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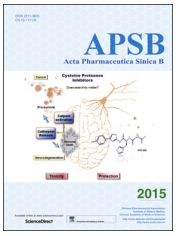

SHORT COMMUNICATION

# 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.