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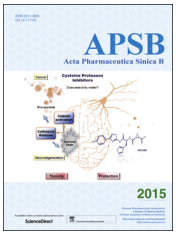

SHORT COMMUNICATION

Prediction of the anti-inflammatory mechanisms of curcumin by module-based protein interaction network analysis

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Abstract Curcumin, the medically active component from *Curcuma longa* (Turmeric), is widely used to treat inflammatory diseases. Protein interaction network (PIN) analysis was used to predict its mechanisms of molecular action. Targets of curcumin were obtained based on ChEMBL and STITCH databases. Protein–protein interactions (PPIs) were extracted from the String database. The PIN of curcumin was constructed by Cytoscape and the function modules identified by gene ontology (GO) enrichment analysis based on molecular complex detection (MCODE). A PIN of curcumin with 482 nodes and 1688 interactions was constructed, which has scale-free, small world and modular properties. Based on analysis of these function modules, the mechanism of curcumin is proposed. Two modules were found to be intimately associated with inflammation. With function modules analysis, the anti-inflammatory effects of curcumin were related to SMAD, ERG and mediation by the TLR family. TLR9 may be a potential target of curcumin to treat inflammation.

Abbreviations: ETS, erythroblast transformation-specific; GO, gene ontology; IFNs, interferons; IL, interleukin; JAK-STAT, Janus kinase-STAT; MAPK, mitogen-activated protein kinase; MCODE, molecular complex detection; NF-κB, nuclear factor kappa B; PIN, protein interaction network; PPIs, protein–protein interactions; STATs, signal transducer and activator of transcription complexes; TLR, toll-like receptor

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1. Introduction

Curcumin, derived from *Curcuma longa* (Turmeric), is not only known as a spice that gives a yellow color to food, but also a traditional medicine that has been widely used particularly for treating various malignant diseases, arthritis, allergies, Alzheimer's disease, and other inflammatory illnesses[1,2]. The anti-inflammatory effects of curcumin have been shown in clinical and experimental studies[3-6], and analogs and derivatives of curcumin with anti-inflammatory biological activity have been developed[7,8]. To make new derivatives as effective as possible, the modified structure should be based on the action targets. Therefore, research into the molecular mechanism of curcumin is important for both new drug design and clinical treatment. Although the anti-inflammatory mechanism of curcumin has been partly unraveled[7-9], it needs to be further clarified at the molecular level.

Proteins perform a vast array of functions within living organisms, but they rarely act alone. Signaling proteins often form dynamic protein–protein interaction (PPI) complexes to achieve multi-functionality and constitute cellular signaling pathways and cell morphogenesis[10,11]. PPIs are pivotal for many biological processes[12-15]. The gene ontology (GO) project[16] is a collaborative effort to construct ontologies which facilitate biologically meaningful annotation of gene products. It provides a collection of well-defined biological terms, spanning biological processes, molecular functions and cellular components. GO enrichment is a common statistical method used to identify shared associations between proteins and annotations to GO. Module-network and GO analysis may provide an efficient way to illustrate the molecular mechanism of anti-inflammatory action for curcumin.

This paper aims to further elucidate the anti-inflammatory molecular mechanism of curcumin, and provide reference for its clinical application and further drug development. A network pharmacology approach was applied to analyze the anti-inflammatory mechanisms of curcumin, as a network analysis approach has the advantage of evaluating the pharmacological effect of a drug as a whole at the molecular level[16]. The protein interaction networks (PINs) of curcumin were constructed by Cytoscape, and the properties of the scale-free, small-world network and module were analyzed based on topological parameters. Functional modules were identified by gene ontology (GO) enrichment analysis based on molecular complex detection (MCODE).

2. Methods

2.1. Network construction

Targets of curcumin were extracted from ChEMBL (https://www.ebi.ac.uk/chembl/#) and STITCH4.0 (http://stitch.embl.de/). ChEMBL[17] is a manually curated chemical database of bioactive molecules with drug-like properties whose data are manually abstracted on a regular basis from the primary published literature, then further curated and standardized. STITCH[18] is a database of protein–chemical interactions that integrates many sources of experimental and manually-curated evidence with text-mining information and interaction predictions.

The PPI information was obtained from the online databases of String 9.1 (http://string-db.org) which was used to retrieve the predicted interactions for the targets[19]. All associations available in String are provided with a probabilistic confidence score. Targets with a confidence score greater than 0.7 were selected to construct the PPI network.

2.2. Network analysis

Topological properties have become very popular to gain an insight into the organization and the structure of the resultant large complex networks[20-22]. Therefore, topological parameters such as the clustering coefficient, connected components, degree distribution and average shortest path were analyzed by Network Analyzer[17] in Cytoscape software. Compared with the random network, the properties of scale-free, small world and modularity of the PIN were also investigated based on the topological parameters.

The MCODE was used to further divide the PPI into modules, using a cutoff value for the connectivity degree of nodes (proteins in the network) greater than 3. The algorithm has the advantage over other graph clustering methods of having a directed mode that allows fine-tuning of clusters of interest without considering the rest of the network and allows examination of cluster interconnectivity, which is relevant for protein networks[23]. Based on the identified modules, GO functional annotation and enrichment analysis were performed using the BinGO[24] plugin in Cytoscape with a threshold of $P<0.05$ based on a hypergeometric test.

3. Results and discussion

3.1. Construction of the network

Ten human proteins from STITCH 4.0 and 68 human proteins from ChEMBL (data accessed in August 2014) were extracted. 67 human proteins as curcumin targets were obtained after removing a repeat protein. The binding affinities (IC_{50}) of ALPI and TLR9 were, respectively, 100 and 8.36 μmol/L. The IC_{50} values of the remaining targets were not available because curcumin would have inhibited or activated other proteins[25,26]. Information on the targets is listed in Table 1. The PPIs of the targets were imported in Cytoscape[27], union calculations were carried out and the duplicated edges of PPIs were removed using Advanced Network Merge[28] Plugins, and the largest connected subgraph was selected as the PIN of curcumin, which included 482 nodes and 1688 edges, as shown in Fig. 1. The nodes represent proteins and the edges indicate their relations. The gray nodes represent seed nodes and the others are nodes that interact with seed nodes. Due to limits of the current studies, some human protein interactions are still unclear. As a result, the network constructed for this research is not comprehensive and the largest connected subgraph was selected for further analysis.

3.2. Network analysis

3.2.1. Topological analysis

All the topological parameters were calculated, as shown in Table 2.

Degree distribution was computed by counting the number of connections between various proteins of the network[29,30]. As shown in Fig. 2A, the degree distribution of the PIN of curcumin followed the power law distribution and the equation is $y=218.67x^{-1.359}$. The PIN of curcumin is a scale-free network.

Table 1 Proposed curcumin targets.

Target	UniProt ID	Target	UniProt ID	Target	UniProt ID	Target	UniProt ID
ABCG2[a]	Q9UNQ0	AR	P10275	HSD17B10	Q99714	POLB	P06746
AKT1[a]	P31749	ATAD5	Q96QE3	HSPA5	P11021	POLI	Q9UNA4
CASP3[a]	P42574	BACE1	P56817	HTT	P42858	POLK	Q9UBT6
CCND1[a]	P24385	BAZ2B	Q9UIF8	IDH1	O75874	PPARD	Q03181
HMOX1[a]	P09601	BRCA1	P38398	IL-8	P10145	RORC	P51449
JUN[a]	P05412	CASP1	P29466	KCNH2	Q12809	RXRA	P19793
MMP9[a]	P14780	CASP7	P55210	KDM4A	O75164	SMAD3	P84022
PPARG[b]	P37231	CYP3A4	P08684	KDM4DL	B2RXH2	SNCA	P37840
PTGS2[a]	P35354	EHMT2	Q96KQ7	LMNA	P02545	TARDBP	Q13148
STAT3[a]	P40763	ERG	P11308	MAPK1	P28482	TDP1	Q9NUW8
AHR	P35869	ESR1	P03372	MAPT	P10636	THRB	P10828
ALDH1A1	P00352	FEN1	P39748	MBNL1	Q9NR56	TLR9	Q9NR96
ALOX12	P18054	GAA	P10253	MLL	Q03164	TP53	P04637
ALOX15	P16050	GBA	P04062	NFE2L2	Q16236	TSG101	Q99816
ALOX15B	O15296	GLS	O94925	NFKB1	P19838	TSHR	P16473
ALPI	P09923	GMNN	O75496	NPSR1	Q6W5P4	USP1	O94782
ALPL	P05186	GNAS	P63092	NR1H4	Q96RI1	VDR	P11473
ALPPL2	P10696	HBB	P68871	NR3C1	P04150		
APOBEC3F	Q8IUX4	HIF1A	Q16665	PIN1	Q13526		
APOBEC3G	Q9HC16	HPGD	P15428	PKM2	P14618		

[a]Targets were obtained from STITCH.
[b]Targets were extracted from both ChEMBL and STITCH. The remaining targets were obtained from ChEMBL.

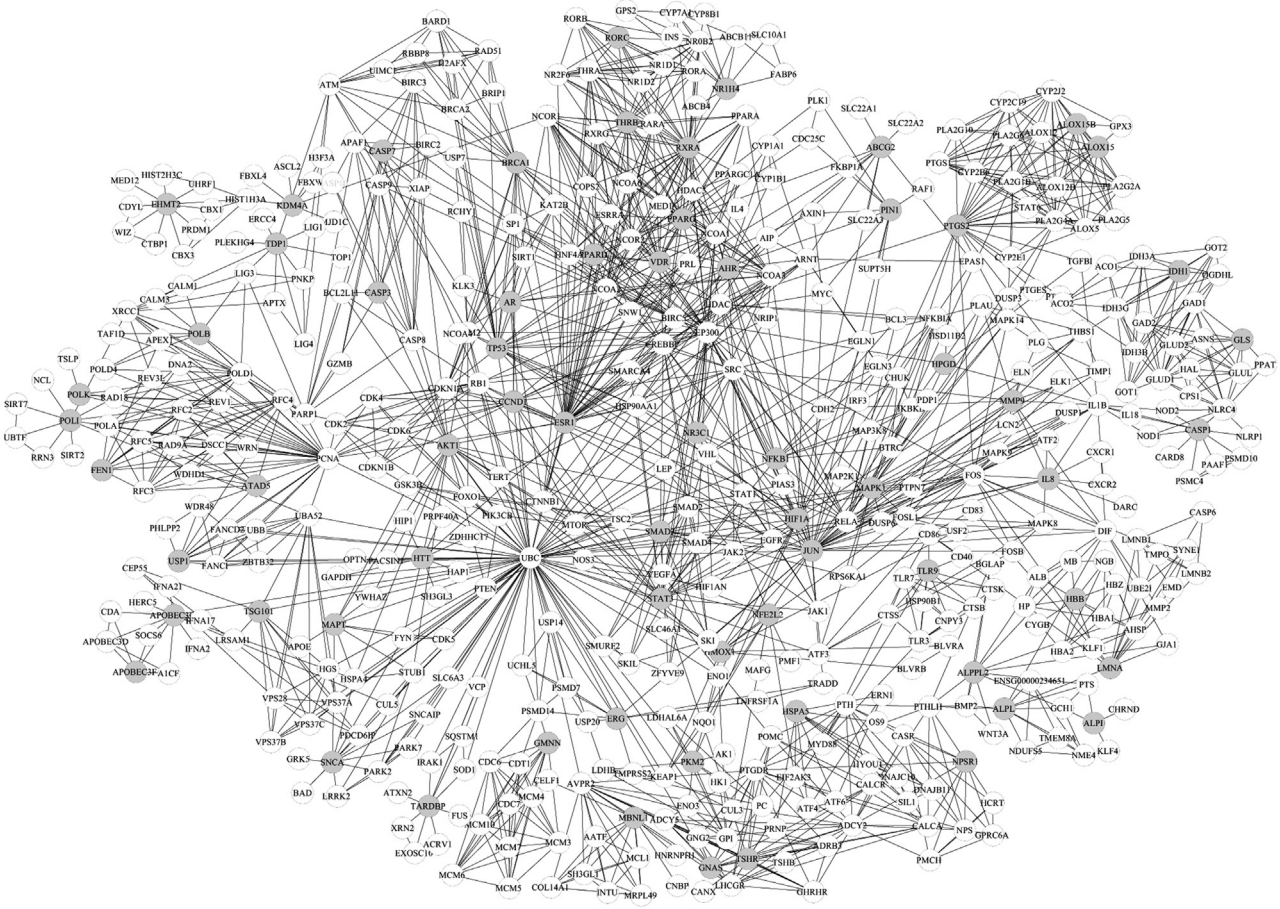

Figure 1 The protein network of curcumin. The nodes and edges indicate the proteins and their relationships. The gray nodes represent seed nodes and the white ones are nodes that interact with the seed nodes.

Average shortest path refers to the average density of the shortest paths between all pairs of nodes[29,30]. As shown in Fig. 2B, network path length was mostly concentrated in steps 3–5. The shortest path length between any two proteins was 4.394. This meant that most proteins were very closely linked and the PIN of curcumin was a small world network.

Clustering coefficient refers to the average density of the node neighborhoods[29,30]. The higher the clustering coefficient, the more modular the network is. Compared with a random network whose number of nodes and edges are the same as the PIN of curcumin, the PIN clustering coefficient for curcumin was higher. This indicates that the PIN of curcumin possesses the property of modularity. This result suggests that the network possesses the scale-free property, a small world property and modular properties.

3.2.2. *Clustering and GO enrichment analysis*

As shown in Fig. 3, 19 modules were identified from the network through the MCODE algorithm. The gray nodes indicate seed nodes and the others are nodes that interact with seed nodes.

Table 2 The topological parameters of the protein interaction network of curcumin.

Parameter	Network	
	PIN of curcumin	Random network
Clustering coefficient	0.641	0.016
Connected component	1	1
Network diameter	11	4
Network centralization	0.165	0.017
Shortest path	231,842 (100%)	231,842 (100%)
Characteristic path length	4.394	3.390
Network heterogeneity	0.995	0.376

The connected component is 1, indicating that the network has no other subgraphs. The network diameter is the greatest distance between any pair of vertices. Network centralization is a network index that measures the degree of dispersion of all node centrality scores in a network. Network heterogeneity measures the degree of uneven distribution of the network.

The results of functional enrichment analysis using BinGO are shown in Table 3. The result shows that curcumin has pharmacodynamic interactions with several biological processes, including regulation of transcription, cell-cycle processing, negative regulation of thrombin, hydrogen peroxide metabolic processing, and anti-inflammatory mechanisms. Module 10 and module 13 are related to anti-inflammatory actions.

Module 10 contains proteins such as interleukin (IL)-8, Nuclear factor kappa B (NF-κB), signal transducer and activator of transcription complex (STAT)3, SMAD3 and ERG. IL-8 is a key indicator of localized inflammation[31]. NF-κB is a key signaling molecule in the elaboration of the inflammatory response. STAT3 is activated in response to various cytokines and growth factors including IL-6 and IL-10. Curcumin was previously reported to exhibit anti-inflammatory actions by decreasing IL-8 levels[32], acting as an NF-κB inhibitor[25,26] and suppressing STAT3[33]. Therefore, the predicted results based on network analysis were consistent with these previous findings. The expression of SMAD3 is related to mitogen-activated protein kinase (MAPK)[34]. It has been reported that curcumin demonstrates anti-inflammatory activity by inhibiting MAPK[35]. Consequently, the anti-inflammatory activity of curcumin would be related to the predicted interaction with SMAD3. ERG is a member of the erythroblast transformation-specific (ETS) family of transcription factors which regulate inflammation[36]. ERG also has been shown to interact with c-Jun (activated through double phosphorylation by the JNK pathway) which contributes to inflammation[37]. At the same time, curcumin was anti-inflammatory by inhibiting JNK activity[32], indicating that the anti-inflammatory effects of curcumin would be related to ERG. Hence, the analysis of module 10 indicated that the anti-inflammatory actions of curcumin may be associated with SMAD3 and ERG.

Module 13 is closely related to the toll-like receptor (TLR) family, including TLR3, TLR7 and TLR9. TLR3[38] leads to the activation of IRF3, which ultimately induces the production of type I interferons (IFNs). IFNs activate STATs, suggesting that the Janus kinase-STAT (JAK-STAT) signaling pathway was initiated[39]. There is evidence that the JAK-STAT pathway is involved in the anti-inflammatory reaction[40]. TLR7 and TLR9 also led to activation of the cells that initiated pro-inflammatory reactions resulting the production of cytokines, such as, type-I interferon[41]. Moreover, TLR9 was the seed node and the binding affinity (IC_{50}) with curcumin is 8.36 μmol/L. This indicates that

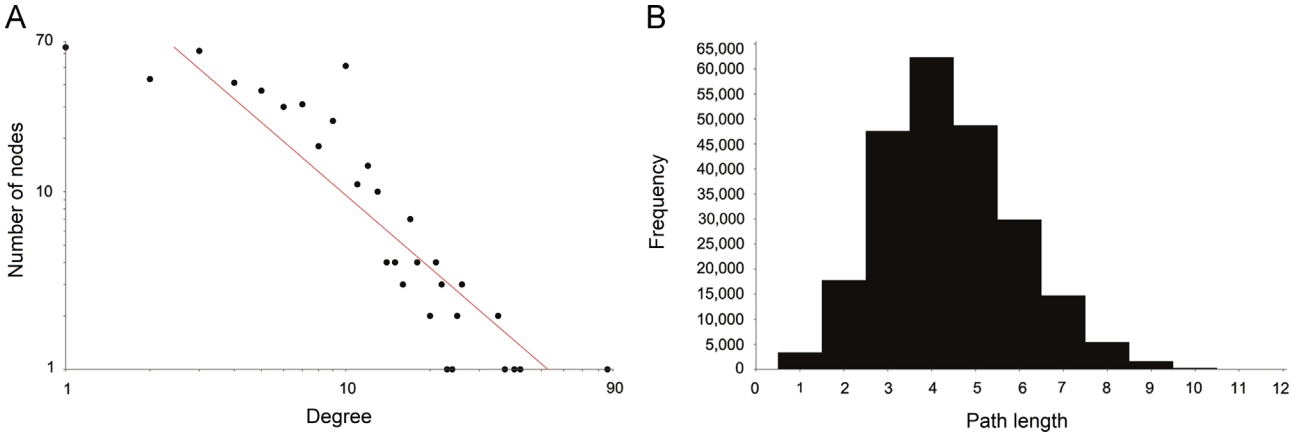

Figure 2 Topological properties of the network. (A) Degree distribution of the curcumin network; (B) shortest path length distribution of the curcumin network.

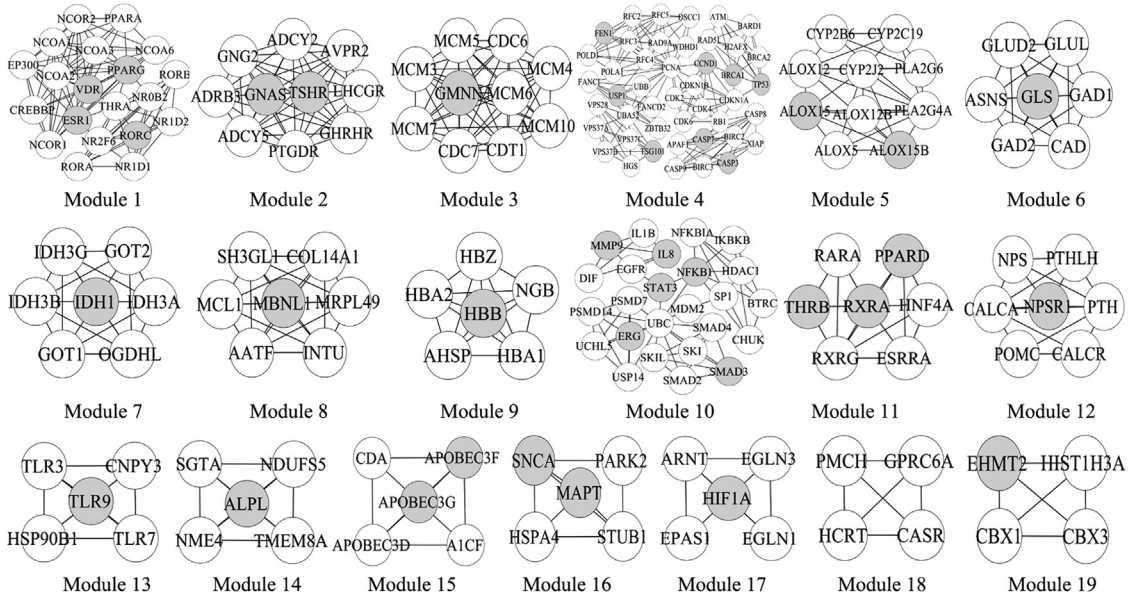

Figure 3 Modules in the PIN of curcumin. With the MCODE algorithm, 19 modules were extracted from the network. The gray nodes present seed nodes and the white ones are nodes that interact with the seed nodes.

Table 3 GO biological process terms of the modules.

Module	GO term	*P* value
Module 1	Transcription initiation from RNA polymerase II promoter	7.6587×10^{-32}
Module 2	G-protein coupled receptor signaling pathway, coupled to cyclic nucleotide second messenger	3.8329×10^{-20}
Module 3	M/G1 transition of mitotic cell cycle	7.7133×10^{-27}
Module 4	Response to DNA damage stimulus	2.0766×10^{-32}
Module 5	Fatty acid derivative metabolic process	2.0128×10^{-16}
Module 6	Glutamine family amino acid catabolic process	5.7668×10^{-18}
Module 7	Tricarboxylic acid cycle	1.6501×10^{-13}
Module 8	Negative regulation of superoxide anion generation	2.30×10^{-4}
Module 9	Hydrogen peroxide catabolic process	1.5518×10^{-8}
Module 10	Cellular response to growth factor stimulus	4.6078×10^{-16}
Module 11	Transcription initiation from RNA polymerase II promoter	6.9789×10^{-16}
Module 12	G-protein coupled receptor signaling pathway	2.3039×10^{-10}
Module 13	Toll-like receptor signaling pathway	5.23×10^{-7}
Module 14	Cementum mineralization	1.31×10^{-4}
Module 15	DNA cytosine deamination	2.1272×10^{-11}
Module 16	Negative regulation of thrombin receptor signaling pathway	1.64×10^{-4}
Module 17	Regulation of transcription from RNA polymerase II promoter in response to hypoxia	4.5178×10^{-16}
Module 18	G-protein coupled receptor signaling pathway	6.04×10^{-5}
Module 19	Chromatin organization	4.23×10^{-5}

P value is the probability of obtaining the observed effect, a very small *P* value indicates that the observed effect is very unlikely to have arisen purely by chance, and therefore provides evidence against the null hypothesis.

TLR9 may be a potential target of curcumin to treat inflammation and curcumin may exert anti-inflammatory properties through the TLR family.

4. Conclusions

In this paper, the PIN of curcumin possesses scale-free, small world properties and modular properties based on analysis of its topological parameters. A module-based network analysis approach was proposed to highlight the anti-inflammatory mechanisms of curcumin. The anti-inflammatory effects of curcumin may be related to SMAD and ERG, and mediated by the TLR family. TLR9 may be a potential target of curcumin to treat inflammation. However, further experiments are needed to confirm these conclusions.

Although the present analysis is restricted to *in silico* analysis, this study provides an efficient way to elucidate possible mechanisms of curcumin, and provides reference for its clinical application and further drug development.

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References

1. Leitman IM. Curcumin for the prevention of acute lung injury in sepsis: is it more than the flavor of the month?. *J Surg Res* 2012;**176**:e5–7.
2. Yilmaz Savcun G, Ozkan E, Dulundu E, Topaloglu U, Sehirli AO, Tok OE, et al. Antioxidant and anti-inflammatory effects of curcumin against hepatorenal oxidative injury in an experimental sepsis model in rats. *Ulus Travma Acil Cerrahi Derg* 2013;**19**:507–15.
3. Menon VP, Sudheer AR. Antioxidant and anti-inflammatory properties of curcumin. *Adv Exp Med Biol* 2007;**595**:105–25.
4. Hemeida RA, Mohafez OM. Curcumin attenuates methotraxate-induced hepatic oxidative damage in rats. *J Egypt Natl Cancer Inst* 2008;**20**:141–8.
5. Jacob A, Wu R, Zhou M, Wang P. Mechanism of the anti-inflammatory effect of curcumin: PPAR-γ activation. *PPAR Res* 2007;**2007**:89369–73.
6. Kumar P, Padi SS, Naidu PS, Kumar A. Possible neuroprotective mechanisms of curcumin in attenuating 3-nitropropionic acid-induced neurotoxicity. *Methods Find Exp Clin Pharmacol* 2007;**29**:19–25.
7. Zhang Y, Liang D, Dong L, Ge X, Xu F, Chen W, et al. Anti-inflammatory effects of novel curcumin analogs in experimental acute lung injury. *Respir Res* 2015;**16**:43–55.
8. Zhao C, Zhang Y, Zou P, Wang J, He W, Shi D, et al. Synthesis and biological evaluation of a novel class of curcumin analogs as anti-inflammatory agents for prevention and treatment of sepsis in mouse model. *Drug Des Dev Ther* 2015;**9**:1663–78.
9. Fajardo AM, Piazza GA. Anti-inflammatory approaches for colorectal cancer chemoprevention. *Am J Physiol Gastrointest Liver Physiol* 2015;**309**:G59–70.
10. Yeh JT, Binari R, Gocha T, Dasgupta R, Perrimon N. PAPTi: a peptide aptamer interference toolkit for perturbation of protein–protein interaction networks. *Sci Rep* 2013;**3**:1156–43.
11. Wang T, Ming Z, Xiaochun W, Hong W. Rab7: role of its protein interaction cascades in endo-lysosomal traffic. *Cell Signal* 2011;**23**:516–21.
12. Maetschke SR, Simonsen M, Davis MJ, Ragan MA. Gene ontology-driven inference of protein–protein interactions using inducers. *Bioinformatics* 2012;**28**:69–75.
13. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, et al. Gene ontology: tool for the unification of biology, the gene ontology consortium. *Nat Genet* 2000;**25**:25–9.
14. Zhang QC, Petrey D, Deng L, Qiang L, Shi Y, Thu CA, et al. Structure-based prediction of protein–protein interactions on a genome-wide scale. *Nature* 2012;**490**:556–60.
15. Ren Z, Wang X, Wang S, Zhai C, He Y, Zhang Y, et al. Mechanism of action of salvianolic acid B by module-based network analysis. *Biomed Mater Eng* 2014;**24**:1333–40.
16. Janga SC, Tzakos A. Structure and organization of drug-target networks: insights from genomic approaches for drug discovery. *Mol Biosyst* 2009;**5**:1536–48.
17. Gaulton A, Bellis LJ, Bento AP, Chambers J, Davies M, Hersey A, et al. ChEMBL: a large-scale bioactivity database for drug discovery. *Nucleic Acids Res* 2012;**40**:D1100–7.
18. Kuhn M, Szklarczyk D, Pletscher-Frankild S, Blicher TH, von Mering C, Jensen LJ, et al. STITCH 4: integration of protein–chemical interactions with user data. *Nucleic Acids Res* 2014;**42**:D401–7.
19. Franceschini A, Szklarczyk D, Frankild S, Kuhn M, Simonovic M, Roth A, et al. STRING v9.1: protein–protein interaction networks, with increased coverage and integration. *Nucleic Acids Res* 2013;**41**:D808–15.
20. Albert R. Scale-free networks in cell biology. *J Cell Sci* 2005;**118**:4947–57.
21. Almaas E. Biological impacts and context of network theory. *J Exp Biol* 2007;**210**:1548–58.
22. Barabasi AL, Oltvai ZN. Network biology: understanding the cell's functional organization. *Nat Rev Genet* 2004;**5**:101–13.
23. Bader GD, Hogue CW. An automated method for finding molecular complexes in large protein interaction networks. *BMC Bioinform* 2003;**4**:2–28.
24. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res* 2003;**13**:2498–504.
25. Jin W, Wang J, Zhu T, Yuan B, Ni H, Jiang J, et al. Anti-inflammatory effects of curcumin in experimental spinal cord injury in rats. *Inflamm Res* 2014;**63**:381–7.
26. Kloesch B, Becker T, Dietersdorfer E, Kiener H, Steiner G. Anti-inflammatory and apoptotic effects of the polyphenol curcumin on human fibroblast-like synoviocytes. *Int Immunopharmacol* 2013;**15**:400–5.
27. Assenov Y, Ramirez F, Schelhorn SE, Lengauer T, Albrecht M. Computing topological parameters of biological networks. *Bioinformatics* 2008;**24**:282–4.
28. Manke T, Demetrius L, Vingron M. Lethality and entropy of protein interaction networks. *Genome Inform* 2005;**16**:159–63.
29. Przulj N. Biological network comparison using graphlet degree distribution. *Bioinformatics* 2007;**23**:e177–83.
30. Estrada E. Virtual identification of essential proteins within the protein interaction network of yeast. *Proteomics* 2006;**6**:35–40.
31. Vlahopoulos S, Boldogh I, Casola A, Brasier AR. Nuclear factor-κB-dependent induction of interleukin-8 gene expression by tumor necrosis factor alpha: evidence for an antioxidant sensitive activating pathway distinct from nuclear translocation. *Blood* 1999;**94**:1878–89.
32. Klawitter M, Quero L, Klasen J, Gloess AN, Klopprogge B, Hausmann O, et al. Curcuma DMSO extracts and curcumin exhibit an anti-inflammatory and anti-catabolic effect on human intervertebral disc cells, possibly by influencing TLR2 expression and JNK activity. *J Inflamm* 2012;**9**:29–42.
33. Shehzad A, Ha T, Subhan F, Lee YS. New mechanisms and the anti-inflammatory role of curcumin in obesity and obesity-related metabolic diseases. *Eur J Nutr* 2011;**50**:151–61.
34. Ross KR, Corey DA, Dunn JM, Kelley TJ. SMAD3 expression is regulated by mitogen-activated protein kinase kinase-1 in epithelial and smooth muscle cells. *Cell Signal* 2007;**19**:923–31.
35. Patwardhan RS, Checker R, Sharma D, Kohli , V, Priyadarsini KI, Sandur SK. Dimethoxycurcumin, a metabolically stable analogue of curcumin, exhibits anti-inflammatory activities in murine and human lymphocytes. *Biochem Pharmacol* 2011;**82**:642–57.
36. Murakami K, Mavrothalassitis G, Bhat NK, Fisher RJ, Papas TS. Human ERG-2 protein is a phosphorylated DNA-binding protein—a distinct member of the ets family. *Oncogene* 1993;**8**:1559–66.
37. Verger A, Buisine E, Carrere S, Wintjens R, Flourens A, Coll J, et al. Identification of amino acid residues in the ETS transcription factor Erg that mediate Erg-Jun/Fos-DNA ternary complex formation. *J Biol Chem* 2001;**276**:17181–9.
38. Alexopoulou L, Holt AC, Medzhitov R, Flavell RA. Recognition of double-stranded RNA and activation of NF-κB by toll-like receptor 3. *Nature* 2001;**413**:732–8.
39. Platanias LC. Mechanisms of type-I- and type-II-interferon-mediated signalling. *Nat Rev Immunol* 2005;**5**:375–86.
40. Busch-Dienstfertig M, Gonzalez-Rodriguez S. IL-4 JAK-STAT signaling, and pain. *JAKSTAT* 2013;**2**:e27638.
41. Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. *Cell* 2006;**124**:783–801.