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Original article

Bioinformatics Identification of Green Tea Anticancer Properties: a Network-Based Approach

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Abstract

Background and objectives: Promising anticancer properties are associated with the consumption of green tea. On the other hand, lung cancer has been showing to possess the highest number of death compared to other types of cancer. The aim of this study was to understand the mechanisms by which green tea shows this effect; bioinformatics study of proteome profile could be essential. For this reason, the proteomics analysis of human lung adenocarcinoma A-549 cells treated with green tea extract was chosen for protein-protein interaction (PPI) network analysis. **Methods:** Cytoscape v.3.8.2 and its applications analyzed a number of 14 differentially expressed proteins (DEPs) from green tea treatment experiment as two networks. The biological annotations and action type exploration of the hub-bottlenecks of the PPI network were then carried out. **Results.** The investigation indicated that among 14 queries DEPs, HNRNPA2B1, PCBP1, and HNRNPC may show substantial role. Moreover, HSPA8 was the top hub-bottleneck and half of the central protein groups were enriched with heterogeneous nuclear ribonucleoproteins complex family (HNRNPs). **Conclusion.** The anticancer bioinformatics study of green tea suggests a complex nature for green tea. This finding urges complementary evaluations to validate whether green tea is applicable as an anticancer agent in medicine.

Keywords: adenocarcinoma of lung; bioinformatics; cancer suppressor genes; humans; tea

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Introduction

Cancer, a genetic-related disease, accounts for 13% causes of death in the world [1,2]. Primarily, treatment approaches are chemotherapy, radiation, immunotherapy, and surgery [3,4]. In spite of being effective, there are many major side effects accompanying application of these therapeutic alternatives [5]. Therefore, discovery of optimized methods with lower side effects is a requirement in cancer management [6]. Natural sources and herbal medicines could be safer candidates for cancer therapy strategies considering less side effects [6]; however, optimization is required for drug discovery [7] with the aid of target therapy via molecular examinations [6]. As in some studies of herbal medicine in cancer treatment, it has been understood that lavender, cocoa, and rose could provide medicinal role [8-11]. In addition, green tea (*Camellia sinensis* (L.) Ktze. leaves) has been known beneficent in health care especially as a

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protective and therapeutic agent in cancer therapy [3,12].

Camellia sinensis, (synonym *Thea sinensis* L.), Theaceae family, is cultivated in many parts of the world for the leaves to provide the popular beverage known as "tea". If the leaves are fermented they are called black tea while non-fermented leaves are known as green tea. Phytochemicals of green tea have some influence on cancers of liver, colon, breast, esophagus, prostate, and oral cancer [13-16]. Furthermore, the risk of diseases including cardiovascular diseases, liver, Alzheimer's and Parkinson's diseases, diabetes, and cancer, can be diminished by the use of green tea [17-20]. In fact, the antioxidant properties of green tea compounds are known for their preventive activities against cancer [21]. For instance, increment of oxidative stress is responsible for the development of any type of malignancies [22]. Polyphenol compounds in green tea, on the other hand, could assist reduction of these vicious products. Green tea, besides being suggested as effective, could also a safe therapy role in comparison with many chemical agents [23]. One of the human organs that is influenced by green tea intake, is lung. Anticancer activity of green tea against lung cancer cells is reported by several researchers [24,25]. One of the ingredients of green tea that has shown promising chemopreventive activity is epigallocatechin-3-gallate (EGCG). The suppressive effects of this component is by targeting many signaling pathways and important proteins of different types of cancers such as lung cancer [26]. As a part of EGCG mechanism of action on lung cancer, NF-κB down-regulation takes parts [27]. Molecular studies such as proteomics proved potents in regards to identifying mechanisms of different types of cancers and their candidate treatments [28]. Moreover, corresponding bioinformatics studies of proteome data in terms of (PPI) network analysis, could develop discovery of biomarkers that underline disease progression mechanisms [29]. Consequently, to elucidate how green tea shrinks cancer risk, protein-protein interaction network analysis of differentially expressed proteins in lung cancer cells in treatment with green tea was evaluated in this study.

Materials and Methods

Ethical considerations

This project is confirmed by Shahid Beheshti University of Medical Sciences ethic committee (IR.SBMU.RETECH.REC.1397.1341).

Proteomics data of human lung adenocarcinoma cell line (A-549) which was treated with green tea extract was investigated in this study for protein-protein interaction network analysis. In the main study, the green tea extract was from *Camellia sinensis* leaves. The cells were treated with 0, 20, and 40 mg/mL of green tea extract for duration of 24 h. The proteomics investigation of these treatments were then proceeded by two dimensional electrophoresis (2-DE) and followed by HPLC-Tandem mass spectrometry analysis [21].

Protein-protein interaction network analysis

A total of 14 protein spots were detected statistically differentially expressed considering fold change ≥2 in the treated samples from the proteomics study. In our study, using the Cytoscape 3.8.2. (http://www.cytoscape.org) and STRING database, the 14 DEP with their Uniprot identifiers were searched for their interaction patterns [30] as listed in Table 1. Centrality computation (highest degree and betweenness values distribution) of the second network was concluded by Network Analyzer 4.4.63-application of Cytoscape software [31]. In this interaction network, nodes are the interacting proteins and edges are the interaction exist among them [32]. The nodes with highest values of degree and betweenness are referred as hubs and bottlenecks, respectively. Hub-bottlenecks are protein nodes that have both features of hubs and bottlenecks [33]. Additionally, ClueGO v.2.5.7 and CluePedia v.1.5.7, the Cytosacpe plug-ins [34,35] were applied for the hub-bottlenecks enrichment in terms of biological process (BP) identification.

Statistical analysis

Kappa score statistics were used as statistical method for grouping analysis of biological terms; the cut off was set as 0.5. In addition, the number and percentage of proteins per term was assigned 2 and 3, respectively. The enrichment/depletion test = two-sided (enrichment/depletion) based on hypergeometric was considered. Finally, Bonferroni step down (the p-value correction method for the assigned *p*-value) ≤0.05 was applied to validate data. The final analysis was to investigate edge actions of the hub-bottlenecks via CluePedia. The CluePedia_STRING_ACTION_v11.0_9606_27.02.2019.txt.gz was the source file for this

analysis.Kappa scoring (0-1) was used for this purpose and a cut off ≥ 0.5 was set for the all types of actions.

Results and Discussion

In order to analyze the topological parameters of green tea extract impact on lung adenocarcinoma cell line (A-549) interaction map, two networks of DEPs were retrieved from STRING, Cytoscape application. First PPI network was built of 14 DEPs without any addition neighbor proteins (Figure1). The second network that was considered for centrality analysis included the query proteins plus 50 neighbor proteins (data not shown).

In the second network, a number of 64 nodes and 1018 edges were present; among them, some were more central than the other elements.

NetworkAnalyzer assigned topological measurements for the query proteins and neighbor proteins of the second network (Table 2).

According to NetworkAnalyzer, among the top ten hub-bottlenecks, three were from DEPs including P22626, Q15365, and P07910. Degree of these three nodes were 49, 48, and 46 while the betweenness centrality was 0.02, 0.02, and 0.01. HSPA8 which was an added first neighbor (proteins that were added to the query ones), displayed the highest degree and betweenness values, 58 and 0.07, respectively.

Enrichment analysis regarding biological process (BP) of hub-bottlenecks conducted by ClueGO+ CluePedia is shown in Figure 2. Groups of terms are prioritized based on the most involved proteins in a group.

Table 2. Top ranked hub-bottlenecks based on first 20% degree (K) and Betweenness centrality (BC) values. The list is ranked based on degree value

Row	Display Name	K	BC	Query Proteins
1	HSPA8	58	0.07	
2	HNRNPA2B1	49	0.02	P22626
3	PCBP1	48	0.02	Q15365
4	HNRNPA1	47	0.01	
5	ELAVL1	46	0.01	
6	HNRNPC	46	0.01	P07910
7	HNRNPD	45	0.02	
8	HNRNPK	45	0.01	
9	EEF2	43	0.03	
10	DDX39B	40	0.02	

Table 1. The list of differentially expressed proteins under exploration in this bioinformatics analysis

Row	Gene name	Protein name	Protein Code
1	ANXA1	Annexin I	P04083
2	ANXA2	Annexin II	P07355
3	FSCN1	Fascin	Q16658
4	LMNA	Lamin A/C	P02545
5	YWHAH	14-3-3 protein	Q04917
6	GPI	Glucose-6-phosphate isomerase	P06744
7	ATP5F1A	ATPsynthaseachain	P25705
8	AHCY	Adenosylhomocysteinase	P23526
9	PRDX1	Peroxiredoxin1	Q06830
10	HNRNPA2B1	hn ribonucleoproteins A2/B1	P22626
11	HNRNPC	hn ribonucleoproteins C1/C2	P07910
12	PCBP1	Poly(rC)-binding protein 1	Q15365
13	FUBP1	FUSE binding protein 1	Q96AE4
14	ETFA	Electron transfer flavoprotein a-subunit	P13804

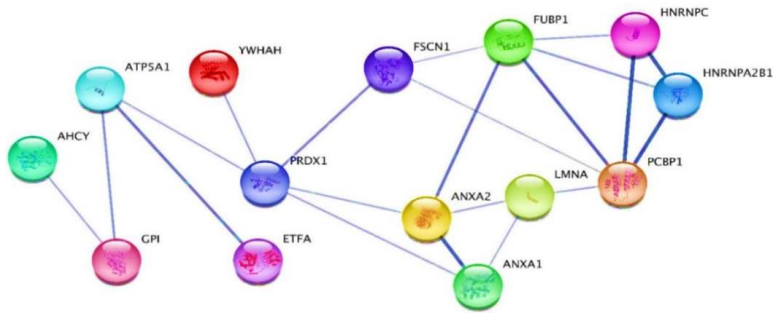

Figure 1. Protein interaction network of comparison between untreated lung cancer cell line and treated with green tea extract. Number of nodes: 14, Number of links: 2

Regulation of DNA biosynthetic process (35.71%), negative regulation of mRNA metabolic process (35.71%), and regulation of mRNA splicing, via spliceosome (28.57%) are the highlighted groups related to the hub-bottlenecks. However, not all queries were retrieved due to the considered statistical parameters. As a matter of fact, eight of hub-bottlenecks were detected while two of them including PCBP1 and EEF2 were not found in this annotation. The first two groups contained five of nodes while the last one was only linked to three of hub-bottlenecks. HNRNPA2B1 showed contribution in all three groups. Moreover, HSPA8, ELAVL1, and DDX39B only belonged to one individual group based on our findings.

Action linkage between hub-bottlenecks assigning kappa scoring was calculated by ClueGO+CluePedia application in Figure 3.

Green tea has been suggested to have inhibitory effects on lung tumor developments [21]. Molecular study could provide additional understanding about the mechanisms by which green tea could be effective. In this view, complementary analysis such as protein-protein interaction network examination of DEPs in green tea treatment is valuable to be studied.

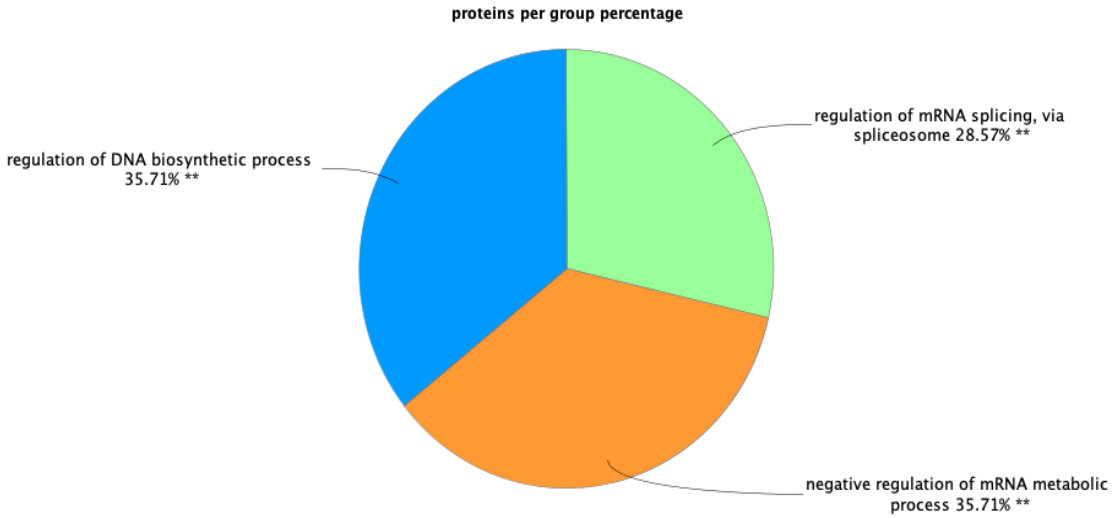

Figure 2. Pie chart visualization of enriched hub-bottlenecks for BP via ClueGO+ CluePedia analysis; grouping is based on protein per group percentage and assigned with different colors; two asterisks represent the p value≤0.01 annotation

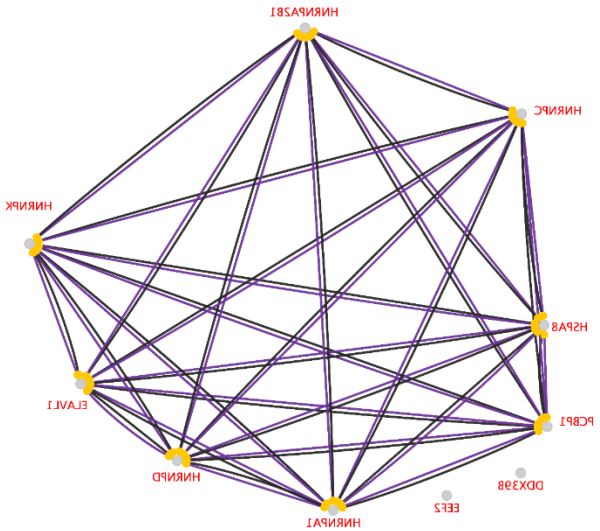

Figure 3. Action view of hub-bottlenecks regarding catalysis: purple and black: reaction. Two nodes including DDX39B and EEF2 remained without interactions in this analysis; Kappa score cut off≥ 0.5

Protein expression data of human lung adenocarcinoma cell line (A-549) in the presence of different concentrations of green tea was used for protein-protein interaction network analysis in this study. A number of 14 DEPs involved in green tea treatment were considered for this evaluation and the network analysis showed that all DEPs were linked as a whole interactive map and no individual node remained. Hence, this analysis indicated that these proteins were in a dense relationship.

Further analysis of these candidates as a network of interacting with surrounding proteins highlights the potentials of some the nodes as the main foundation of the network stability. These central nodes could be both from query nodes and neighbor ones. Among the 10 top hub-bottlenecks, three are from the query including HNRNPA2B1, HNRNPC, and PCBP1. The half of hub-bottlenecks are from the heterogeneous nuclear ribonucleoprotein complex (HNRNPs). These proteins dominantly participate in RNA processing [36,37] are known to have diverse associations in carcinogens and metastasis [38]. In the original study, two members of HNRNP family were among the significant differentially expressed proteins [21]. Yet, their role in green tea regulatory properties was not highlighted.

Biological process ontology of hub-bottlenecks showed that the first two ranked groups of terms indicated the highest number of associated proteins. These biological processes as regulatory function may be a part of green tea extract role in fighting against cancer expansion. Action analysis of these hub-bottlenecks indicated that the most significant interactions between them were catalysis and reaction considering medium scoring cut off.

Lastly, the top five as the highest ranked hub-bottleneck (HSPA8, HNRNPA2B1, HNRNPC, PCBP1, HNRNPA1, and ELAVL1) were then designated for literature review to gain more knowledge about their possible correlations in green tea treatment. The first protein, heat shock protein 8 (HSPA8), according to one study, has been reported as a differentially expressed element at gene level in lung cancer therapy via green tea extract in mice. In fact, green tea showed regulatory effect on this protein expression [39]. In another study by a proteomics approach, this protein was indicated as one of the differentially expressed elements likely involved in increasing egg white quality after treating with green tea extract [40]. These findings indicate that HSPA8 could be part of the green tea underlying regulatory mechanism. The next hub-bottleneck is heterogeneous nuclear ribonucleoproteins A2/B1 (HNRNPA2B1), which is a DEPs in both patients' tissue and blood samples comparing with healthy cases [41]. By exposure to green tea, the expression of this protein changes that could intricates in chemo-preventive properties of green tea extract. What is more, this protein may participate in green tea regulatory effect as lnc-HC/hnRNPA2B1complex on cholesterol metabolism by bile acid biosynthesis reduction and exporting cholesterol [42]. The next hub-bottleneck is poly(C)-binding protein 1 (PCBP1). Its pathogenicity in lung cancer has been suggested by other studies [43,44]. Geuens et al. reported the role of PCBP1 in neurodegenerative diseases [45]. Heterogeneous nuclear ribonucleoprotein A1 (HNRNPA1) is the fourth hub-bottleneck that is not from DEPs while is reported as therapeutic agent in tumor prevention of green tea. Indeed, quercetin, flavonoid in green tea, targets this protein [46]. The fifth hub-bottleneck is ELAV-like protein 1 (ELAVL1), RNA binding protein that acts as a regulatory mediator in inflammatory system [47]. Besides, studies showed that this culprit protein is responsible for development of cancers including ovarian, breast, colon, bladder and lung cancer [48-50]. It is also reported by N Melling et al, that cytoplasmic accumulation of ELAVL1 is correlated with prostate cancer [51]. Likewise, GU et al. also reported up-regulation of miR-324-5p targets ELAVL1 to inhibit proliferation and invasion of cells of colorectal cancer [52]. On the contrary, green tea could increase the level of this protein [53] and consequently, may also act as a stimulating factor in cancer development it may be depended on dosage of consemption. Apparently, ELAVL1 is associated in cancer promotion, which should be considered to be examined as a possible harmful aspect and side effect of green tea.

The introduced central proteins may play more significant roles in the network and thus are novel agents in green tea protective effect mechanisms; still complexity is obvious (the undesirable side effects). Thus, the aberrant expression of HSPA8, HNRNPA1, PCBP1, ELAVL1, HNRNPC, HNRNPD, HNRNPK, EEF2, and DDX39B in lung cancer is important to be studied in the treatment of green tea

thoroughly. In other words, although antitumor effects of green tea have been suggested by many studies; the associations are not yet well established. As a final point, while green tea could improve metabolic processes in liver [54], its cancer fighting essences should be studied further.

Conclusion

In brief, green tea, which serves as phytopharmaceutical agent, can play a chemo-preventive role against cancer; nevertheless, with complex effects that has to be clarified. It seems more investigation is required to analyze the complex nature of green tea to establish its potential anticancer entity prior to clinical applications.

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Author contributions

Mostafa Rezaei Tavirani designed and supervised the study; Somayeh Esmaeili was involved in data collection; Mona Zamanian-Azodi and Majid Rezaei Tavirani were involved in data collection and data 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: protein-protein interaction; DEPs: differentially expressed proteins; HNRNPs: heterogeneous nuclear ribonucleoproteins; 2-DE: two dimensional electrophoresis; EGCG: epigallocatechin-3-gallate; BP: biological process; K: degree; BC: Betweenness centrality