate that the gene expression profiles of treated samples and controls are comparable. The PPI network was constructed and analyzed to find the limited numbers of DEGs among 157 queried individuals. Hub nodes can be considered as critical elements of the PPI network (12). However, the bottleneck nodes carry valuable information (13).

Based on the results of network analysis, 12 hubs and 6 bottleneck nodes were identified as the central elements of the network. Hub-bottleneck nodes, those common hubs and bottlenecks, are known as important elements of a PPI network and have been highlighted in previous investigations (14, 15). JUN, DUSP1, and SQSTM1 were identified as the hub-bottleneck nodes that discriminate cells treated with toxin-A from control cells. As depicted in Table 1, JUN is a potent hub relative to the other hub-bottlenecks; it has a degree value about 2-fold greater than the other hub-bottlenecks. Interesting data is presented in Figure 4. The co-expression of JUN with both DUSP1 and SQSTM1 is illustrated in Figure 4. It can be concluded that there are two categories of hub-bottlenecks: first, JUN, and second, DUSP1 and SQSTM1.

The Jun proto-oncogene, AP-1 transcription factor subunit (JUN) family includes JUN-B, c-JUN, and JUN-D (16). Investigation indicated that activator protein-1 (AP1) (which is a collection of several JUN and FOS family members, such as c-Fos, Fos-B, Fra1, Fra2, c-Jun, Jun-B, and Jun-D) is activated in response to toxin-A in intestinal epithelial cells, which further leads to MAPK activation. Evidence indicates that toxin-A *C difficile* induces secretion of IL-8 through MAPK (17).

There is evidence of the involvement of dual specificity phosphatase 1 (DUSP1) in colonic inflammation in response to toxin-B of *C difficile*. Based on an evaluation by Ying Li et al., TRIM46 plays a prominent role in toxin-B-induced colonic inflammation through the regulation of DUSP1/MAPKs and NF-κB signaling pathways (18). Researchers have shown that DUSPs are able to inhibit MAPKs in mammalian cells. It has been indicated that DUSP1 is an inhibitor of all MAPKs pathways (19).

Sequestosome 1 (SQSTM1) (P62) is the third hub-bottleneck of the analyzed network. Toxin-B of *C difficile* has been reported to induce autophagic cell death in cultured human colonocytes by increasing the formation of LC3+ autophagosomes and decreasing levels of the autophagic substrate p62/SQSTM1 (20). As described, the significant role of the three introduced hub-bottlenecks in response to the toxins of *C difficile* has been highlighted in previous studies, and

Table 1. Queried hub-bottleneck nodes and the related centrality parameters; descriptions extracted from STRING database.

No.	Gene	Description	Degree	Betweenness centrality
1	JUN	Jun proto-oncogene, AP-1 transcription factor subunit	97	0.040
2	DUSP1	dual specificity phosphatase 1	54	0.030
3	SQSTM1	sequestosome 1	43	0.014

the current findings correspond with the literature. The importance of the current analysis lies in the highlighting of JUN, DUSP1, and SQSTM1 as key genes among numerous DEGs that are dysregulated in response to toxin-A of *C difficile*. The introduced key DEGs can be suitable drug targets to prevent *C difficile* infection.

Conclusion

There are limited possible drug targets (JUN, DUSP1, and SQSTM1) to prevent and treat *C difficile* infection. Because the findings of our bioinformatics analysis correspond with those of previous experimental investigations, it seems that the data is ready to be used in clinical trial investigations.

Acknowledgement

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Conflict of interests

The authors declare they have no conflicts of interest.

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