Advances in Bioresearch
Adv. Biores., Vol 9 (5) September 2018: 40-45

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.9.5.4045

Advances in Bioresearch

ORIGINAL ARTICLE

Pharmacodynamics approach to predict the capsaicin targets in cancer by module-based protein-protein interaction (PPI) network analysis

B S Sharath, Shivananda Kandagalla, Pavan Gollapalli, Manjunatha H*
Department of PG Studies and Research in Biotechnology and Bioinformatics, Kuvempu University, JnanaSahyadri, Shankaraghatta, Shimoga, Karnataka-577 451, India.
Corresponding author :Manjunatha H
E-mail: manjunathh@kuvempu.ac.in ; manjunatha75@gmail.com

ABSTRACT
Capsaicin, a natural phenomenon extracted from Capsicum (hot pepper), extensively used as an anti-inflammatory, anti-carcinogenic, anti-metastatic, anti-angiogenic and chemo-preventive drug in the field of pharmacology. Protein-protein interaction network (PPIN) analysis was used to predict the mechanism of action of capsaicin on cancer target genes/proteins. Targets were curated based on ChEMBL and STITCH databases and their individual interactions were extracted from STRING database at high confidence. The functional enrichment analysis was performed to identify the cancer targets of capsaicin in disease module and the core interactome (PPIN)of capsaicin with 403 nodes and 1597 edgeswas constructed using Cytoscape. Furthermore, module/sub-graph identified based on molecular complex detection (MCODE) algorithm was subjected to gene ontology (GO) analysis to distinguish the biologically enriched pathway in cancer. Based on this analysis, the mechanism of capsaicin was hypothetically proposed by identifying essential bottleneck/hub nodes SRC, TAC1, PTGS2, ERBB3 and CREBBP, may be a probable target of capsaicin in cancer condition and needs further elucidation in molecular level.
Keywords :*Capsaicin, Protein-protein interaction (PPI) network, STRING, MCODE, Bottleneck nodes.*

Received 12.05.2018 Revised 11.06.2018 Accepted 23.08.2018

How to cite this article:
B S Sharath, Shivananda Kandagalla, Pavan Gollapalli, Manjunatha H. Pharmacodynamics approach to predict the capsaicin targets in cancer by module-based protein-protein interaction (PPI) network analysis, Uttar Pradesh. Adv. Biores. Vol 9 [5] September 2018: 40-45.

INTRODUCTION

Capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide)is an alkaloid of Capsicum and belongs to the family Solanaceae, is known to exhibit ananti-inflammatory, anti-carcinogenic, anti-metastatic, anti-angiogenicand chemopreventive properties by inducing apoptosis[1,2]. Nearly, 25% of the world population using capsicum as a natural food ingredient and consumption depends on region, culture and genetics. The extract of capsaicin has been used long ago as a traditional medicine to treat burning sensations and analgesics. It is well known that capsaicin-modifies intracellular signalling in certain cells via receptors found on the surface of the cells and it was confirmed to have shown apoptosis in certain cancer cell linesas well [3]. Recently, derivatives and analogues of capsaicin with efficient anti-inflammatory property are being developed for specific targets [4]. Thus, understanding molecular mechanism of capsaicin is important for developing and designing new molecules.

Biological reactions can be presented in many different ways. In general, protein-protein interaction complexes (PPI)/signalling pathways are the set of genes/proteins that interact with each other to achieve a given biological process in a coordinated mode. For instance,signaling pathways in KEGG (Kyoto Encyclopaedia of Genes and Genomes) uses nodes to represent genes/protein and edges to represent signals, such as activation/repression, that transmits signals from one gene to another[5]. In recent years, an *in silico* validation and analysis of biological networks play a vital role in identifying hub nodes. The nodes are the well-connected functional proteins maintaining the global network and cell-cell communication in the complex biological system; these nodes are linked to each other via physical

interactions [6].The topological parameters explain the role of node in the interactome. In scale-free PPI network, the gene ontology (GO) of protein networks/modules provides biological meaning to the signaling pathways. GO analysis is a method to evaluate protein clusters involved in the biological process of certain diseases[7]. In the present study, we constructed the PPIN of capsaicin target genes associated with the cancer to elucidate the anti-cancer mechanism of capsaicin. The network analysis was performed in order to study the relative topological analysis of core interactometo identify the hub node based on bottleneck score. In addition, sub-graph/module functional enrichment analysis was done for probable enriched pathways.

MATERIAL AND METHODS

Curation of capsaicin targets

For the identification of target genes/proteins of capsaicin, comprehensive literature survey was carried out using ChEMBL database (https://www.ebi.ac.uk/chembl/#)and STICH5.0 database (http://stitch.embl.de/), which are the part of EMBL-EBI used to manually curate the drug-gene information of bioactive molecules [8, 9].

The physical and functional interactions for each gene was obtained in STRING v10 database (http://string-db.org), which aims to provide critical evaluation of predicted protein–protein interactions derived from genomic resources, experimental evidences, text mining, co-occurrence and co-expression [10].

Functional enrichment analysis

To examine the functional association of capsaicin genes associated with cancer signaling, we performed functional enrichment analysis through "Database for Annotation, Visualization and Integrated Discovery" (DAVID) functional enrichment analysis (https://david.ncifcrf.gov/) [11], an online tool to understand the biological significance which defines the role of cancer associated gene targets of capsaicin.

Construction and analysis of PPIs network

The PPIs network of candidate genes associated with the capsaicin molecule was constructed. To explore the important hub protein responsible for regulating the core interactome, bottleneck method of cytohubba was used[12]. The top 10 genes with shortest path were considered as key regulators of core PPI network [13, 14]. The PPI Network analysis performed in the present study was followed based on our previous report[15]. The PPIN was visualized and analyzed using open-source Cytoscape v 3.2.1 software [16].The scale free evaluation of core interactions were investigated and analyzed using Network Analyzer v.3.3.1 by means of power law fit of the form $y = ax^b$[17].

In addition, Module analysis was performed using MCODE clustering algorithm[18] by parameters keeping degree cut-off = 2, node score cut-off = 0.3, k-core = 4 and maximum depth upto 100 and the clusters less than 8 nodes were discarded[19, 15].

Sub-graph/Module construction

The gene ontology of modules was performed using BinGO plugin of cytoscape to examine the minimum number of significant genes associated with the capsaicin in the core interactome. For the analysis, the threshold of p-value was set at <0.05 based on a hypergeometric test[20].

RESULTS AND DISCUSSION

We found 110curated genes associated with capsaicin molecule. The curated genes were further subjected to functional enrichment analysis to get the cancer associated capsaicin targets. In cancer condition, the capsaicin has sensitized over 56 differentially expressed genes (Table 1) and the capsaicin genes associated with cancer signalling were subjected for network construction and analysis to identify possible drug target.

Network construction and analysis

Based on the STRING database evidence, the cancer associated capsaicin genes interactions at high confidence (0.7) was considered to build core protein–protein interaction using Cytoscape 3.2, resultant network has 403 nodes and 1597 edges (Figure. 1). The nodes represent proteins and the edges indicate their relations. The network topology analysis has identified 10 hub proteins via bottleneck method. The hub proteins in the protein network is a distinct scale-free networks characterized by a power-law distribution which contains a small number of highly connected proteins known as hubs and large number of poorly connected proteins non-hubs. Thus, hub proteins in the network are more likely involved inregulating cellular functions in the body than non-hub proteins.As a consequence of limitations of the present approach, some human protein interactions are indistinct. Therefore, the

protein interactions constructed for this study is not comprehensive and the major connected modules was selected for further analysis.

The protein network of capsaicin is a scale-free network. To evaluate the confidence of protein network, topological analysis was calculated using Network Analyzer v.3.3.1.Topological parameters like Betweenness centrality (BC), Degree distribution (D) and topological co-efficient were computed to find the hub node in the interactome, while the explanation for each topological parameters was explained in our previous report based on power law distribution of the form $y = ax^b$[17, 15]. In general, Betweenness centrality is treated as bottleneck protein regulating the whole interactome. Hence, the bottleneck node in the interactomewas identified as SRC having highest BC value of 0.25 and largest degree of 40 as well. The network generated by this cut-off value fits better with a scale-free network topology and the mainstream signalling, which pass through this bottleneck node.This indicates the scale free network of capsaicin interactions in cancer condition and possesses the property of modularity. All the topological parameters of the core interactome of capsaicin were presented in Table 2 and some important parameters like Betweeness Centrality, Degree Distribution and Topological co-efficient were shown in the graphical plot (Figure 2). The study was further extended to identify the sub-graphs/modules to enriched biological process acting on cancer network of capsaicin.

Sub-graph and enrichment analysis

Sub-graphs/modules are the highly interconnected clusters derived from the core interactome. Modules provide concrete hypothesis with regard to disease-related biological process at certain threshold and are more significant [21]. Based on this hypothesis and to overcome the immense interactions, the network was divided into four modules through MCODE algorithm (Figure 3). The colour node in the modules indicates seed node and the others are nodes interacting with seed node.

The functional enrichment result of modules shows that capsaicin haspharmacodynamics interaction with several biological processes, including G-protein coupled receptor protein signaling pathway, Prostanoid metabolic process, Response to hormone and chemical stimulus, Signaling etc.as shown in Table 3. Apart from identified bottlenecks in core interactome, module analysis open up seed proteins TAC1, PTGS2, ERBB3, CREBBP responsible for regulating cancer signaling and acts as bottleneck nodes in specific biological processes.

Table 1 The list of capsaicin genes targets retrieved from ChEMBL and STITCH databases.

Gene targets	UniProt ID	Gene targets	UniProt ID	Gene targets	UniProt ID
ACHE	P22303	CYP2D6	P10635	MAPT	P10636
ADRB2	P07550	CYP2E1	P05181	MC4R	P32245
ADRB3	P13945	CYP3A4	P08684	MMP1	P03956
AGTR2	P50052	CYSLTR1	Q9Y271	MMP9	P14780
APEX1	P27695	DRD1	P21728	NFE2L2	Q16236
AR	P10275	DRD2	P14416	NR3C1	P04150
CALCR	P30988	DRD3	P35462	OPRD1	P41143
CASP1	P29466	DRD4	P21917	OPRK1	P41145
CCKAR	P32238	EGFR	P00533	OPRM1	P35372
CCR2	P41597	ELANE	P08246	POLI	Q9UNA4
CCR4	P51679	ERBB2	P04626	PPARD	Q03181
CCR5	Q9UK39	ESR1	P03372	PPARG	P37231
CNR1	Q9ULM6	ESR2	Q92731	PRKCA	P17252
CXCR1	P51681	FLT1	P17948	PTGS1	P23219
CXCR2	P21554	GBA	P04062	PTGS2	P35354
CYP1A2	P25024	GLS	O94925	SLC6A3	Q01959
CYP2A6	P25025	HMGCR	P04035	SLC6A4	P31645
CYP2C9	P05177	MAOA	P21397	TBXAS1	P24557
CYP2C19	P11509	MAPK1	P28482		

Table 2 The topological analysis of the protein interaction network of Capsaicin interactomes

Network parameters	Core interactome	Backbone network (shortest path length)
Number of nodes/edges	392/1558	164/633
Clustering co-efficient	0.708	0.776
Network centralization	0.121	0.294
Avg. number of neighbors	7.949	7.720
Network density	0.020	0.047
Shortest path (%)	100% (153272)	100% (26732)
Network diameter	10	6
Characteristic path length	4.528	3.252

Table 3 Gene Ontology (GO) biological process terms of the modules.

Modules	GO-ID	Description	p-value	Clustering frequency (%)
Module 1	7186	G-protein coupled receptor protein signaling pathway	2.2302E-39	30/35 85.7%
	7189	Activation of adenylatecyclase activity by G-protein signaling pathway	1.2228E-30	15/35 42.8%
Module 2	6692	Prostaglandin metabolic process	4.6856E-24	8/8: 100.0%
	46457	Prostaglandin biosynthetic process	5.6479E-22	7/8 87.5%
Module 3	9725	Response to hormone stimulus	1.9473E-16	16/32: 50.0%
	9719	Response to organic substance	1.0453E-	19/32 59.3%
Module 4	42221	Response to chemical stimulus	5.7181E-14	20/29 68.9%
	23052	Signaling	5.0112E-12	24/29 82.7%

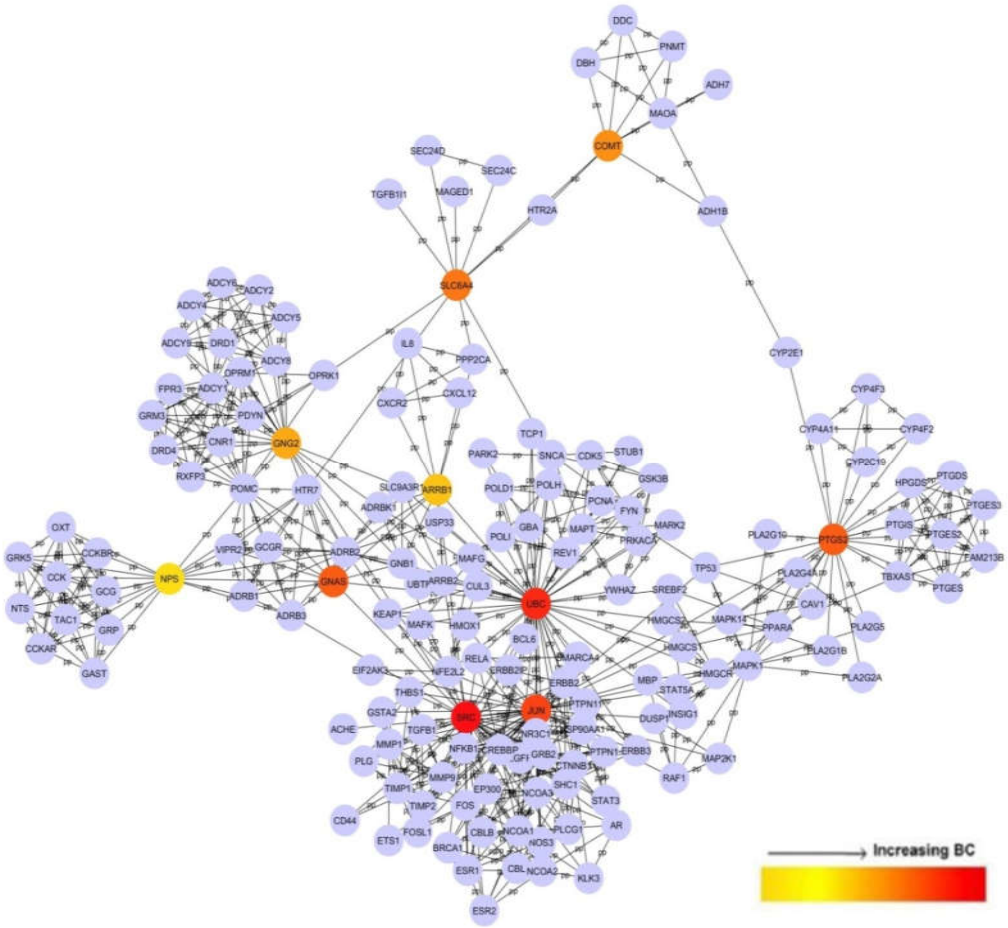

Figure 1 The PPI network of capsaicin target genes. The nodes and edges specify the genes and their relationships. The bottleneck node colour ranges from yellow to red with increasing BC.

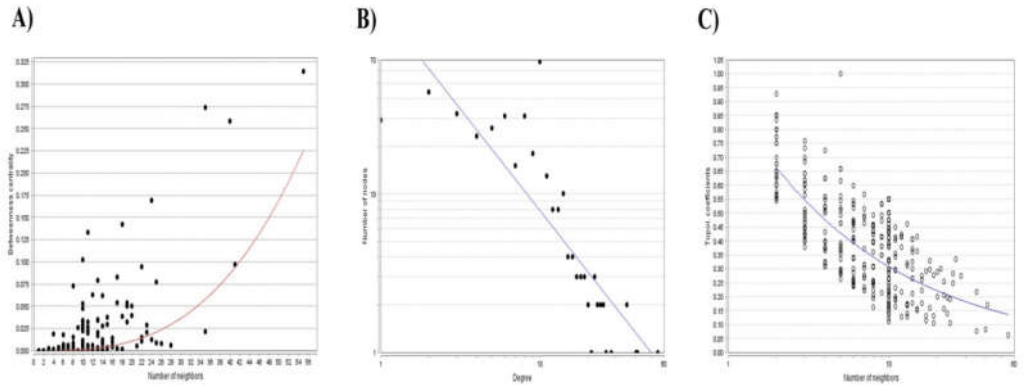

Figure 2 The graphical plot presenting the topological properties of capsaicin network. A) Betweeness Centrality B) Degree Distribution and C) Topological co-efficient.

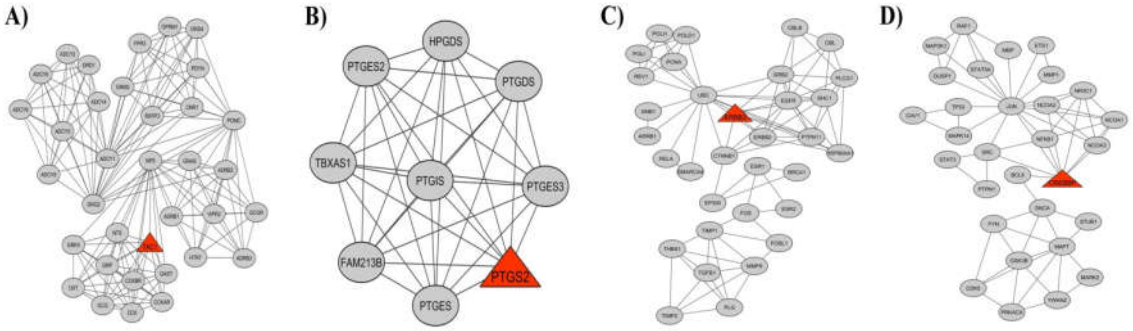

Figure 3 MCODE analyses of modules in PPIN of capsaicin. Four modules were extracted. Red colour nodes represents seed protein and other gray colour nodes that interacts with seed proteins.

CONCLUSION

Protein interaction network analysis of capsaicin possesses scale-free network and modularity properties based on topological parameters. This scale-free network has led to the detection of topologically significant nodes known as hubs/bottlenecks proteins. This method was employed for the analysis of core interactome as well as modules/sub-graphs to identify the key regulators involved in cancer through topological measure. The topological analysis identifies SRC, TAC1, PTGS2, ERBB3 and CREBBPas essential bottleneck proteins in regulating the cancer signaling. Furthermore, functional enrichment analysis of modules confirmed that the bottleneck proteins interact directly/indirectly with the non-hub proteins involved in regulating cancer. Hence, the present study provides potential biomarkers known as bottleneck nodesas a therapeutic target for cancer treatment.However, further experimental validations are needed to confirm these conclusions. Though the present analysis is hypothetical, this study may provide a well-organized way to elucidate mechanism of capsaicin for its experimental applications and future drug developments.

ACKNOWLEDGMENT

The authors are thankful to Registrar, Kuvempu University for providing all the facilities to complete this work.

CONFLICT OF INTERESTS

The authors declare no conflict of Interests regarding this paper publication.

REFERENCES

1. Manjunatha H, Srinivasan K. (2006). Protective effect of dietary curcumin and capsaicin on induced oxidation of low-density lipoprotein, iron-induced hepatotoxicity and carrageenan-induced inflammation in experimental rats. *FEBS J.*, **273** (4): 528–37.

2. Kempaiah RK, Manjunatha H, Srinivasan K. (2005). Protective effect of dietary capsaicin on induced oxidation of low-density lipoprotein in rats. *Mol. Cell. Biochem.*,**275**: 7–13.
3. Zhang Y, Deng X, Lei T, Yu C, Wang Y, Zhao G, Luo X, Tang K, Quan Z, Jiang D. (2017). Capsaicin inhibits proliferation and induces apoptosis in osteosarcoma cell lines via the mitogen-activated protein kinase pathway. *Oncol. Rep.*, **38**: 2685–96.
4. Huang X-F, Xue J-Y, Jiang A-Q, Zhu H-L. (2013). Capsaicin and Its Analogues: Structure-Activity Relationship Study. Curr. Med. Chem., 20: 2661–72.
5. Kanehisa M, Goto S. (2000). KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Res.*, **28**: 27–30.
6. Rual J-F, Venkatesan K, Hao T, Hirozane-Kishikawa T, Dricot A, Li N, Berriz GF, Gibbons FD, Dreze M, Ayivi-Guedehoussou N, Klitgord N, Simon C, Boxem M, Milstein S, Rosenberg J, Goldberg DS, Zhang L V., Wong SL, Franklin G, Li S, Albala JS, Lim J, Fraughton C, Llamosas E, Cevik S, Bex C, Lamesch P, Sikorski RS, Vandenhaute J, Zoghbi HY, Smolyar A, Bosak S, Sequerra R, Doucette-Stamm L, Cusick ME, Hill DE, Roth FP, Vidal M. (2005). Towards a proteome-scale map of the human protein–protein interaction network.*Nature*, **437**: 1173–8.
7. Camon E, Magrane M, Barrell D, Binns D, Fleischmann W, Kersey P, Mulder N, Oinn T, Maslen J, Cox A, Apweiler R. (2003). The Gene Ontology Annotation (GOA) Project: Implementation of GO in SWISS-PROT, TrEMBL, and InterPro. *Genome Res.*, **13**: 662–72.
8. Gaulton A, Bellis LJ, Bento AP, Chambers J, Davies M, Hersey A, Light Y, McGlinchey S, Michalovich D, Al-Lazikani B, Overington JP. (2012). ChEMBL: a large-scale bioactivity database for drug discovery. *Nucleic Acids Res.*, **40**: D1100–7.
9. Szklarczyk D, Santos A, von Mering C, Jensen LJ, Bork P, Kuhn M. (2016). STITCH 5: augmenting protein-chemical interaction networks with tissue and affinity data. Nucleic Acids Res., 44: D380-4.
10. Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerta-Cepas J, Simonovic M, Roth A, Santos A, Tsafou KP, Kuhn M, Bork P, Jensen LJ, von Mering C. (2015). STRING v10: protein-protein interaction networks, integrated over the tree of life. *Nucleic Acids Res.*, **43**: D447-52.
11. Huang DW, Sherman BT, Tan Q, Collins JR, Alvord WG, Roayaei J, Stephens R, Baseler MW, Lane HC, Lempicki RA. (2007). The DAVID Gene Functional Classification Tool: a novel biological module-centric algorithm to functionally analyze large gene lists. *Genome Biol.*, **8**: R183.
12. Chin C-H, Chen S-H, Wu H-H, Ho C-W, Ko M-T, Lin C-Y. (2014). cytoHubba: identifying hub objects and sub-networks from complex interactome. *BMC Syst. Biol.*, **8 Suppl 4**: S11.
13. Yu H, Kim PM, Sprecher E, Trifonov V, Gerstein M. (2007). The Importance of Bottlenecks in Protein Networks: Correlation with Gene Essentiality and Expression Dynamics. *PLoSComput. Biol.*, **3**: e59.
14. Rolland T, Taşan M, Charloteaux B, Pevzner SJ, Zhong Q, Sahni N, Yi S, Lemmens I, Fontanillo C, Mosca R, Kamburov A, Ghiassian SD, Yang X, Ghamsari L, Balcha D, Begg BE, Braun P, Brehme M, Broly MP, Carvunis A-R, Convery-Zupan D, Corominas R, Coulombe-Huntington J, Dann E, Dreze M, Dricot A, Fan C, Franzosa E, Gebreab F, Gutierrez BJ, Hardy MF, Jin M, Kang S, Kiros R, Lin GN, Luck K, MacWilliams A, Menche J, Murray RR, Palagi A, Poulin MM, Rambout X, Rasla J, Reichert P, Romero V, Ruyssinck E, Sahalie JM, Scholz A, Shah AA, Sharma A, Shen Y, Spirohn K, Tam S, Tejeda AO, Trigg SA, Twizere J-C, Vega K, Walsh J, Cusick ME, Xia Y, Barabási A-L, Iakoucheva LM, Aloy P, De Las Rivas J, Tavernier J, Calderwood MA, Hill DE, Hao T, Roth FP, Vidal M. (2014). A Proteome-Scale Map of the Human Interactome Network.*Cell*, **159**: 1212–26.
15. BelenahalliShekarappa S, Kandagalla S, Gollapalli P, BasavapattanaRudresh B, Hanumanthappa T, Hanumanthappa M. (2017). Topology of protein–protein interaction network and edge reduction co-efficiency in VEGF signaling of breast cancer.*Netw.Model.Anal. Heal. Informatics Bioinforma.*,**6:** 17.
16. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T. (2003). Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks. *Genome Res.*, **13**: 2498–504.
17. Assenov Y, Ramírez F, Schelhorn S-E, Lengauer T, Albrecht M. (2008).Computing topological parameters of biological networks.*Bioinformatics*, **24**: 282–4.
18. Bader GD, Hogue CW V. (2003).An automated method for finding molecular complexes in large protein interaction networks.*BMC Bioinformatics*, **4**: 2.
19. Zhuang D-Y, Jiang L, He Q-Q, Zhou P, Yue T. (2015). Identification of hub subnetwork based on topological features of genes in breast cancer. *Int. J. Mol. Med.*, **35**: 664–74.
20. Maere S, Heymans K, Kuiper M. (2005). BiNGO: a Cytoscape plugin to assess overrepresentation of Gene Ontology categories in Biological Networks. *Bioinformatics*, **21**: 3448–9.
21. Khurana V, Peng J, Chung CY, Auluck PK, Fanning S, Tardiff DF, Bartels T, Koeva M, Eichhorn SW, Benyamini H, Lou Y, Nutter-Upham A, Baru V, Freyzon Y, Tuncbag N, Costanzo M, San Luis B-J, Schöndorf DC, Barrasa MI, Ehsani S, Sanjana N, Zhong Q, Gasser T, Bartel DP, Vidal M, Deleidi M, Boone C, Fraenkel E, Berger B, Lindquist S. (2017).Genome-Scale Networks Link Neurodegenerative Disease Genes to α-Synuclein through Specific Molecular Pathways.*Cell Syst.*, **4**: 157–170.e14.