Original article

Evaluation of Anticancer Effect of Ghost Pepper: A Bioinformatics Assessment

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Abstract

Background and objectives: Natural sources can be effective in treating diverse pathological conditions especially cancer. Molecular evaluations of pepper on renal cancer could provide further information about its anticancer property. **Methods:** To achieve a clear understanding of pepper effect on cancer cells, protein-protein interaction network analysis of differentially expressed proteins (DEPs) in human renal adenocarcinoma cells treated with ghost pepper were evaluated. Cytoscape V. 3.8.2 and its applications were applied to analyze the DEPs. **Results:** Centrality study showed CYCS and CAT as DEPs were the hub-bottlenecks that were essential for the network stability. Among the 10 introduced central proteins, eight individuals belonged to the added first neighbors from STRING database. The finding indicated that the main central proteins belonged to the first neighbors of the queried proteins and were involved in the anticancer activity. **Conclusion:** Analysis highlighted anticancer property of ghost pepper on the human renal adenocarcinoma cells and also antioxidant effect which was associated with catalase activity.

Keywords: bioinformatics; cell line; ghost pepper; human renal adenocarcinoma; protein

Introduction

The progression of cancer cells can be hindered by the application of phytochemicals that exist in plants such as pepper [1,2]. This plant can have health-boosting effects on different conditions including inflammatory, microbial and fungus diseases [3-5]. The effect of pepper has been investigated in breast, colon, renal and prostate cancers [6-8]. The main component of pepper that shows health benefits is capsaicin which is responsible for its hot taste [9]. One of the hottest peppers around the world is ghost pepper, *Capsicum chinense* Jacq. (Solanaceae), indicating antioxidant and anti-inflammatory effects by isolated capsaicinoids [7,10]. Molecular studies of plant-based treatments could promote understanding the mechanisms by which these candidates play anticancer roles [11,12]. Proteomics is a promising approach that

identifies novel protein biomarkers related to the studied condition [13]. Moreover, through bioinformatics analysis, it is possible to gain more view of the molecular associations with the disease statues. On the other hand, by exploring protein-protein interaction network analysis, biomarkers that have substantial participation in the interaction strength could be detected. These candidates are more valuable in the therapeutic investigations [14]. In a network analysis, it is possible that the central proteins such as hub-bottlenecks be determined. These nodes are the key elements for the network stability and strength [15].

Materials and Methods
Ethical considerations
This project is approved by Shahid Beheshti University of Medical Sciences ethic committee (IR.SBMU.RETECH.REC. 1399.754).

Data collection
To validate the cancer fighting quality of ghost pepper, protein-protein interaction network analysis of human renal adenoma proteome in the treatment of ghost pepper was performed to identify the potential biomarkers. The data was extracted from original article that is published by Venu Perla et al [7]. These candidates may be important to understand the malignancy mechanisms and as molecular targets for clinical approaches.

Network analysis
A number of 107 differentially expressed proteins (DEPs) were detected via proteomics in the treatment of human renal adenocarcinoma cells with pepper [7]. Protein-protein interaction network analysis was performed for these candidate proteins by the use of Cytoscape v. 3.8.2 [16]. This network building platform applies STRING database to conduct network construction from four sources including PubMed, protein query, disease, and Stitch compound query [17]. Here, protein query was carried out and the designated DEPs were subjected to the query. Centrality analysis of the constructed network of DEPs was handled via "Network Analyzer" plug-in integrated in Cytoscape using graph algorithms [18]. Degree and betweenness centrality are the topological parameters that are assessed for the identification of hub-bottlenecks. Proteins with the highest

value of degree and betweenness centrality are called hub-bottleneck elements [19]. Furthermore, CluePedia analyzed pathway and action relationship between the hub-bottlenecks [20]. The pathways of queries were explored based on corresponding gene per term: 2 and percentage of gene in term of 3. The number of chosen pathways was set to 10.

Results and Discussion
Among the differentially expressed proteins, 88 significantly changed with the fold change ≥ 2. These 88 proteins were subjected to the protein-protein interaction network analysis, and only 82 individuals were realized as contributors in the network scale. Two networks were concluded from the query including first network with no additional first neighbor proteins and the second with 50 added surrounding proteins. In the first network, 82 original nodes with 174 edges were present based on cut off of 0.4 as the default option. The second network, however, had 132 nodes with 1647 connections when 50 additional proteins participating with the same designated statistical cut off. In the first network, 13 query nodes remained as individuals that were not included in the main network while in the second network only 3 individuals were not involved in the chief network (data not shown).

"NetworkAnalyzer" was applied to detect the significant nodes of the network with regards to high values of degree and betweenness centrality. These nodes as mentioned in the methods are recognized as hubs and bottlenecks, respectively. The nodes with the highest amount of degree and betweenness centrality are called hub-bottlenecks. To achieve hub-bottlenecks of the network, the second network was considered for this evaluation. In a way that, the common top 20% of nodes with both degree and betweenness centrality values were assigned as hub-bottlenecks in Table1.

Among the hub-bottlenecks, two proteins are from the queried ones including CYCS and CAT. Pathway annotation of 10 hub-bottlenecks was then performed using CluePedia plug-in with designated cut off in Figure 1.

A number of seven pathways were detected that are related to the hub-bottlenecks in the CluePedia analysis. These annotations include histone kinase activity, cyclin binding, regulation of telomerase activity, disordered domain biding, RNA-directed DNA polymerase activity,

disordered domain specific binding, and protein phosphatase 2A binding.

**Table1.** The list of 10 top hub-bottlenecks of pepper network with the related degree and betweenness centrality

Row	Display Name	K	BC	Query proteins
1	TP53	65	1.00	-
2	MYC	65	0.57	-
3	GAPDH	64	0.71	-
4	JUN	57	0.00	-
5	CDK2	50	0.00	-
6	AKT1	51	0.71	-
7	MAPK14	49	0.29	-
8	CDK1	47	0.14	-
9	CYCS	42	0.14	CYCS
10	CAT	42	0.00	CAT

K: degree; BC: betweenness centrality. The proteins are prioritized based on degree values. BC values are presented as normalized amounts.

CluePedia via STRING database analyzed the action types which were presented between the hub-bottleneck nodes in Figure 2.

In the action analysis, two of the hub-bottlenecks including MYC and CYCS were not participating in the interactions considering the selected cut off. TP53 was the most contributing protein in the actin relations. This protein participated in all kinds of action of queries.

Molecular analysis refers to beneficial properties of pharmaceutical substances including plant-based drugs especially in cancer treatment [21]. In this respect, investigation of protein-protein interaction network of renal adenocarcinoma cells treated with ghost pepper was conducted [7]. Two networks of the query DEPs were built and the second network with the related first neighbor proteins was assigned for centrality analysis. Centrality identification recognized 10 hub-bottleneck proteins among them two (CYCS and CAT) were from the DEPs. The top hub-bottleneck nodes were TP53 and MYC which are well-known proteins in relationship with cancers [22]. The hub-bottleneck nodes are known as central nodes that play critical roles in the interactome unit [23]. Since most of the first neighbors that play role as central nodes are the proteins which are highlighted in association with cancers [24], we focused in the function of the two queried central nodes. As it is displayed in Figure 2, CYCS was not involved in the action map; therefore, the single queried central node (CAT) was selected as the key differentially expressed protein that, based on network analysis and its role in the action map, is a critical protein in response to pepper treatment.
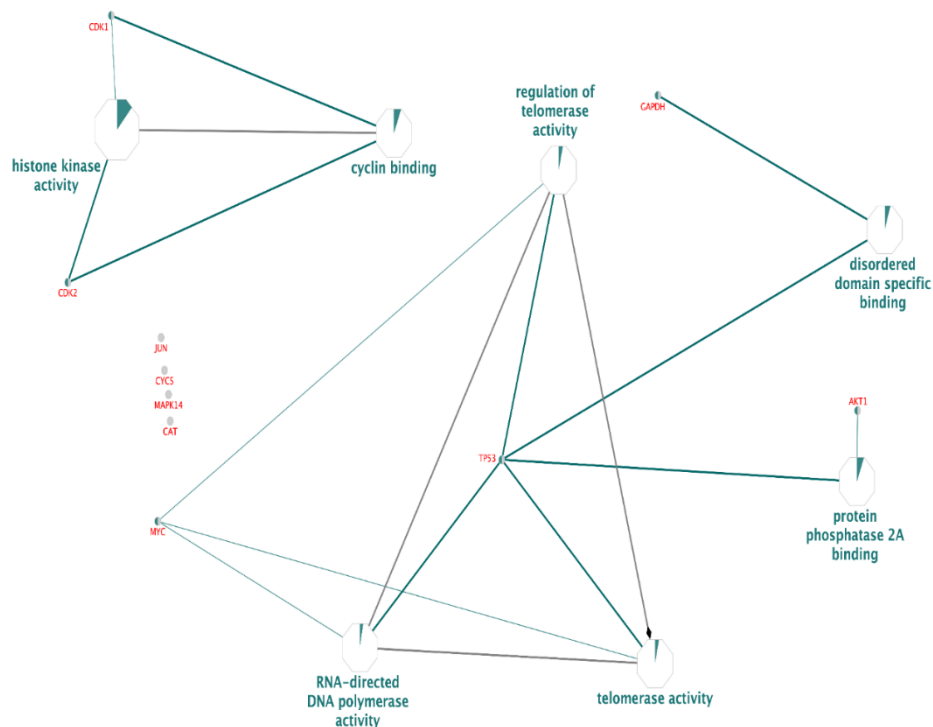
Figure 1. Pathway analysis of hub-bottlenecks with CluePedia application of cytoscape. Gene per group: 2, percentage of gene per term: 3 were considered. The assessed proteins are presented as red colored nodes.
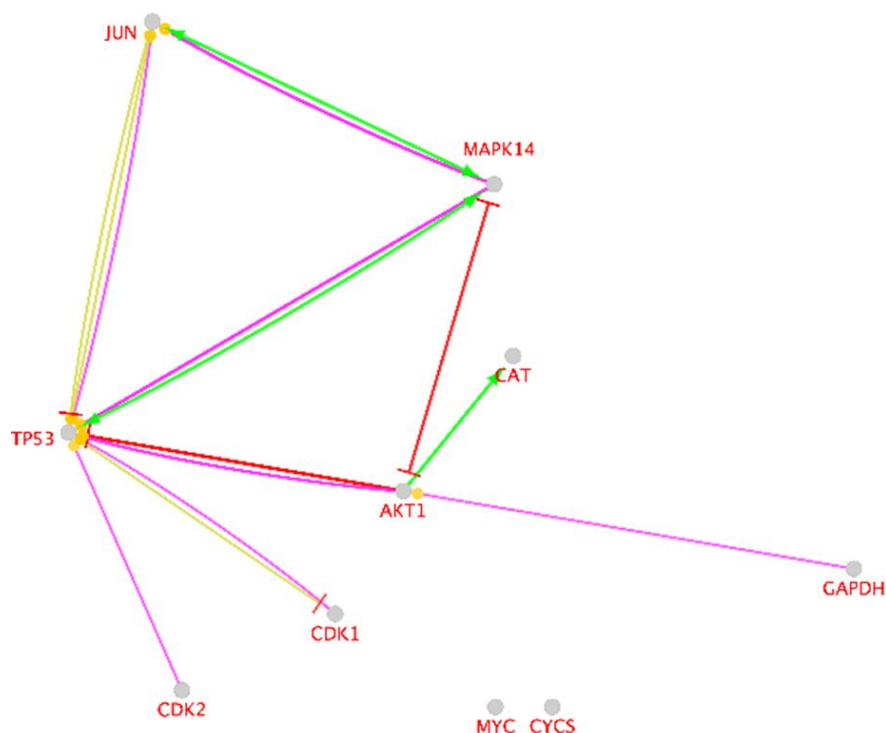
Figure 2. Action view of hub-bottlenecks including activation (green), expression (yellow), inhibition (red), and post-translation modification (purple) relations. Kappa score cut off ≥ 0.5 was considered. Straight arrow refers to direction of activation. The round and bar tips refer to positive regulation and inhibition, respectively.

As it is depicted in the gene ontology analysis, the queried central nodes were remained isolated and the central first neighbors were related to the biological terms that are known as regulation of cell cycle and cell proliferation, the main associated terms to cancers. Cyclin binding and telomerase activity which are related to cell cycle and cancers [25,26] are connected directly to P53 and MYC. As like the two mentioned biological terms, the other terms (RNA-directed DNA polymerase activity, protein phosphatase 2A binding, histone kinase activity, regulation of telomerase activity, and disordered domain specific binding) are related mainly to the DNA regulation.

CAT action in Figure 2 is depicted as the single connection with AKT1. Based on this action, AKT1 activates CAT and no other action is issued by CAT in relationship with other central nodes. Catalase (CAT) is an important enzyme that is studied for about 100 years by many researchers from different aspects such as function and structure in different types of biological organisms [27]. Hydrogen peroxidase (H_2O_2) is the CAT substrate; investigations indicate that this substrate induces an inactive form of enzyme slowly and presence of NADPH prevents this event [27]. Hydrogen peroxide is known as a cytotoxic agent in human body; therefore, activity of antioxidant system in body reduces its level to prevent body from its damages [28]. While high concentration of hydrogen peroxide is known to be toxic, low concentration of this substrate plays role in modulation of several biological processes such as apoptosis, cell proliferation, platelet activation, and carbohydrate metabolism. It is reported that catalase mutation is associated with vitiligo, diabetes mellitus, and hypertension. Decrease of catalase synthesis is reported in tumors in patients [29]. Our finding revealed the prominent role of pepper consumption in prevention of cancer events which is expressed mainly by the first neighbor added proteins and also leading to introduce catalase as the main regulated enzyme among the queried proteins which were regulated by pepper.

Conclusion

Anticancer property of pepper was highlighted in this research project via the first neighbors of the queried proteins. Antioxidant activity of pepper

was also determined as the associated process to the role of queried proteins. It seems that a careful investigation considering dosage and pattern of consumption of pepper in a suitable population can provide valuable information about consumption protocol of pepper. However, the findings are corresponded to ghost pepper and the ratio of capsaicinoids is considerable.

Acknowledgment

Author contributions

Mostafa Rezaei-Tavirani desighned and supervised the study; Mona Zamanian-Azodi, Vahid Mansouri and Somayeh Esmaeili 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

DEPs: differentially expressed proteins; K: degree; BC: betweenness centrality