Analysis of Potential Human Targets and Pathways of Sulforaphane Based on Network Pharmacology

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Abstract. Sulforaphane (SFN) is a naturally occurring bioactive compound widely present in cruciferous vegetables, and its anti-tumor, anti-inflammatory, and other activities have been extensively studied. This study adopts a network pharmacology approach, aiming to analyze and elucidate the core targets and pathways of SFN across these four pharmacological effects. In this study, relevant targets were first obtained using SwissTargetPrediction and PharmMapper. Subsequently, GO analysis and KEGG enrichment analysis were performed to uncover its action sites in the human body, the functions of the associated target genes, the biological processes involved, and the resulting effects. Further interpretation of the PPI network provides additional insight into its pharmacological actions from the levels of targets and pathways. Based on this, the present paper offers a systematic summary of SFN's anti-tumor, anti-inflammatory, adjunctive therapy for diabetes, and neuroprotective effects by integrating targets and pathways, and compares these findings with previous studies. In conclusion, sulforaphane, as a promising natural compound with therapeutic and intervention potential in multiple areas, holds promise for contributing to the treatment of related diseases in the future and bringing new hope and prospects to patients undergoing treatment or individuals aiming to prevent these conditions.

Keywords: Sulforaphane, Network Pharmacology, Anti-tumor, Anti-inflammatory, Neuroprotective.

1. Introduction

With the improvement of people's living standards, people are increasingly willing to obtain components from nature that have health-preserving and even therapeutic effects. Coincidentally some related components in plants do possess corresponding therapeutic effects. Among these, there are some components with relatively strong efficacy and a wide range of effects, such as curcumin, flavonoids, and sulforaphane.

Sulforaphane (SFN) is a natural active ingredient widely found in cruciferous plants, especially in broccoli buds. Current research has confirmed its excellent effects in anti-tumor, antioxidant and anti-inflammatory aspects, and it may have potential effects in the treatment of neurological diseases. However, due to its benefits and safety in various body surface and animal studies, it is still in the clinical trial stage [1]. Related studies have shown that SFN is strongly correlated with Nuclear Factor Erythroid 2-Related Factor 2 Signaling Pathway (Nrf2) and Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB). It is worth noting that these two related pathways are related to anti-tumor, anti-inflammatory and antioxidant aspects. This confirms the efficacy of SFN in these three aspects. However, inflammation is an inducing factor for many diseases, including neurodegenerative diseases (NDDs) such as Parkinson's disease and Alzheimer's disease. Related studies have also confirmed its neuroprotective effect in spinal cord injury, which shows its therapeutic potential in neurological diseases and will become a new drug in this field[2].

Network pharmacology was first proposed by British pharmacologist Andrew L. Hopkins in 2007, aiming to provide a theoretical basis for the identification of new drugs through computer networks and a large number of biological databases. The core purpose of this field is to explore the molecular mechanism of action of multi-target drugs. Network pharmacology transforms the traditional "single-target, single-drug" approach into a more comprehensive "multi-target, multi-component" approach in the network.

Based on this, this study uses relevant tools in network pharmacology to conduct target analysis on SFN, thereby revealing and analyzing its potential targets and pathways in the human body, aiming to further elucidate its disease-fighting function and further confirm its function.

2. Data Acquisition and Preprocessing Workflow

2.1. Data Acquisition and Filtering

At the beginning of this study, the SMILES structure of SFN was obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov) as its basic information.

After that, the targets of SFN in the human body were searched and filtered using the SwissTargetPrediction (http://www.swisstargetprediction.ch) and PharmMapper (http://www.lilab-ecust.cn/pharmmapper) databases. A total of 101 targets were obtained through the search of the SwissTargetPrediction database. After removing the targets with a Probability value of 0, 38 relevant targets were finally obtained [3-5]. Then, the MOL of SFN was obtained using TCMSP (https://www.tcmsp-e.com/tcmsp.php) for target prediction in PharmMapper. The number of targets obtained was 200, and the Normalized Fit Score ranged from 0.9429 to 0.1531. The median value of 0.5 was taken as the lower limit, and 37 targets were finally obtained [6-8]. After obtaining its Gene Name, it was integrated with the previously obtained 38 targets and deduplicated, resulting in a final total of 70 targets.

2.2. Data Processing

After obtaining the list of potential targets predicted by SFN in the human body, it was first imported into Metascape for GO and KEGG analysis, and the results were used for subsequent research and analysis on its function [9]. Then the target list was imported into STRING (https://string-db.org) to obtain the protein-protein interaction (PPI) network [10], and the protein interaction data was then imported into Cytoscape to obtain the PPI network diagram [11], connecting its function and pathway relationship.

3. Result

3.1. GO Analysis and KEGG Analysis

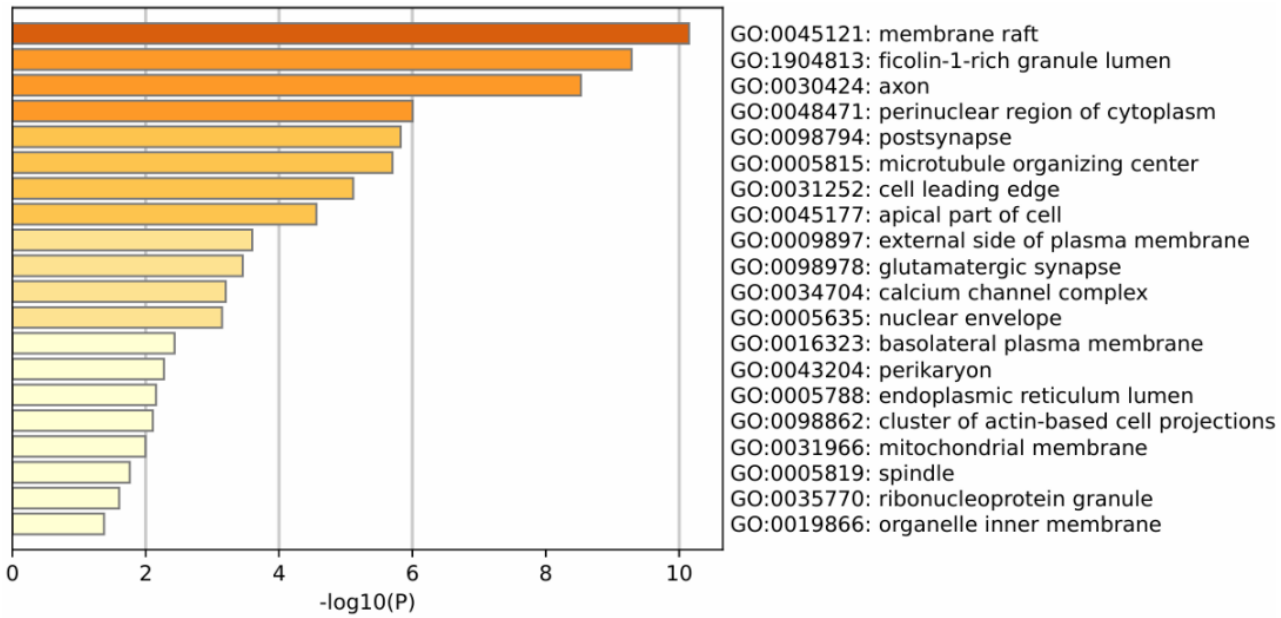


Fig. 1 GO Cellular Components Analysis (Picture credit: Original)

Fig. 1 shows the Gene Ontology Cellular Components corresponding to the 18 SFN. The horizontal axis represents the -log 10 (P) value, used to reflect the enrichment confidence. The region with the highest enrichment is the membrane raft, followed by neuron-related structural regions such as axon, postsynapse, and glutamatergic synapse.

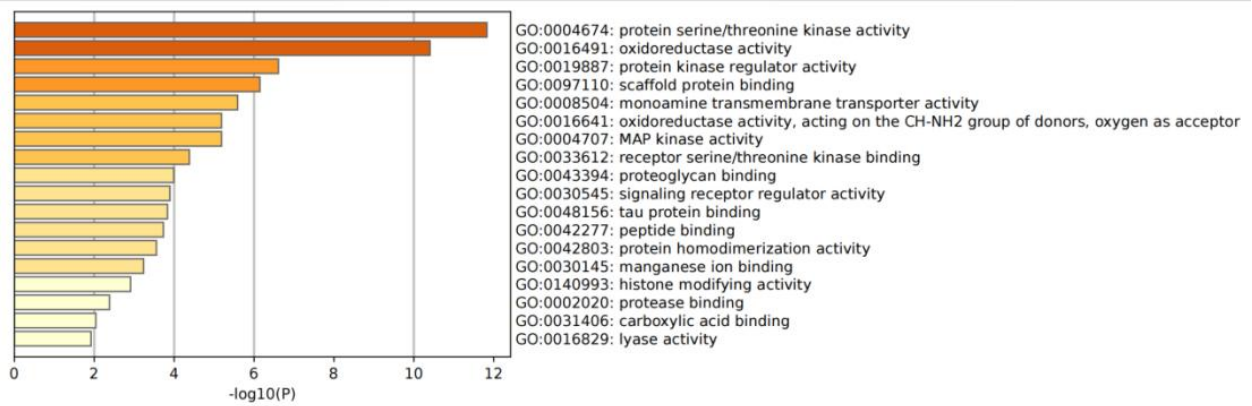


Fig. 2 GO Molecular Functions Analysis (Picture credit: Original)

Fig. 2 shows the Gene Ontology Molecular Functions corresponding to the 18 SFN. The horizontal axis represents the -log 10 (P) value, used to indicate the enrichment confidence level. The highest enrichment levels were observed for protein serine/threonine kinase activity and oxidoreductase activity, with MAP kinase activity also showing significant enrichment. This indicates that the potential targets of SFN primarily assemble kinase signaling complexes in the membrane raft, thereby participating in phosphorylation-mediated signal transduction and redox metabolism in the endoplasmic reticulum, mitochondria, and neuronal regions. This is consistent with the results of the GO Cellular Components analysis.

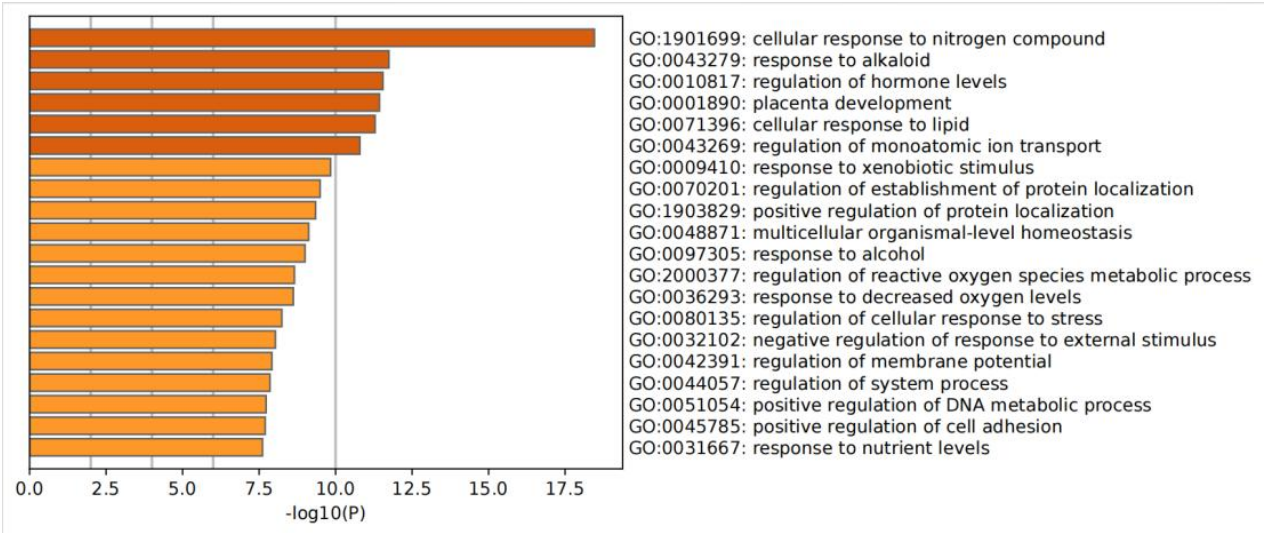


Fig. 3 GO Biological Processes Analysis (Picture credit: Original)

Fig. 3 shows the Gene Ontology Biological Processes corresponding to 20 SFN. The horizontal axis represents the -log 10 (P) value, used to indicate the enrichment confidence level. The highest enrichment levels were for cellular response to nitrogen compound and response to alkaloid, indicating that SFN mainly regulate phosphorylation-mediated signal transduction by regulating membrane proteins located on the membrane raft, and then carry out redox metabolic processes in the endoplasmic reticulum, mitochondria, and neurons, thereby responding to exogenous substances, oxidative stress, maintaining homeostasis, ion transport, and hormonal regulation.

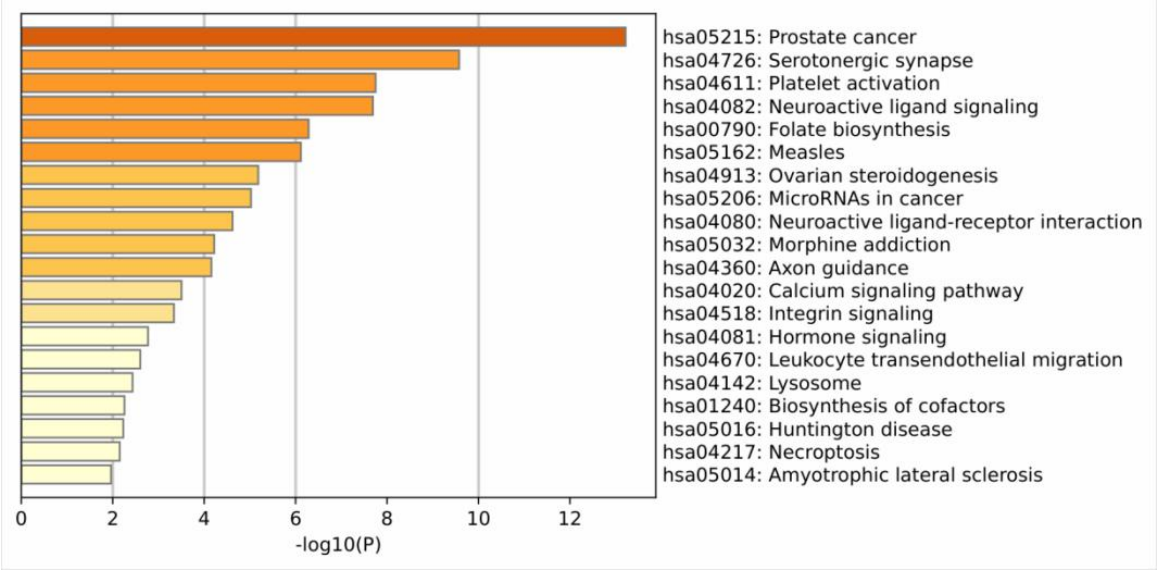


Fig. 4 KEGG Analysis (Picture credit: Original)

Fig. 4 shows the KEGG pathways corresponding to 20 SFN. The horizontal axis represents the -log 10 (P) value, used to indicate the enrichment confidence level. The highest enrichment level was observed in prostate cancer, followed by multiple related neural signaling pathways and calcium signaling pathways. Combining the results of GO analysis and KEGG enrichment analysis, the target genes of SFN mainly regulate tumor proliferation, neural signal transduction, cell death, and homeostasis in the human body through pathways such as prostate cancer, calcium signaling, neural synapses, hormones, and programmed necrosis.

3.2. PPI Network Diagram4

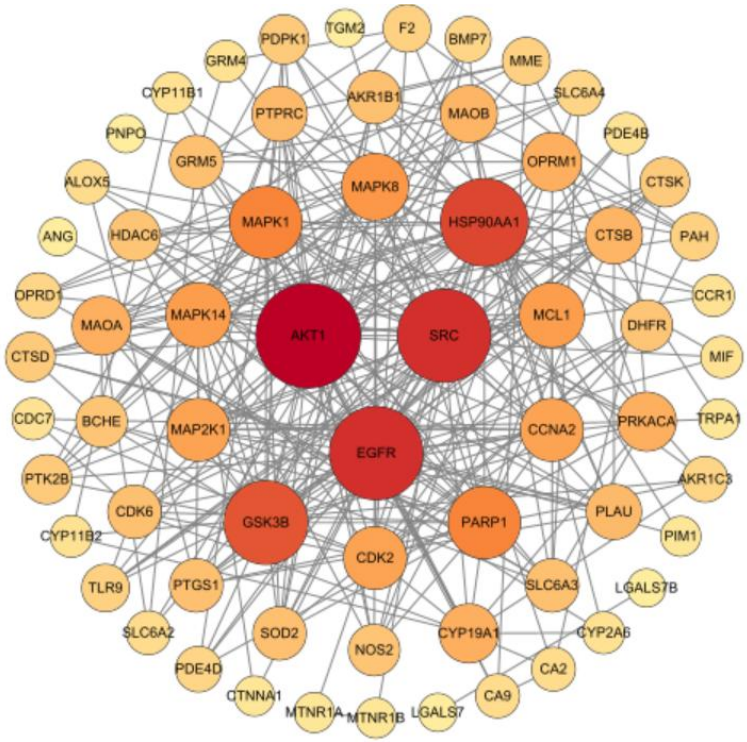


Fig. 5 PPI Network Diagram (Picture credit: Original)

Fig. 5 illustrates the interactions between potential SFN targets in the human body. Larger nodes and darker-colored targets indicate higher connectivity. The top five connectivity targets are AKT1, EGFR, SRC, HSP90AA1, and GSK3B. Serine/threonine-protein kinase (AKT1) and Epidermal Growth Factor Receptor (EGFR) are key regulatory target genes mediating calcium signaling,

hormone regulation, and tumor proliferation. AKT1 is a target gene closely related to cell survival and anti-apoptosis, while EGFR is a target gene closely related to cell proliferation, cell survival, and anti-apoptosis. SRC is a key regulatory target gene for membrane signaling, cell adhesion, migration, and synaptic signaling. This further confirms that the corresponding SFN target genes play a role in regulating tumor proliferation, neural signaling, cell death, and maintaining homeostasis in the human body.

4. Discussion

Based on the results, the functions of SFN can be categorized into the following four aspects according to their mechanisms of action: Anti-tumor and Anti-cancer effect, anti-inflammatory and antioxidant effects, adjunctive therapy for diabetes, possesses neuroprotective effects.

SFN can disrupt tumor cell proliferation signals by regulating target genes such as AKT1 and EGFR, which are related to cell proliferation and growth, thereby inducing cell cycle arrest and apoptosis. At the same time, SFN can also inhibit HSP90AA1, which interacts with a variety of oncogenic proteins, thus playing an anti-cancer role. Chen et al. also demonstrated the efficacy of SFN in inhibiting the proliferation of pancreatic cancer cells, enhancing the sensitivity of cells to treatment, and affecting various cancer control characteristics in their related study in 2018 [12].

SFN inhibits the release of inflammatory factors by regulating the MAPK14 target pathway, and as a potent activator of the Nrf2 pathway, it also activates the intracellular antioxidant defense system to scavenge free radicals and reduce oxidative damage. This is consistent with the findings of Sonia Medina et al. in related studies that SFN exerts an immunomodulatory effect by reducing pro-inflammatory interleukins and enhancing the anti-inflammatory response of epithelial-macrophage cells in vitro [13].

SFN induces downstream molecules such as glucose transporters by regulating the phosphorylation of insulin receptor substrate-1 mediated by AKT1 target genes, thereby enhancing glucose uptake and improving insulin resistance . Existing studies have confirmed that SFN can effectively alleviate kidney damage in diabetic mice by activating the AMPK and SIRT1 signaling pathways and inhibiting the NF-κB signaling pathway [14]. In this study, AKT1, GSK3B, MAPK14, and SOD2 are key hubs connecting the AMPK and SIRT1 pathways.

The pathology of many NDDs may be related to the regulation of the Nrf2 pathway. In previous studies, SFN was used to treat mice after knocking out the Nrf2 pathway target. The results showed that the neuroprotective effect of SFN disappeared. This result revealed that SFN exerts its neuroprotective effect by regulating the Nrf2 signaling pathway [15]. In this study, the PPI protein interaction network and PPI network diagram were used to find that SFN indirectly regulates the Nrf2 pathway through the targets of downstream antioxidant enzymes such as SOD2 and NOS2, as well as upstream regulatory factors such as MAPK14, AKT1, and GSK3B. SFN also affects the metabolism and transport of neurotransmitters by regulating target genes such as MAOA and MAOB, which are related to the oxidation and concentration regulation of catalytic hormones and neurotransmitters, and target genes such as OPRM1, which are closely related to pain and mood regulation. All of these evidences suggest that SFN may have potential regulatory functions and neuroprotective effects in NDDs. This is consistent with current research showing that SFN is becoming a promising substance for neuroprotection and treatment of NDDs.

5. Conclusion

This study conducted GO analysis, KEGG enrichment analysis, and PPI protein interaction network diagrams on potential human targets of SFN predicted from the SwissTargetPrediction and PharmMapper databases. Based on the obtained data and results, this paper analyzes and explains the core targets and pathways of SFN in four major pharmacological effects from a network pharmacology perspective: antitumor and anticancer, anti-inflammatory and antioxidant, adjuvant

therapy for diabetes, and neuroprotective effects. It systematically summarizes the molecular targets and pathways regulated by SFN in these four major pharmacological effects. The aim is to provide theoretical support and a reference for subsequent basic experimental and clinical research related to SFN.

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