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Research Paper

The Main Targets of Okadaic Acid Toxin in Human Intestinal Caco-2 Cells: An Investigation of Biological Systems

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ABSTRACT

Background: Okadaic acid (OA) is a toxin of polluted shellfish. Consuming the contaminated shellfish is accompanied by diarrhea and paralytic and amnesic disorders. There is a correlation between diarrhea and the consumed OA. Determining the critical targeted genes by OA was the aim of this study.

Methods: The transcriptomic data about the effect of OA on human intestinal caco-2 cells were extracted from gene expression omnibus (GEO) and evaluated via the GEO2R program. The significant differentially expressed genes (DEGs) were included in a protein-protein interaction (PPI) network and the central nodes were enriched via gene ontology to find the crucial affected biological terms.

Results: Among the 178 significant DEGs plus 50 added first neighbors, four hub-bottleneck genes (ALB, FOS, JUN, and MYC) were determined. Twenty-eight critical biological terms were identified as the dysregulated individuals in response to the presence of OA. "ERK1/2-activator protein-1 (AP-1) complex binds KDM6B promoter" was highlighted as the major class of biological terms.

Conclusion: It can be concluded that down-regulation of ALB as a potent central gene leads to impairment of blood homeostasis in the presence of OA. Up-regulation of the other three central genes (JUN, FOS, and MYC) grossly affects the vital pathways in the human body.

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1. Introduction

The poisoning character of shellfish has been considered for many years. Paralytic and diarrhetic, neurotoxic, and amnesic shellfish poisoning are related to saxitoxin and derivatives, okadaic acid (OA), dinophysistoxins, brevetoxins, and domoic acid, respectively [1]. OA is a polyether toxin produced by marine microalgae. The accumulated OA in the digestive glands of the shellfish is a source of poison that acts as a highly selective inhibitor of protein phosphatases type 1 (PP1) and 2A (PP2A). This effect on the mentioned enzymes leads to dramatic increases in the phosphorylation of numerous proteins in the body. This property of OA made it a useful probe to investigate cellular processes, such as cell division and memory [2]. The molecular mechanism of the poisoning effect of OA has attracted the attention of many scientists. Evidence provides new knowledge about the role of OA in tumor progression [3].

High-throughput methods, such as genomics and proteomics are introduced as suitable tools in medicine in the fields of new drug discovery, diseases, and toxicology [4, 5]. High-throughput data analysis is tied to bioinformatics. A combination of genomics and bioinformatics is applied to investigate many subjects in biology and medicine. Toxicogenomics is a field dealing with bioinformatics [6]. One of the branches of bioinformatics that is applied in proteomics and genomics is the network analysis method. Protein-protein interaction (PPI) network analysis is based on interactions between the studied genes or proteins. In such analyses, the genes or proteins interact with the neighbors in many ways to form a network. Each element of the PPI network has a specific property, such as the connected neighbor's position and pattern. Based on the topology of the studied network, the nodes that make more connections with the first neighbors are called hubs while the others that participate in the shortest paths are known as bottleneck nodes. The common hubs and bottlenecks are recognized as hub bottlenecks. The hub-bottleneck genes are known as the central nodes, which control the main processes related to the molecular events in the studied systems [7-9]. In the present study, transcriptomic data about the effect of OA on human intestinal Caco-2 cells were extracted from gene expression omnibus (GEO) and analyzed via PPI network analysis and gene ontology evaluation to find the prominent targeted genes by OA. The findings can be used as possible new targets in the treatment protocol for patients caused by the consumption of pollutant shellfish.

2. Materials and Methods

Transcriptomic data about the effects of OA on human intestinal Caco-2 cells were recorded in GSE159293 of GEO. The treated cells and the controls were characterized by GSM4826163-8 and GSM4826157-62, respectively, and the data were published by Huguet et al. for toxins (Basel) in 2020 [10]. The transcriptomic profiles were assessed via box plot analysis to find the comparability of the expressed individuals. Adjusted $P \leq 0.05$ (Padj) was considered to find the significantly expressed genes, including up- and down-regulated genes, and the results were visualized via volcano plot. The possible separation of groups by the dysregulated genes was assessed via a uniform manifold approximation and projection (UMAP) plot.

A total of 250 top DEGs were selected for analysis. After screening, the recognized genes by the STRING database were included in a PPI network via Cytoscape software, version 3.7.2. STRING enables the researcher to add the required numbers of the first neighbor to the queried DEGs to maximize the elements of the main connected component of the studied PPI. The optimum number of first neighbors was added to the queried DEGs to reduce the number of isolated DEGs and make maximum connections between the nodes of the main connected component of the constructed network. The network was assessed via the "Network Analyzer" application of Cytoscape software, version 3.7.2 to evaluate centrality parameters, such as degree and betweenness centrality. The top 10% of the connected principal component nodes that belonged to the examined DEGs based on the value of degree and betweenness were identified as hubs and bottlenecks. The common hubs and bottlenecks were introduced as the hub-bottleneck nodes.

Since the hub-bottlenecks play a crucial role in cell function, the hub-bottleneck-related biological terms were investigated via ClueGO software, version 2.5.7. All default sources of gene ontology were selected for analysis and Medium≤Network specificity≤Detailed was considered to determine the biological terms and groups. Term P, term P corrected with Bonferroni step down, group P, and group P corrected with Bonferroni step down ≤0.01 were painstaking.

3. Results

The distribution of the expressed genes of the human intestinal Caco-2 cells exposed to OA and the controls is shown in Figure 1. The data are median-centric and comparable. The volcano plot (Figure 2) indicated that

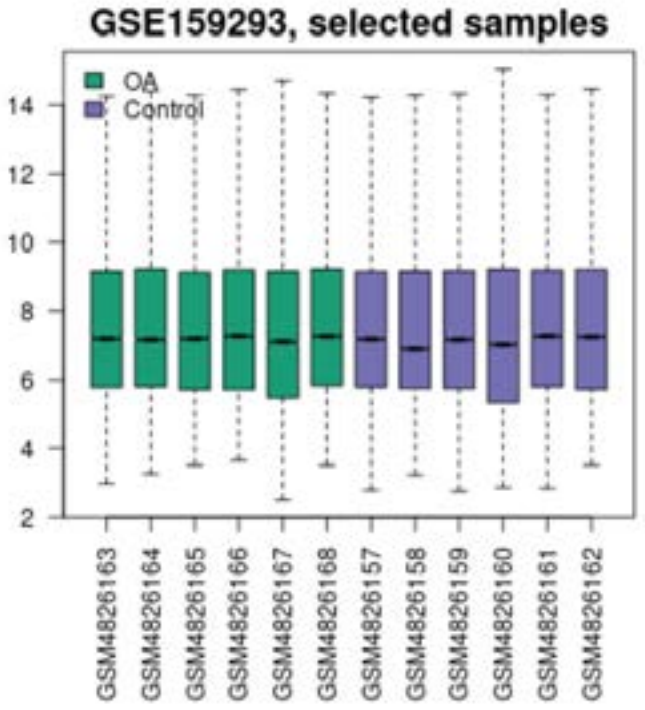

Figure 1. Box plot representation of the studied transcriptomic profiles of the treated human intestinal Caco-2 cells treated with OA and the control cells

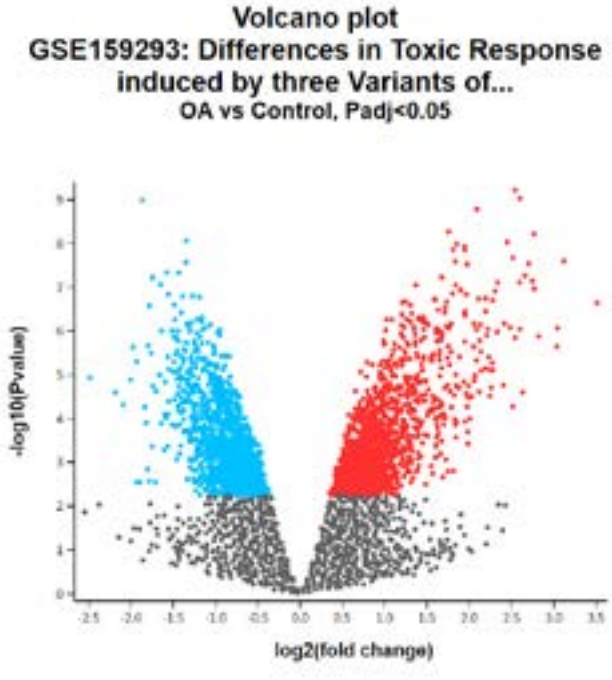

Figure 2. Volcano plot related to the studied transcriptomic profiles of the treated human intestinal Caco-2 cells that are treated with OA versus the control cells

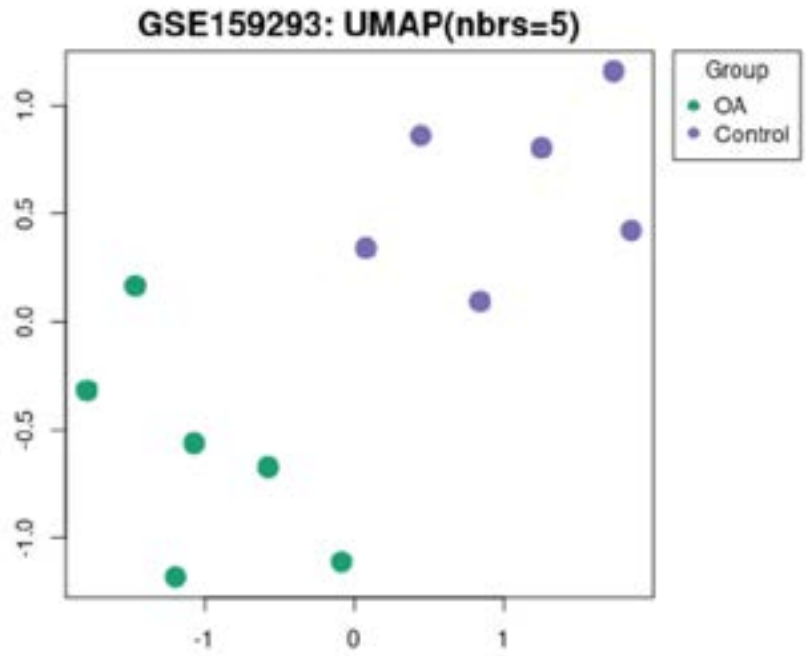

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Figure 3. Umap plot related to the studied transcriptomic profiles of the treated human intestinal Caco-2 cells treated with OA versus the control cells

many genes were significantly up- or down-regulated. The results of the UMAP evaluation are shown in Figure 3. Based on UMAP findings, the treated cells with OA were separated from the control cells completely.

Screening of the top 250 DEGs led to 178 significant genes, of which 170 cases were recognized by the STRING database. The network was created by the 170 recognized queried DEGs plus 50 added first neighbors. The network includes 36 isolated DEGs, six paired genes, and a main connected component of 178 nodes that were connected with 2769 edges. Four hub-bottleneck nodes (including ALB, FOS, JUN, and MYC) were determined among the queried DEGs (Table 1).

In total, 28 biological terms associated with the four hub bottlenecks were identified. The terms were clustered into four groups (Figure 4 and Table 2). "ERK1/2-activated AP-1complex binds KDM6B promoter" and "expression of STAT3-upregulated nuclear proteins" appeared as the largest and smallest classes of biological terms, respectively. The biological terms were extracted from REACTOME_Reactions_08.05.2020, WikiPathways_08.05.2020, GO_BiologicalProcess-EBI-UniProt-GOA-ACAP-ARAP_08.05.2020_00h00, REACTOME_Pathways_08.05.2020, and REACTOME_Reactions_08.05.2020 as the sources of ontology.

4. Discussion

Gene expression profiling is introduced as a valuable method to assess the toxicity of foods [11]. Zhang et al. designed a project to evaluate the toxic effect of polystyrene nanoplastic on *Daphnia pulex* as an organism model for ecotoxicity. Based on RNA sequencing

Table 1. Hub-bottleneck nodes of the main connected component of PPI network of the treated cells versus controls

Name	Degree	Betweenness Centrality	Log (Fold Change)
ALB	83	0.098	-1.49
FOS	81	0.016	2.54
JUN	85	0.015	1.77
MYC	86	0.037	1.96

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Table 2. The biological terms related to the hub-bottleneck nodes (bold terms are the names of groups)

GO Term	Groups	% Associated Genes	Associated Genes
Expression of STAT3-upregulated nuclear proteins	1	10.00	[*FOS, MYC*]
IL-5 signaling pathway	2	7.50	[*FOS, JUN, MYC*]
IL-2 signaling pathway		7.14	[*FOS, JUN, MYC*]
Mononuclear cell differentiation	3	5.26	[*JUN, MYC*]
Monocyte differentiation		5.26	[*JUN, MYC*]
Regulation of monocyte differentiation		10.00	[*JUN, MYC*]
FCERI-mediated MAPK activation	4	6.25	[*FOS, JUN*]
ERK1/2-activated AP-1complex binds KDM6B promoter		100.00	[*FOS, JUN*]
AP-1 complex stimulates KDM6B transcription		66.67	[*FOS, JUN*]
AP-1 transcription factor binds IGFBP7 promoter		100.00	[*FOS, JUN*]
AP-1 stimulates transcription of IGFBP7		66.67	[*FOS, JUN*]
MAPK targets/ Nuclear events mediated by MAP kinases		6.45	[*FOS, JUN*]
Formation of activated protein 1 (AP-1) complex (cFOS/c-JUN heterodimer).		100.00	[*FOS, JUN*]
Activation of the AP-1 family of transcription factors		20.00	[*FOS, JUN*]
AP-1 transcription factor binds IL1A promoter		100.00	[*FOS, JUN*]
AP-1 stimulates IL1A transcription		66.67	[*FOS, JUN*]
TP53 and AP-1 stimulate MSH2 expression		40.00	[*FOS, JUN*]
TP53 and AP-1 bind the MSH2 promoter		50.00	[*FOS, JUN*]
Physiological and pathological hypertrophy of the heart		8.00	[*FOS, JUN*]
PDGF pathway		5.00	[*FOS, JUN*]
Signaling of hepatocyte growth factor receptor		5.88	[*FOS, JUN*]
Photodynamic therapy-induced NFE2L2 (NRF2) survival signaling		8.33	[*FOS, JUN*]
PDGFR-beta pathway		6.90	[*FOS, JUN*]
Type I interferon induction and signaling during SARS-CoV-2 infection		6.90	[*FOS, JUN*]
Host-pathogen interaction of human coronaviruses-MAPK signaling		5.56	[*FOS, JUN*]
Host-pathogen interaction of human coronaviruses-interferon induction		6.06	[*FOS, JUN*]
Estrogen signaling pathway		8.70	[*FOS, JUN*]
Cellular response to cadmium ion		5.00	[*FOS, JUN*]

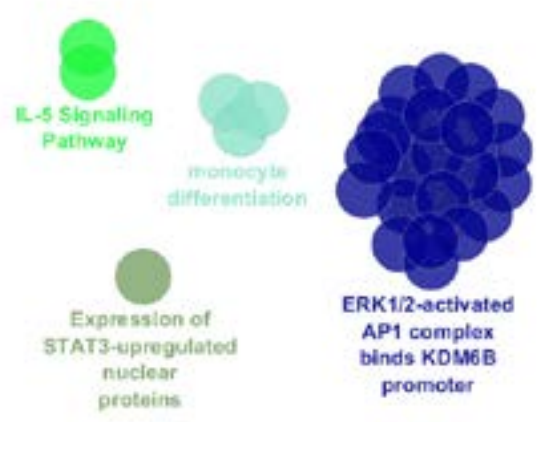

Figure 4. The biological terms related to the hub-bottleneck nodes

Mediumnetwork specificity ≤ detailed was considered.

results, they detected 244 DEGs, which were characterized by P≤0.05 and fold change of >2 [12]. In the present study, transcriptomic data about the toxic effect of OA on human intestinal Caco-2 cells was studied via bioinformatic tools. As shown in Figure 1, gene expression profiles are suitable subjects to be investigated for more understanding of the molecular mechanism of the mentioned toxin on human cells. It seems that many genes were affected by OA toxin (see the volcano plot). The application of genomics and bioinformatics in toxicology has attracted the attention of many scientists. Yang et al. investigated the molecular regulatory mechanism and toxicology of 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine/probenecid (MPTP/p)-induced progressive Parkinson's disease in mice via transcriptomic responses and bioinformatic analysis [13].

UMAP plot indicated that the dysregulated genes were suitable tools to separate the treated cells from the controls. As it is shown in Figure 3, the two sets of samples were differentiated by the studied DEGs. After validation of the comparison between treated and untreated cells, the top DEGs were included in a PPI network. ALB, FOS, JUN, and MYC were introduced as the key genes in response to the OA toxin. As shown in Table 1, ALB was down-regulated while the other 3 DEGs were up-regulated genes. Albumin plays a crucial role in the human body. Serum albumin is involved in plasma oncotic pressure plus several key functions, such as the transport of endogenous and exogenous ligands [14]. Roopenian et al. elevated the levels of lipase, total bilirubin, total cholesterol, calcium, alanine aminotransferase, low-density lipoprotein, high-density lipoprotein, total triglyceride, iron, and aspartate aminotransferase in albumin-deficient mouse models null mice [15] and showed the significant effect of albumin on human health. Albumin not only is a hub-bottleneck node regarding the queried DEGs, but it is ranked as the first hub among all elements (including the queried DEGs and the added first neighbors) of the network.

As it is depicted in Table 2 and Figure 4, 28 biological terms were related to JUN, FOS, and MYC. The biological terms were classified into four clusters, including 22, 3, 2, and 1 terms. The larger cluster was "ERK1/2-activated AP-1complex binds KDM6B promoter". JUN, FOS, and MYC were associated with 27, 25, and 6 biological terms, respectively. JUN was related to all biological terms except "Expression of STAT3-upregulated nuclear proteins". FOS was the second hub-bottleneck node that was related to the most biological terms. The "ERK1/2-activated AP-1complex binds KDM6B promoter" term is the largest group of biological terms including a term, which is involved in the function of "activator protein – 1". It is reported that this gene is complicated in almost all cellular and physiological functions [16]. Another element of this biological term was KDM6B. KDM6B as a histone demethylase can act as both a tumor suppressor and an oncogene conditional in the cellular setting [17]. Induction of AP-1 by OA was carried out about 30 years ago [18]. A significant increase in transcription factor AP-1 (JUN) and proto-oncogene protein c-fos (FOS) in chicken embryos exposed to OA has been reported by researchers [19].

5. Conclusion

In conclusion, ALB, JUN, FOS, and MYC are the critical targets of OA toxin. Down-regulation of albumin, which is a major controller of blood homeostasis, can

affect the normal function of the human body. Since JUN and FOS participate in the crucial pathways that are involved in vital functions and physiological operations in the human body, the up-regulation of these two genes may be a potent response to the attack of OA toxin in the body.

Ethical Considerations

Compliance with ethical guidelines

This project was approved by the Ethics Committee Shahid Beheshti University of Medical Sciences (Code: IR.SBMU.RETECH.REC.1402.138).

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Authors' contributions

Conceptualization: Babak Arjmand, Mostafa Rezaei Tavirani and Mohammad Rostami Nejad;Methodology and software: Mona Zamanian Azodi and Zahra Razzaghi; Project administration: Babak Arjmand, Mostafa Rezaei Tavirani; Resources: Zahra Razzaghi; Supervision: Mostafa Rezaei Tavirani, Reza M Robati and Mohammad Rostami Nejad; Data curation: Mona Zamanian Azodi; Funding acquisition and formal analysis: Mostafa Rezaei Tavirani; Investigation: Mona Zamanian Azodi, Reza M Robati, and Mitra Rezaei; Validation: Zahra Razzaghi; Visualization: Mona Zamanian Azodi; Writing–original draft: Mostafa Rezaei Tavirani, Mona Zamanian Azodi, Mitra Rezaei; Review & editing: Babak Arjmand, Zahra Razzaghi and Reza M Robati.

Conflict of interest

The authors declared no conflict of interest.

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