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Original article

Network Analysis of 20S-Ginsenoside Rg3 Effect on Human Colorectal Adenocarcinoma Cell Line HT-29

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Abstract

Background and objectives: 20S-Ginsenoside Rg3 is a pharmacological active compound of ginseng. Evidences indicate that S20-Rg3 as an anti-cancer factor plays role in prevention and treatment of cancer. In the present study, proteomic data of 20S-ginsenoside Rg3 effect on human colorectal adenocarcinoma cell line HT-29 was analyzed via network analysis to understand more details about the molecular events. **Methods:** The differentially expressed proteins (DEPs) related to the effect of 20S-ginsenoside Rg3 on human colorectal adenocarcinoma cell line HT-29 were extracted from literature and analyzed via protein-protein interaction (PPI) network. The central nodes of the network were determined based on degree value and betweenness centrality. **Results:** Eight DEPS plus 100 added first neighbors were included in the PPI network. Five central nodes as hub-bottlenecks including ACTB, GAPDH, TP53, AKT1, and ALB among the added first neighbors and ANXA5 as hub-bottleneck and GSTP1 and PCNA as bottlenecks among the queried DEPs were introduced. **Conclusion:** PCNA, GSTP1, and ANxA5 as cell protective proteins are the crucial targeted proteins by 20S-ginsenoside Rg3 in the treated cell line HT-29. Up-regulation of GSTP1 and ANXA5 is correspondent to the cell protective property of 20S-ginsenoside Rg3, and down-regulation of PCNA refers to the opposite effect. It seems that cell protective roles of 20S-ginsenoside Rg3 are accompanied with the possible side effects.

Keywords: bioinformatics; differentially expressed proteins; ginseng; network analysis

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Introduction

Root of *Panax ginseng* C.A.Mey. (Araliaceae), is known as ginseng. Ginsenosides, secondary metabolites, and triterpene glycosides are the pharmacologically active components of ginseng. 20S-ginsenoside Rg3 and 20R-Rg3 are the two optical isomers of ginsenoside Rg3 [1].

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Investigations indicate that ginsenoside Rg3 can be considered as a cancer preventive factor. It also plays role as a therapeutic agent in cancer treatment. Role of ginsenoside Rg3 in inhibition of proliferation, induction of apoptosis, promotion of immunity, and inhibition of metastasis and angiogenesis is mentioned in literature [2]. Anticancer property of ginsenoside Rg3 has been investigated and confirmed in the human hepatocellular carcinoma cells. Although molecular mechanism of ginsenoside Rg3 (20S-Rg3) is discussed in the administrated projects, better understanding of this molecular event need more investigation [3,4].

Experience shows that high throughput methods such as genomics, proteomics, metabolomics, in combination with computational approaches and bioinformatics are useful means to explore the unknown aspects of molecular mechanism in the fields of pharmacology, biology and medicine. Many diseases are assessed via proteomics and bioinformatics to introduce the related biomarkers. Pharmaceutical researches via proteomics and bioinformatics provide useful findings about herbal foods and drugs. In such investigations effects of herbal extracts or certain compounds on the biological samples such as different human cell lines are evaluated based on expression change of the proteins or genes of the studied samples. The significant DEPs or differentially expressed genes are studied via bioinformatical analysis such as network approaches [5-7].

PPI network analysis is a network evaluation of the interacted elements such as the queried proteins by considering the roles of the proteins (nodes) in the network. Centrality property of a node (degree, betweenness centrality, or the other centrality parameters) determines importance of protein role in integrity of the constructed network [8,9]. Two crucial central nodes are known as hubs and bottlenecks. The hubs make large numbers of connections with the first neighbors while the bottlenecks are mainly involved in the shortest paths [10-12]. The common hubs and bottlenecks which are known as hub-bottlenecks are the potent central nodes and play the key role in the network. In the present project, the DEPs from effect of 100 µM of ginsenoside 20S-ginsenoside Rg3 on human colorectal adenocarcinoma cell line HT-29 were extracted from published data by Seo Young Lee [1] and evaluated via PPI network analysis to introduce the critical targeted proteins.

Material and Methods

Ethical considerations

This project is confirmed by IR.SBMU.RETECH.REC.1400.678 ethical code.

Data collection

Data was extracted from published document entitled "Proteomic analysis of the anti-cancer effect of 20S-ginsenoside Rg3 in human colon cancer cell lines" by Seo Young Lee et al. [1]. As it is described in the original research, proteome of human colorectal adenocarcinoma cell line HT-29 that was treated with 20S-Rg3, for 24 h versus controls was investigated. Eight DEPs were introduced as targeted protein by 20S-Rg3.

Network analysis

The 8 queried DEPs plus 100 first neighbors from STRING database [13] were interacted by cytoscape software [14] via "protein query" of STRING. Number of 108 nodes were linked via 1758 undirected edges. The formed PPI network was analyzed by "NetworkAnalyzer" application of cytoscape software and the network was visualized by degree value. Two centrality parameters, degree and betweenness centrality, were assessed to determine the hubs and bottlenecks. The top 10% of the nodes based on degree value and betweenness centrality were identified as hubs and bottlenecks, respectively. The common hubs and bottlenecks were introduced as hub-bottlenecks. The central nodes including hubs, bottlenecks, and hub-bottlenecks were evaluated to explore the related functions and pathways.

Results and Discussion

Fold change and mode of dysregulation of the queried 8 DEPs are illustrated in the Figure 1. As it is shown in the figure thee DEPs are down-regulated while five individuals are up-regulated. STRAP and RBP4 are extremely down-regulated and up-regulated, respectively. The PPI network including the eight queried DEPs is shown in the Figure 2. As it is depicted, there is no linkage between the node and all proteins remained as isolated nodes. The PPI network including the 8 queried DEPs and 100 added first neighbors is presented in Figure 3. The 108 nodes are

connected by 1758 edges and all queried DEPs are connected.

Results of network analysis are tabulated in Table 1 and 2. Eleven hubs including ANXA5 as a single queried DEP and ten added first neighbors are shown in Table 1. Bottleneck nodes (GSTP1, ANXA5, and PCNA as queried DEPs and 8 added first neighbors) are presented in Table 2. Evaluation showed that minimum number of added first neighbors to make all queried DEP connected in network was 20 individuals (Figure 4).

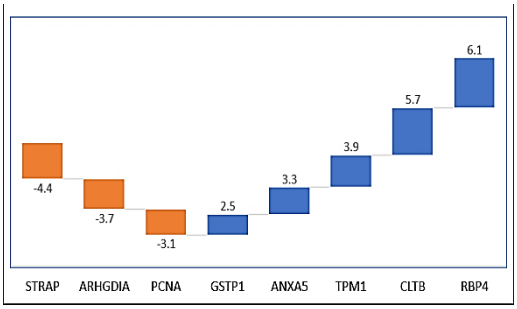

Figure 1. Fold change (expression value of treated cells/expression value of controls) of the 8 queried DEPs that discriminate the HT-29 cells treated with 100 µM of 20S-ginsenoside Rg3 for 24 h from controls; range and blue colors refer to down-regulation and up-regulation respectively.

Figure 2. PPI network including the 8 queried DEPs that discriminate the HT-29 cells that were treated with 100 µM of 20S-ginsenoside Rg3 for 24h from controls

To assess the most related first neighbors, combination of the queried 8 DEPs and 10 added first neighbors was analyzed via PPI network analysis (Figure 5). Protein expression change of biological samples is studied to find the dysregulated individuals under the studied condition to find the involved proteins in the altered situation [15]. As it is shown in Figure 1, eight significant DEPs with different amounts of fold change are introduced as targeted proteins by 20S-ginsenoside Rg3 in human colorectal adenocarcinoma cell line HT-29. STRAP and RBP4 as the most down-regulated and up-regulated proteins are appeared in the two ends of the spectrum of fold change values, respectively.

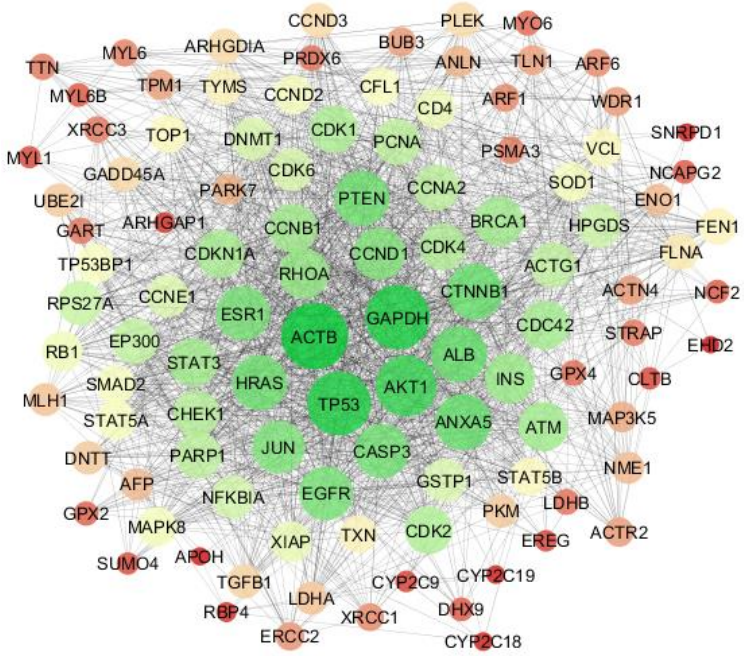

Figure 3. PPI network including the eight queried DEPs that discriminate the HT-29 cells that were treated with 100 µM of 20S-ginsenoside Rg3 for 24 h from controls plus 100 added first neighbors; nodes are layout by degree value (are not similar in size and color) and are picked manually; brown to green and small to large refer to increase of degree value

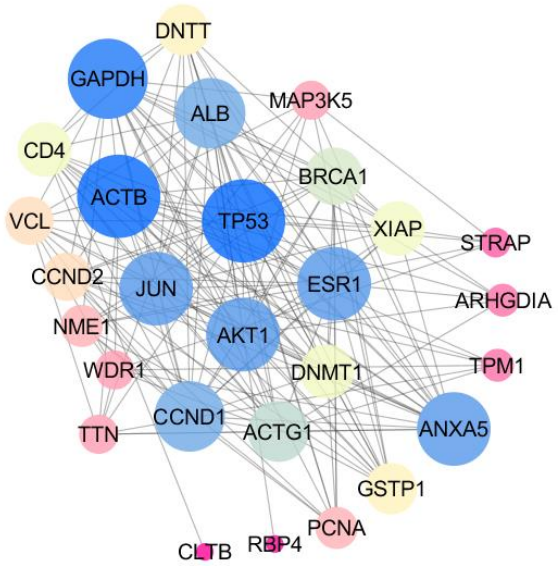

Figure 4. PPI network including the eight queried DEPs related to HT-29 cells that were treated with 100 µM of 20S-ginsenoside Rg3 for 24 h from controls plus 20 added first neighbors; nodes are layout by degree value; red to green and small to large size refer to increase of degree value

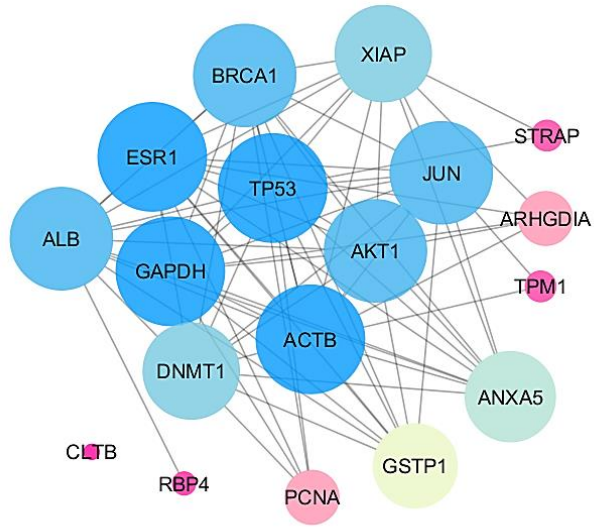

Figure 5. Network counting the queried DEPs of HT-29 cells (treated with 100 µM of 20S-ginsenoside Rg3 for 24 h) relative to controls plus 10 added first neighbors; nodes are layout by degree value; red to green and small to large size refer to increase of degree value

PPI network analysis revealed that there in no connection between the queried proteins. Adding 100 first neighbors led to the construction of a scale free PPI network. Network analysis led to introduce 11 hubs, 11 bottlenecks, and 1 hub-bottleneck. ANXA5 the single queried hub appeared as hub-bottleneck node. The 5 top hub nodes are first neighbors and also the 5 top bottlenecks are among the first neighbors. As it is depicted in tables 1 and 2, the top 5 hubs and bottlenecks including actin beta (ACTB), glyceraldehyde 3-phosphate dehydrogenase (GAPDH), tumor protein p53 (TP53), AKT serine/threonine kinase 1 (AKT1), and albumin (ALB) are hub-bottlenecks.

Table 1. The hub nodes of the analyzed PPI of the HT-29 cells that were treated with 100 µM of 20S-ginsenoside Rg3 for 24 h versus controls

No.	Display name	Query term	Degree
1	ACTB	-	83
2	GAPDH	-	79
3	TP53	-	78
4	AKT1	-	73
5	ALB	-	64
6	ANXA5	anxa5	64
7	CTNNB1	-	63
8	HRAS	-	62
9	PTEN	-	62
10	CASP3	-	61
11	EGFR	-	60

Table 2. The bottleneck nodes of the analyzed PPI of the HT-29 cells that were treated with 100 µM of 20S-ginsenoside Rg3 for 24h versus controls

No.	Display name	Query term	Betweenness centrality
1	ACTB	-	0.063
2	ALB	-	0.044
3	TP53	-	0.041
4	GAPDH	-	0.040
5	AKT1	-	0.032
6	GSTP1	GSTP1	0.028
7	ANXA5	ANXA5	0.026
8	PCNA	PCNA	0.023
9	HPGDS	-	0.022
10	RHOA	-	0.021
11	HRAS	-	0.019

Gu et al. published data about role of ACTB in 33 tumors based on the datasets of gene expression omnibus and the cancer genome atlas [16]. Nicholls et al. published a document entitled "GAPDH: a common enzyme with uncommon function" and Guo et al. presented evidence about role of GAPDH in tumor [17,18]. TP53 is a well-known tumor suppressor protein [19].

Relationship between AKT1 up-regulation and human tumors via suppressing apoptosis and accelerated proliferation is reported by researchers [20]. Dysregulation of albumin expression in cancers is led to introduction of CRP/ALB ration as an inflammation based score in pancreatic cancer [21]. It can be concluded that the critical added first neighbors are the cancer related proteins. These crucial central first

neighbor proteins were investigated among the two sub-networks (Figures 4 and5); first the subnetwork counting the eight queried DEPS and at least numbers of the added first neighbors to make connected all DEPs and the other subnetwork including the DEPs and 10 added first neighbors. Presence of the five first neighbor hub-bottlenecks is illustrated in Figures 4 and 5.

As it was discussed, ANXA5 is a single hub-bottleneck DEP while GSTP1 and PCNA were introduced as the queried DEP bottlenecks. Based on literature, glutathione S-transferases including GSTM1, GSTT1 and GSTP1 are known as multifunctional enzymes which play roles in the detoxification process of a various reactive oxygen species that are produced through synthesis of melanin and also oxidative stress processes [22]. It is reported that GSTP1 is involved in cellular protection against toxic foreign chemicals and oxidative stress in lung epithelium [23]. Significant role of proliferating cell nuclear antigen (PCNA) the other bottleneck DEP in the DNA replication and repair is confirmed by researchers [24]. Bhardwaj et al. published a document about investigation related to the native and the mutated PCNA forms. Their finding indicates that PCNA mutations leads to DNA mismatch repair process [25]. Anti-apoptotic and anti-inflammatory properties of annexin A5 (ANXA5) are the well-knwon roles of this protein in promotion of related biological processes [26]. Anti-oxidant role of ANXA5 through ERK/Nrf2 pathway is highlighted in literature [27].

Returning to the results in Figure 1, fold change for PCNA, GSTP1, and ANXA5 are -3.1, 2.5, and 3.3, respectively. Over-expression of GSTP1 and ANXA5 are corresponded to the useful properties of 20S-ginsenoside Rg3 effect on the treated cell line. The finding confirmed protective role of 20S-ginsenoside Rg3 against stresses. As it was described, PCNA plays crucial role in DNA repair and replication processes. It seems that down-regulation of PCNA refers to the opposite property of 20S-ginsenoside Rg3 effect on the studied cell line. Our finding indicates that ginseng contains set of components with different properties and it cannot be considered as an absolutely safe nutrient.

Conclusion

Findings indicate that PCNA, GSTP1, and ANxA5 as cell protective proteins are the crucial targeted proteins by 20S-ginsenoside Rg3 in the human colorectal adenocarcinoma cell line HT-29. However, up-regulation of GSTP1 and ANXA5 refers to the cell protective property of 20S-ginsenoside Rg3, down-regulation of PCNA is corresponded to the opposite effect. Relationship between the five central first neighbors and cancer was highlighted. It seems that positive roles of 20S-ginsenoside Rg3 are accompanied with the side effects that should be considered in consuming ginseng. More analysis is required to detect complete properties of ginseng as a useful nutrient.

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Author contributions

Mona Zamanian Azodi designed and supervised the study; Babak Arjmand, Mostafa Rezaei Tavirani, Mahmood Khodadoost, Mohammad Rostami Nejad and Nayebali Ahmadi 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.

References

[1] Lee SY, Kim GT, Roh SH, Song JS, Kim HJ, Hong SS, Kwon SW, Park JH. Proteomic analysis of the anti-cancer effect of 20 S-ginsenoside Rg3 in human colon cancer cell lines. *Biosci Biotechnol Biochem*. 2009; 73(4): 811–816.

[2] Sun M, Ye Y, Xiao L, Duan X, Zhang Y, Zhang H. Anticancer effects of ginsenoside Rg3. *Int J Mol Med*. 2017; 39(3): 507–518.

[3] Zhang C, Liu L, Yu Y, Chen B, Tang C, Li X. Antitumor effects of ginsenoside Rg3 on human hepatocellular carcinoma cells. *Mol Med Rep*. 2012; 5(5): 1295–1298.

[4] Zhang F, Li M, Wu X, Hu Y, Cao Y, Wang X, Xiang S, Li H, Jiang L, Tan Z, Lu W, Weng H, Shu Y, Gong W, Wang X, Zhang Y, Shi W, Dong P, Gu J, Liu Y. 20 (S)-ginsenoside Rg3 promotes senescence and apoptosis in gallbladder cancer cells via the p53 pathway. *Drug Des Devel Ther*. 2015; 9: 3969–3987.

[5] Arjmand B, Zamanian Azodi M, Rezaei

Tavirani M, Khodadoost M, Rostami Nejad M, Ahmadi N. Tumorigenesis inhibition of carnosol from rosemary: an insight to molecular targeting. *Res J Pharmacogn*. 2022; 9(1): 103–110.

[6] Rostami-Nejad M, Razzaghi M, Esmaeili S, Zali A, Rezaei-Tavirani M, Heidari MH, Rezaei-Tavirani M, Zamanian-Azodi M, Ahmadi N. Evaluation of anticancer and neuroprotective properties of curcumin: a network analysis. *Res J Pharmacogn*. 2020; 7(3): 85–92.

[7] Guingab-Cagmat J, Cagmat E, Hayes RL, Anagli J. Integration of proteomics, bioinformatics, and systems biology in traumatic brain injury biomarker discovery. *Front Neurol*. 2013; 4: 1–12.

[8] Vella D, Marini S, Vitali F, Di Silvestre D, Mauri G, Bellazzi R. MTGO: PPI network analysis via topological and functional module identification. *Sci Rep*. 2018; 8(1): 1–13.

[9] Ghasemi M, Seidkhani H, Tamimi F, Rahgozar M, Masoudi-Nejad A. Centrality measures in biological networks. *Curr Bioinform*. 2014; 9(4): 426–441.

[10] He X, Zhang J. Why do hubs tend to be essential in protein networks? *PLoS Genet*. 2006; 2(6): 1–9.

[11] Dietz KJ, Jacquot JP, Harris G. Hubs and bottlenecks in plant molecular signalling networks. *New Phytol*. 2010; 188(4): 919–938.

[12] Bahia W, Soltani I, Abidi A, Haddad A, Ferchichi S, Menif S, Almawi WY. Identification of genes and miRNA associated with idiopathic recurrent pregnancy loss: an exploratory data mining study. *BMC Med Genomics*. 2020; 13(1): 1–13.

[13] Szklarczyk D, Morris JH, Cook H, Kuhn M, Wyder S, Simonovic M, Santos A, Doncheva NT, Roth A, Bork P, Jensen LJ, von Mering C. The STRING database in 2017: quality-controlled protein–protein association networks, made broadly accessible. *Nucleic Acids Res*. 2016; 45(1): 362–368.

[14] Doncheva NT, Morris JH, Gorodkin J, Jensen LJ. Cytoscape stringapp: network analysis and visualization of proteomics data. *J Proteome Res*. 2018; 18(2): 623–632.

[15] Leary S, Gaudieri S, Parker MD, Chopra A, James I, Pakala S, Alves E, John M, Lindsey BB, Keeley AJ, Rowland-Jones SL, Swanson MS, Ostrov DA, Bubenik JL, Das SR, Sidney J, Sette A, COVID-19 genomics UK (COG-UK) consortium, de Silva TI, Phillips E, Mallal S. Generation of a novel SARS-CoV-2 sub-genomic RNA due to the R203K/G204R variant in nucleocapsid: homologous recombination has potential to change SARS-CoV-2 at both protein and RNA level. *Pathog Immun*. 2021; 6(2): 1–23.

[16] Gu Y, Tang S, Wang Z, Cai L, Lian H, Shen Y, Zhou Y. A pan-cancer analysis of the prognostic and immunological role of β-actin (ACTB) in human cancers. *Bioengineered*. 2021; 12(1): 6166–6185.

[17] Nicholls C, Li H, Liu JP. GAPDH: a common enzyme with uncommon functions. *Clin Exp Pharmacol Physiol*. 2012; 39(8): 674–679.

[18] Guo C, Liu S, Sun MZ. Novel insight into the role of GAPDH playing in tumor. *Clin Transl Oncol*. 2013; 15(3): 167–172.

[19] Tommasino M, Accardi R, Caldeira S, Dong W, Malanchi I, Smet A, Zehbe I. The role of TP53 in cervical carcinogenesis. *Hum Mutat*. 2003; 21(3): 307–312.

[20] Liu H, Radisky DC, Nelson CM, Zhang H, Fata JE, Roth RA, Bissell MJ. Mechanism of Akt1 inhibition of breast cancer cell invasion reveals a protumorigenic role for TSC2. *Proc Natl Acad Sci USA*. 2006; 103(11): 4134–4139.

[21] Liu Z, Jin K, Guo M, Long J, Liu L, Liu C, Xu J, Ni Q, Luo G, Yu X. Prognostic value of the CRP/Alb ratio, a novel inflammation-based score in pancreatic cancer. *Ann Surgic Oncol*. 2017; 24(2): 561–568.

[22] Ibarrola-Villava M, Martin-Gonzalez M, Lazaro P, Pizarro A, Lluch A, Ribas G. Role of glutathione S-transferases in melanoma susceptibility: association with GSTP1 rs1695 polymorphism. *Br J Dermatol*. 2012; 166(6): 1176–1183.

[23] Aynacioglu AS, Nacak M, Filiz A, Ekinci E, Roots I. Protective role of glutathione S-transferase P1 (GSTP1) Val105Val genotype in patients with bronchial asthma. *Br J Clin Pharmacol*. 2004; 57(2): 213–217.

[24] González-Magaña A, Blanco FJ. Human PCNA structure, function and interactions. *Biomolecules*. 2020; 10(4): 1–19.

[25] Bhardwaj V, Purohit R. Computational investigation on effect of mutations in PCNA

resulting in structural perturbations and inhibition of mismatch repair pathway. *J Biomol Struct Dyn*. 2020; 38(7): 1963–1974.

[26] de Jong R, Pluijmert NJ, de Vries MR, Pettersson K, Atsma DE, Jukema JW, Quax PHA. Annexin A5 reduces infarct size and improves cardiac function after myocardial ischemia-reperfusion injury by suppression of the cardiac inflammatory response. *Sci Rep*. 2018; 8(1): 1–10.

[27] Zhang L, Qin Z, Li R, Wang S, Wang W, Tang M, Zhang W. The role of ANXA5 in DBP-induced oxidative stress through ERK/Nrf2 pathway. *Environ Toxicol Pharmacol*. 2019; 72: 1–9.

Abbreviations

PPI: protein-protein interaction; DEPs: differentially expressed proteins