Down-regulation of TP53 is a highlighted molecular event in gastric ulcer

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ABSTRACT

Aim: The current study explored the crucial dysregulate proteins and biochemical pathways in gastric ulcer as its main aim.

Background: Gastric ulcer as an acid-related gastrointestinal disease is known as one of the most public gastrointestinal disorders.

Methods: A total of 100 proteins from STRING database were analyzed by Cytoscape and its applications to find the central proteins and the related biochemical pathways. Action map analysis was applied to explore regulatory relationships between the critical proteins.

Results: Network analysis and gene ontology revealed that IL6, ALB, TNF, INS, IL1B, IL10, TP53, CXCL8, and PTGS2 are the highlighted proteins related to gastric ulcer. Six clusters of biochemical pathways, namely “response to external stimulus,” “multicellular organismal process,” “regulation of biological quality,” “cellular response to stimulus,” “cellular response to chemical stimulus,” and “transport” were identified as the dysregulated pathway in patients.

Conclusion: Down-regulation of TP53 by IL2, PTGS2, and TNF seems to be a main process occurring in gastric ulcer patients.

Keywords: Gastric ulcer, Network analysis, Protein, TP53, Human.

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Introduction

Gastric ulcer is known as a one of the most common public gastrointestinal disorders (1). Gastric ulcer, gastroesophageal reflux disease, nonsteroidal anti-inflammatory drug–associated ulcers, and nonerosive reflux disease are all acid-related gastrointestinal disorders. Experiments have revealed that treatment with histamine receptor 2 blockers and then proton pump inhibitors (PPIs), which are involved in acid secretion control, has suitable efficacy (2).

Accurate and noninvasive diagnosis and efficient treatment of gastric ulcer imply understanding the molecular mechanism of the disorder (3). In the case of gastric ulcer, its molecular mechanism has been documented; however, more investigations are required to fully understand different aspects of this disorder (4). There is evidence that related biomarkers for gastric ulcer are investigated by omics methods such as metabolomics (5). Gastric ulcer compared to gastric cancer is evaluated by proteomics (6).

Methods such as proteomics, genomics, and metabolomics provide a large set of data about the assessed samples. The produced data is usually available as expression changes. Most data is stored in various databases, such as STRING and Gene Expression Omnibus (7-9). Proteomics coupled with bioinformatics is a suitable tool for exploring the highlighted dysregulated proteins in the evaluated disease (10). Network analysis as a bioinformatic approach is a common method to analyze large numbers of dysregulated proteins. Many diseases are

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investigated through network analysis. Some gastrointestinal diseases, such as inflammatory bowel disease, nonalcoholic steatohepatitis, esophageal cancer, gastric cancer, and colon cancer, are evaluated through network analysis (9, 11).

In network analysis, large numbers of proteins or other biological molecules such as genes or metabolites interact with each other to form a network. Proteins (nodes) link to other proteins through connections (edges). A connection may be directed or nondirected edges. A few nodes make up large numbers of connections with the first neighbor nodes; these nodes are known as hubs. Other types of nodes are those that participate in large numbers of the shortest paths between other nodes; these proteins are called bottlenecks. The hubs that are bottlenecked are known as hub-bottlenecks. Hubs, bottlenecks, and hub-bottlenecks are the central nodes of a network. The central nodes of a network play critical roles in the integrity of a network.

In the present study, the related dysregulated proteins of gastric ulcer were extracted from STRING database to create a network. The central proteins were identified and compared with the nodes involved in related chemical pathways. Regulatory properties of the nodes were assessed through action map analysis. The crucial chemical pathways and central nodes will be introduced for use in applied fields.

### Methods

STRING database includes several queries such as “disease query.” The dysregulated proteins related to various diseases are listed in this query (7, 12). The dysregulated proteins related to gastric ulcer were extracted from STRING database. The queried proteins were included in a protein-protein interaction network using Cytoscape software (v3.7.2), and the created network was analyzed using the “NetworkAnalyzer” plugin of Cytoscape software (13). A confidence (score) cutoff = 0.4 was applied. Centrality parameters of nodes were assessed, and the nodes were ranked based on degree value and betweenness centrality. The Twenty nodes based on degree value and 20 proteins based on betweenness centrality were selected as hubs and bottlenecks, respectively. The hubs and bottlenecks were compared to find the common nodes as hub-bottlenecks.

Elements of the main connected components of the analyzed network were enriched using ClueGO (v2.5.7) to find the related biochemical pathways from Kyoto Encyclopedia of Genes and Genomes (KEGG). Clusters of pathways were determined based on kappa score. Pathways and the associated proteins were identified using the CluePedia (v1.5.7) plugin of Cytoscape software. The expression relationship between the main connected component elements was evaluated, and the interacting individuals were assessed.

| No. | Display name | Degree | Betweenness centrality |
|-----|--------------|--------|------------------------|
| 1   | IL6          | 60     | 1.00                   |
| 2   | ALB          | 57     | 0.98                   |
| 3   | TNF          | 56     | 0.65                   |
| 4   | INS          | 52     | 0.81                   |
| 5   | CD4          | 50     | 0.54                   |
| 6   | IL1B         | 49     | 0.38                   |
| 7   | IL10         | 47     | 0.19                   |
| 8   | TP53         | 44     | 0.77                   |
| 9   | VEGFA        | 43     | 0.04                   |
| 10  | CXCL8        | 42     | 0.00                   |
| 11  | CD8A         | 41     | 0.29                   |
| 12  | PTGS2        | 38     | 0.02                   |
| 13  | GPT          | 36     | 0.40                   |
| 14  | ATP4A        | 35     | 0.54                   |
by action map analysis. Activation, inhibition, reaction, catalysis, binding, and post-translation modification actions for the connected proteins in the expression map were investigated to screen the evaluated nodes.

The final results of network analysis, including the introduced hub-bottlenecks, were compared with the results of action map analysis, and the critical dysregulated proteins were highlighted.

Results

Gastric ulcer was searched from “disease query” of STRING database, and 100 related proteins were downloaded. The queried proteins interacted by undirected edges via Cytoscape software to form a network. A network comprising two paired proteins, 18 isolated nodes, and a main connected component containing 80 proteins was created. The main connected component was analyzed by the “NetworkAnalyzer” application of Cytoscape software. The 20 top nodes (25% of all nodes) based on degree value were identified as hubs, and bottlenecks were determined in a similar manner. Common hub and bottleneck nodes were introduced as hub-bottlenecks (see Table 1).

The 80 elements of the main connected components were assessed by ClueGO-CluPedia to explore the related biochemical pathways (from KEGG source) and expression relationships among the evaluated proteins. Results including six pathway groups (“response to external stimulus,” “multicellular organismal process,” “regulation of biological quality,” “cellular response to stimulus,” “cellular response to chemical stimulus,” and “transport”) are shown in Figure 1.

Further investigation revealed that 45 queried proteins among the 80 elements of the main connected component were connected to each other by expression relationship links (see Figure 2). The connected proteins of the expression map are represented in Figure 3. Evaluation indicated that there are nine common proteins between hub-bottlenecks and

Figure 1. Pathway analysis for 80 elements of main connected component. The large and small circles refer to pathway groups and queried proteins, respectively. The yellow connections refer to expression relationships. The round and bar tips refer to up-regulation and down-regulation effects, respectively. Link colors refer to the related pathways with the same colors.
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Elements of connected proteins in the expression map. These common proteins (including IL6, ALB, TNF, INS, IL1B, IL10, TP53, CXCL8, and PTGS2) and the related expression relationships are shown in Figure 4. To find a clear relationship between the 9 common proteins, activation, inhibition, reaction, catalysis, binding, and post-translation modification associations between these proteins were explored and are shown in Figure 5.

Figure 2. Expression map analysis for 80 elements of main connected component. The round and bar tips refer to up-regulation and down-regulation effects, respectively. The different colors of a node refer to the related pathway groups.

Figure 3. The connected proteins of expression map are presented. The round and bar tips refer to up-regulation and down-regulation effects, respectively. The different colors of a node refer to the related pathway groups.
Discussion

Network analysis revealed that among the 100 queried proteins, 80 individuals participated in interactome creation. Centrality assessment led to the introduction of 14 hub-bottleneck proteins. It is suggested that the hub-bottleneck nodes are the critical
elements of a network and play a crucial role in the integrity of the investigated network (14).

Gene ontology assessment indicated that there are six classes of biochemical pathways which are related to the 80 nodes of the main connected component. Three clusters of the biochemical pathways are highlighted as responses to stimuli. As shown in Figure 1, several pathways of “cellular response to stimulus” are common with the other pathways. Therefore, the introduced pathway clusters are overlapped and related to each other. Transport is another pathway cluster which is pointed. The significant role of the transport pathway in the functioning of a large number of proteins is discussed in the literature (15). As shown in Figure 1, the identified pathways are connected to all 80 queried proteins.

The 80 proteins were investigated by expression map analysis. The number of proteins among the 80 queried proteins showed they had a regulatory effect on each other (see Figures 2 and 3). The nine common proteins between hub-bottlenecks and these connected proteins were selected as critical proteins (see Figure 4). As illustrated in Figure 4, except for CXCL8, the other eight critical proteins participated in all six clusters of the biochemical pathways. CXCL8 participated in five clusters of the pathways. This finding indicates that the introduced critical proteins play a significant role in the control of the explored pathways. The highlighted point in Figure 4 refers to the connections of TP53. It is well known that TP53 is involved in maintaining cellular functions such as cell cycle arrest, DNA repair, and apoptosis (16). TP53 is downregulated by PTGS2, IL6, and TNF. Another key point in Figure 4 is the downregulation of insulin by IL6. Insulin is a hormone tied to the regulation of glucose, and its disorder is related to diabetes (17). Investigation showed that the incidence of gastric cancer is positively associated with plasma insulin levels (18). It seems that IL6 plays two contrasting roles in gastric ulcer by the downregulation of TP53 and insulin.

In Figure 5, activation is a prominent action relative to the other actions. Except for IL6, PTGS2 and TNF activate TP53. This relationship may compensate for a part of the downregulation of TP53. The single inhibitor protein shown in Figure 5 is IL10, which inhibits IL1B, IL6, TNF, and CXCL8. Investigation indicated that polymorphism of IL10 is accompanied by the risk of gastric cancer and atrophic gastritis (19). Data from Figures 4 and 5 indicates the activation and overexpression of albumin. Different functions such as anti-oxidation, molecular transport, anti-thrombotic effects, anti-inflammation, adjustment of capillary permeability, and endothelial stabilization are attributed to albumin (20). There is evidence that the attenuation of IL6 and TNF-α expression levels is associated with anti-ulcer effects (21). This finding corresponds to the current results. Attenuation of IL6 and TNF-α led to a decrement in the downregulation of TP53 (see Figure 4).

**Conclusion**

In conclusion, IL6, ALB, TNF, INS, IL1B, IL10, TP53, CXCL8, and PTGS2 are the key dysregulated proteins associated with gastric ulcer. It seems that the downregulation of TP53 is a main process that occurs in gastric ulcer patients. The roles of IL6 and TNF in promoting disease was highlighted in the present analysis. Experimental evidence is required for validation of this project. Additional investigations using suitable sample sizes of patients are suggested. It is a key point related to gastric ulcer that TP53 downregulation can aid the development of cancer and its related processes.

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**Conflict of interests**

The authors declare that they have no conflict of interest.

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