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Original article

Contradictory Effects of 6-Shogaol on the Human Cervical Cancer Cell Line HeLa Through Network Analysis

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Abstract

Background and objectives: To identify new targets for cancer clinical management, protein-protein interaction (PPI) network analysis of proteome data could accelerate this approach. For this aim, proteins with differential expressions in 6-shogaol exposure from proteomics study underwent protein-protein interaction (PPI) network analysis. **Methods:** Cytoscape version 3.8.2 and its plug-ins including NetworkAnalyzer and ClueGO2.5.8+CluePedia1.5.8 were applied for the construction and the corresponding analysis of the network. **Results:** A number of six differentially expressed proteins (DEPs) were identified as hub-bottlenecks of the PPI network. The critical proteins GAPDH, ENO1, HSP90AB1, ACTG1, RPSA, and CALR were determined as central elements of the analyzed network and their related biological processes were identified as "protein folding chaperones" and "glucose catabolic process". **Conclusion:** Potential candidates may be predicted as both anticancer agents and promoters of side effects of 6-shogaol in cancer treatment; however, complementary studies are required to provide validation and deeper understating of its molecular behavior in this regard.

Keywords: gene network; ginger; HeLa cell; herbal medicine; protein-protein interaction

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Introduction

Herbal medicine has presented capable candidates in the treatment of many diseases including cancer. Natural bioactive compounds from plants own cancer-fighting properties as apoptosis promoting factors, anti-cell growth, immunity regulator, and adjuvant therapy resources [1,2]. Ginger, as a popular plant, has indicated health benefits for human with regards to revealing nausea, menstrual problems, diabetes, and obesity [3-5]. This species has an ancient application in Chinese history as both food and medicine [6]. Ginger has also showed to be effective in breast, colon, and gastric cancer cancers which is related to its phenolic

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substances [6-8]. Different secondary metabolites of ginger including 6-gingerol and 6-, 8-, and 10 shogaols have been evaluated for this purpose and expressed promising results [9-11]. As a bioactive constitute of ginger, 6-shogaol has expressed beneficial effects in neurodegenerative diseases and cancer [12,13]. Pharmaceutical benefits of this component have been proven both in vitro and in vivo for cancers of breast, colon, prostate, and lung [14-17]. The suggested mechanisms by which these components show cancer preventing properties are inhibiting cell proliferation and apoptosis [18,19]. Molecular analysis can help in order to recognize what agents are important and contribute in these processes [20]. Molecular studies can shed lights on how the medical plants such as ginger act as antitumor agents in different kinds of cancers [21]. One of the innovative approaches is proteomics that is applied for biomarker discovery and understanding treatment outcomes [22]. This top-notch line of high throughput evaluation of proteome is in a great consideration for different exploration fields including prognosis, drug targeting, and revealing underlying mechanism of diseases and related treatments [23]. In addition, bioinformatics can assist in providing complementary information by analysis protein-protein interaction (PPI) network of tumor biology. The mechanisms of treatment are better explained by the identification of central biomarkers of PPI network. Central proteins can be explored by using designated centrality parameters including degree and betweenness centrality. These proteins are essential for the network stability and strength [24]. Therefore, biomarkers with centrality values are more prominent in the mechanisms of herbal medicine treatments and as valuable therapeutic targets [25].

Cervical cancer is a common malignancy in women ranked as fourth in the world [26] and is associated with high rates of mortality [27]. Surveys show that linked factors in raising the chance of cervical cancer could be lack of screening methods and programs especially in developing countries [28,29]. Proteomics analysis of cervical HeLa cells treated with 6-shogaol might be a promising approach to accelerate treatment of the cervical cancer [30] and bioinformatics can develop additional information in this area. Thus, in this paper, proteome data of the above mentioned study was subjected for protein-protein interaction network analysis for elucidating ginger therapeutic effects and its possible side effects on cervical cancer.

Materials and Methods

Ethical considerations

This project is approved by ethical committee of Shahid Behheshti university of Medical Sciences (IR.SBMU.RETECH.REC.1400.414).

Data collection

The proteome data from label-free shotgun proteomics study [30] was applied for the protein-protein interaction network analysis in this research. In the main conducted study, cervical HeLa cell line was treated with 15 μM 6-shogaol for 24 hours. The proteomics analysis identified 76 differentially expressed proteins (fold change≥2) and (p-value<0.05), most of them were up-regulated in the presence of 6-shogaol. The original study explored the interaction network analysis; however, not the centrality analysis [30]. By the aid of Cytoscape 3.8.2 V and its applications, a PPI network of differentially expressed proteins (DEP)s was constructed and analyzed [31]. STRING database as the source for predicting the network. Disease query, PubMed query, STITCH query, and protein query are the sources for attainment of interactions based on the design of the study [32]. A protein query was used to screen the DEPs as a connecting network in the Cytoscape platform. For this aim, a confidence score cut off=0.5 was set to provide better statistically significant connections. In the next step, topological features of the PPI network including common Degree (K) and Betweenness centrality (BC) features were computed by NetworkAnalyzer application [33]. Proteins in the network are called nodes and the links between them are considered as edges. Degree is a number of edges connected directly to a node whereas betweenness centrality is the function of shorts paths which a node participates. Hub-bottlenecks, nodes with highest values of degree and betweenness centrality are considered as central elements of the studied PPI network [34]. These parameters are calculated as 10% of highest ranked degree and betweenness centrality values of the proteins. Common nodes are suggested as hub-bottlenecks of the PPI network. These key portions could be important in the network structure stability. Moreover, enrichment analysis by the application ClueGO 2.5.8+CluePedia 1.5.8 was performed for

biological process determination of the hub-bottlenecks [35,36].

Statistical analysis

The designated kappa score cut off which falls between 0-1, was higher than the default option (0.4) and equals to 0.5 for term grouping. Moreover, the number of corresponding genes and percentage per group was assigned as 2 and 3, respectively. Correction p-value method was Bonferroni step-down test. The statistical significance for the grouping was set as $p \leq 0.05$.

Results and Discussion

The PPI network was retrieved via Cytoscape by querying the 76 DEPs from 6-shogaol-treated cervical cancer HeLa cells proteome (Figure 1).
According to the obtained network, DEPs are connected and only one of them, GSTM3, is remained as an individual node. This network query just comprises the DEPs and no additional neighbors.

To screen the hub and bottlenecks of the PPI network, 10% of high ranked degree (K) and betweenness centrality (BC) nodes were assigned. The hub-bottlenecks are the nodes with high values of degree and betweenness centrality that are tabulated in table1.
Among the hub-bottlenecks of the PPI network, HSP90AB1 is down-regulated and the rest are up-regulated. GAPDH with degree of 50 and the betweenness of 0.4 is the top central node. CALR is the least important hub-bottleneck with the degree of 17 and BC of 0.04.
In order to better depict a scale free network, scatter plot of degree and betweenness value distribution is described in figure 2.

Table 1. The list of hub-bottlenecks of the PPI network ranked based on degree values

Row	Display name	Regulation	K	BC
1	GAPDH	Up	50	0.4
2	ENO1	Up	27	0.05
3	HSP90AB1	Down	23	0.07
4	ACTG1	Up	22	0.04
5	RPSA	Up	21	0.04
6	CALR	Up	17	0.04

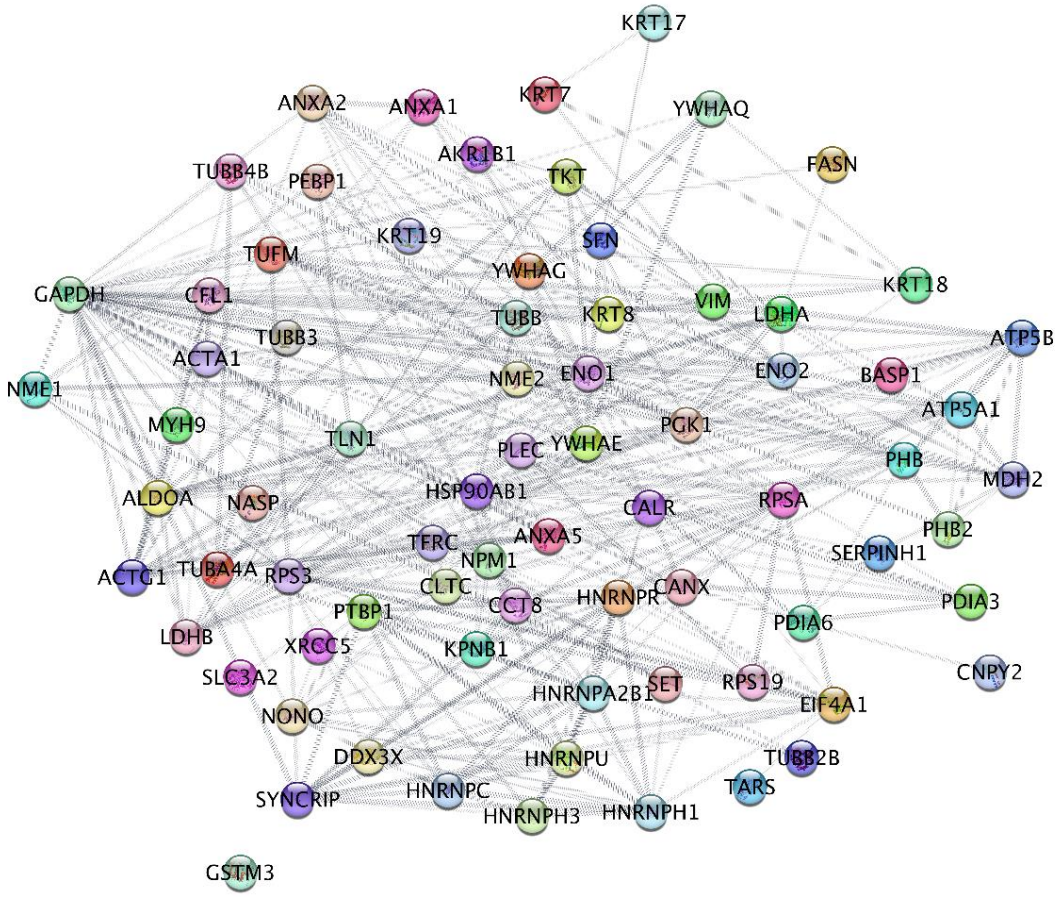

Figure 1. The protein-protein interaction network of 6-shogaol-treated HeLa cervical cancer cells; number of nodes and edges are 76 and 389, respectively.

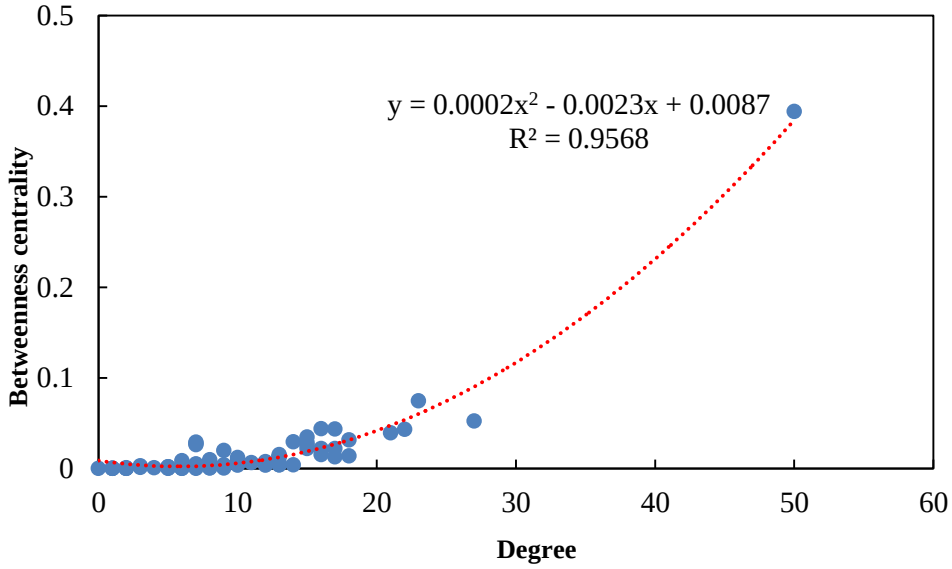

Figure 2. Scatter plot of degree and betweenness values distribution

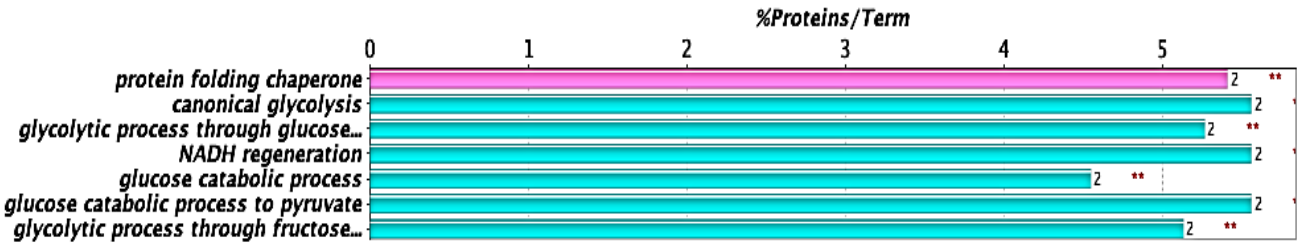

Figure 3. Bar chart view of biological process terms associated with hub-bottlenecks of the protein-protein interaction network, p≤0.05

A scale free network was approved by exhibiting a scatter plot of degree and betweenness centrality distributions. Based on this analysis, few numbers of nodes demonstrated high values of degree and betweenness centrality which is a nature of scale-free network.

In order to identify the gene ontology properties of hub-bottlenecks, biological processes annotation via ClueGO+ CluePedia was handled through Cytoscape (Figure 3).

The Y-axis describes the terms' name and X-axis implies the protein percentage per terms. The numbers in front of term bars show number of linked proteins. Protein folding chaperone and glucose catabolic process are the highlighted leading terms of each group. The two groups are displayed with color codes of blue and pink. Asterisk sign shows the statistical significance of contribution of the correlated term.

Expression analysis in a protein-protein interaction network scale could predict the most fundamental agents of the network stability and thus introduction of the most promising biomarkers. Ginger, on the other hand, accounts as a medicinal plant that shows anticancer properties in different kinds of tumors such as cervical cancer, breast cancer, lung cancer, and colon cancer [14,30,37]. One of the most major potent components of ginger is 6-shogaol that possess antioxidant and anti-inflammatory activities [38]. In the original study, pathways of endoplasmic reticulum stress and mitochondrial pathway were diagnosed as a part of 6-shogaol apoptosis mechanism against cancer development [30].

To shed lights on the underlying mechanisms of 6-shogaol in cancer and probing adequate targets for drug designing, bioinformatics assessment of

proteome information could be beneficial [39]. In this view, PPI analysis of proteome data of 6-shogaol-treated cervical cells via Cytoscape software was handled. The visualized network indicates that DEPs are in significant interactions as they are all in interacting network with the assigned statistical criteria except one DEP. Furthermore, the suggested hub-bottlenecks include GAPDH, ENO1, HSP90AB1, ACTG1, RPSA, and CALR. These nodes are all differentially expressed proteins and almost all of them are up-regulated except HSP90AB1. These six proteins could be as highlights of the cervical cancer proteome feasible targets and fundamentals of ginger biological activity.

Clarification of the role of these proteins in cancer initiation and development can be considered by literature survey. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the top hub-bottleneck is a housekeeping gene that its dysregulation has been reported in many cancers [40]. GADPH is noteworthy in development of cancer and highlights the novel function of glucose metabolism in cancer [41]. There is a controversy about the pivotal role of this enzyme that according to some studies this protein accelerates the metastasis process of cancer whereas others suggest that it may pinpoint as a cell death regulator [41]. The up-regulation of this protein is known in different cancers [42] which is also noticed in cervical cancer [43]. In the main study, GADPH was also reported to be up-regulated in cervical cancer HeLa cells treated with 6-shogaol. It can be inferred based on contradictory reports, this outcome can be due to the either possible side effects or anticancer process of treating with 6-shogaol. The next ranked hub-bottleneck is also a glycolysis enzyme, α-enolase (ENO1), which has promoting effects on many cancers [44-46]. Increases expression of ENO1 induced by 6-shogaol can be consider as an unfavorable effect. It is clear that the first high ranked hub-bottlenecks are prominently involved in glucose metabolism. This process is vital in tumor progression and metastasis in a wide range of cancers [47]. Heat Shock Protein 90 Alpha Family Class B Member 1 (HSP90AB1) is down regulated in exposure to 6-shogaol in the original study and it is reported up-regulated in many solid tumors including cervical cancer [48,49]. It can be concluded that 6-shogaol possess modulatory effects on HSP90AB1. Actin Gamma 1 (ACTG1) is the next protein that its dysregulation has been highlighted in skin cancer, hepatocellular carcinoma, and uterine cancer [50-52]. While this protein is reduced in expression in ovarian cancer [53] in other types of cancers is over-expressed. By up-regulating ACTG1, 6-shogaol may show regulatory properties. Ribosomal Protein SA (RPSA) is also a hub-bottleneck that associates with cervical cancer and pancreas cancer [54,55]. Calreticulin (CALR) as a chaperon protein of ER plays role in many processes of the cells. It participates as a major component in correct folding of protein in ER [56]. Non-small cell lung cancer is one of the reported malignancies that this proteins is one of its prognostic biomarkers [57]. In addition, CALR is important in Notch Signaling in cervical cancer which shows down-regulation in this system [58]. By promoting the up-regulation of this protein, 6-shogaol can thereby regulate its levels. All of the central proteins reported correlations with cervical tumorigenesis [43,45,49,54,58,59).

To investigate the ontology characteristic of hub-bottlenecks, ClueGO+CluePedia combined plug-ins analyzed the proteins. Enrichment analysis specified that there are two statistically significant groups of biological processes related to the hub-bottlenecks. These groups may be the most associated biological processes with the mechanisms of 6-shogaol treatment. Thus, the implication of “glucose catabolic process” and “protein folding chaperone” in cancer could be altered by regulation of hub-bottlenecks.

The proteins acting as hub-bottlenecks and their biological processes could be essential in maintenance of the PPI network stability and consequently in favor of ginger effectiveness. While the first two hub-bottlenecks indicate fundamental roles in cancer development by contributing in glucose metabolism, the others show possible important influences in regulation of protein expression. The feasible contradictory properties of 6-shogaol in cancer therapy could propose presence of its beyond preventing effects which requires additional studies.

Conclusion

The network-based analysis introduced central proteins including GAPDH, ENO1, HSP90AB1, ACTG1, RPSA, and CALR that may represent as either a part of underlying antitumor mechanisms of 6-shogaol or its side effects in cancer treatments. These candidates may serve as

prospective drug targets in the future of cervical cancer treatments. It is suggested that more investigations regarding contradictory effects of ginger to be pursued.

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Author contributions
Babak Arjmand and Mona Zamanian Azodi designed and supervised the study; Majid Rezaei Tavirani, Somayeh Esmaeili and Reza Vafaee were involved in data collection and analysis; all authors approved the final draft of the manuscript.

Declaration of interest
The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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Abbreviations

PPI: protei-protein interaction; DEPs; differentially expressed proteins; K: degree; BC: betweenness centrality