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The key targets and signaling pathways of sulforaphane against colorectal cancer were analyzed based on network pharmacology

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Abstract:

Background: Sulforaphane is a dietary supplement molecule with a wide range of clinical applications, but its anti-colorectal cancer mechanism is not clearly understood. This paper was to systematically studied the molecular mechanism of sulforaphane against colorectal cancer by network pharmacology.

Methods: The protein target was screened by SwissTargetPrediction, and the core target was analyzed by Cytoscape. Enrichment analysis and disease ontology analysis are then performed to generate a "gene-compound-disease" network to intuitively explore the underlying pharmacological mechanisms. Finally, the anti-colorectal cancer effect of sulforaphane was verified by CCK-8 in cell level.

Results: Totally 43 targets were researched and the main anti-colorectal cancer mechanisms of sulforaphane were addressed. *GAPDH*, *SRC*, *MAPK1*, *JAK2*, *GSK3B* were identified as hub targets. The KEGG pathway mainly related to AGW-RAGE pathway and viral carcinogenesis.

Conclusion: This study successfully identified the anti-colorectal cancer mechanism of sulforaphane (10 hub genes). In addition, sulforaphane can be further studied and developed as a lead compound. The findings here provide better guidance for follow-up experiments where we identify potentially active compounds for drug discovery or health promotion.

Keywords: sulforaphane,colorectal cancer,network pharmacology,hub gene, protein-protein network

Introduction

Colorectal cancer (CRC) is the most common cancer in the world. The epidemiology in colorectal cancer varies significantly across regions, ages, genders, and ethnicities (Hou et al., 2022). The treatment for colorectal cancer including cytotoxic chemotherapy, biologic therapies, immunotherapy and its combina-

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tion(Biller & Schrag, 2021). Despite progress in colorectal cancer treatment, there is still a deficiency in clinical trial data. It is essential to validate significant effects and develop more personalized treatment alternatives. (Shang et al., 2023).

TCM (Traditional Chinese medicine) has unique advantages for the treatment of colorectal cancer. Sulforaphane is a type of isothiocyanate present in cruciferous vegetables like cabbage, cauliflower, and broccoli sprouts. (Vanduchova, Anzenbacher, & Anzenbacherova, 2019). Sulforaphane has attracted the attention of researchers in clinical studies of cancer chemopprophylaxis for its natural ability as a phase II enzyme inducer (Iahtisham Ul, Khan, Awan, & Iqbal, 2022). The impact of sulforaphane on colorectal cancer has not yet been researched. Network pharmacology is an integrative approach that merges systems biology with network informatics. In recent years, it has been applied in the creation of new medications. This approach suggests that complex diseases like cancer arise not from mutations in a single gene, but from imbalances in biological network systems due to mutations in several genes. (Nogales et al., 2022).

This research employs network pharmacology to investigate the principal targets and molecular mechanisms through which sulforaphane exerts its effects on colorectal cancer. Additionally, the study validates the inhibitory effects of sulforaphane on colorectal cancer in vitro. The findings of this study offer a theoretical foundation for future investigations into the application of sulforaphane in the treatment of colorectal cancer.

1.Materials and Method

1.1 The Target gene of Sulforaphane

The structural characteristics of sulforaphane were derived from PubChem. Target identification for sulforaphane was conducted utilizing the 100 Target Fishing Database, which includes the STPD (Swiss Target Prediction Database), PMD (PharmMapper Database), and PubChem. The analysis was restricted to homo sapiens, selecting target proteins that exhibited a normal fit score in PharmMapper (≥0.4), a probability score >0 in SWISS. Furthermore, authenticated targets were compiled using the ETCM and PubChem databases. The standardized nomenclature for all targets was sourced from Uniprot.

1.2 Colorectal cancer Target Gene Prediction

Colorectal cancer-associated genes were identified through an examination of three databases: GeneCards, DisGeNET, and OMIM. The shared targets between sulforaphane and colorectal cancer were analyzed using Venny (2.1).

1.3 PPI Network

The analysis of protein interactions was conducted utilizing the String database. The interaction relationships were established based on confidence (> 0.4) for "Homo sapiens" and subsequently exported as a TSV file. The network was imported into Cytoscape (version 3.8.2) to facilitate the construction of PPI network and to identify the hub targets.

1.4 GO and KEGG Analysis

Metascape was employed to conduct GO and KEGG pathway analyses, with a significance threshold set at $P<0.05$. The gene ontology encompasses three primary categories (MF, BP and CC). The analysis utilizes p-values to rank the results, and Metascape is utilized to identify the KEGG and GO pathways (top 20).

1.5 Component-Target-Pathway Network

We developed a CTP network utilizing Cytoscape version 3.7.2.

1.6 CCK-8

HCT-116 cells were inoculated in 96-well plates of 1.91 x 10^5 cells/mL per well and were subsequently cultured in a temperature-controlled incubator until they adhered. Following this, HCT-116 were exposed to varying concentrations of sulforaphane (100, 50, 25, 12.5, and 6.25 mg/mL) for a duration of 24 hours. After treatment, the medium was replaced with DMEM containing 10% CCK8, and the cells were incubated in a temperature-controlled environment for 1.5 hours. The OD (450 nm) were then measured using an enzyme-linked instrument.

2. Results

2.1 Potential Targets Against colorectal cancer Identification

A total of 100 potential genes for sulforaphane were identified through searches conducted in the STP and PubChem databases. Additionally, 1,026 colorectal cancer genes were compiled from the GeneCards, OMIM, and DisGeNET, with duplicates removed. Following this, 43 targets that intersect between sulforaphane and colorectal cancer-related genes were identified and these were deemed genes for sulforaphane in the context of colorectal cancer for further investigation (see Figure 1).

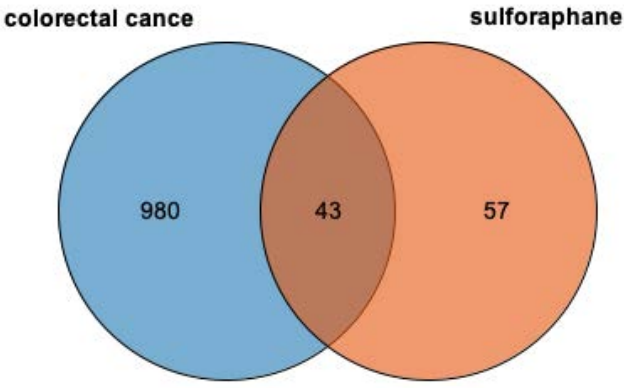

Figure 1. Venn for sulforaphane against colorectal cancer

2.2 PPI Network Analysis

The 43 genes associated with sulforaphane were imported into the String to analyze PPI, followed by their transfer to Cytoscape (version 3.7.2). The analysis network represents the target as concentric circles, comprising 37 nodes and 65 edges (Figure 2). The dimensions and coloration of the nodes vary according to their degree; specifically, nodes with a higher degree are depicted as larger. Notably, *GAPDH, SRC, MAPK1, JAK2* and *GSK3B* were identified as hub targets based on their degree and compact centrality, constituting the innermost layer of the concentric circles.

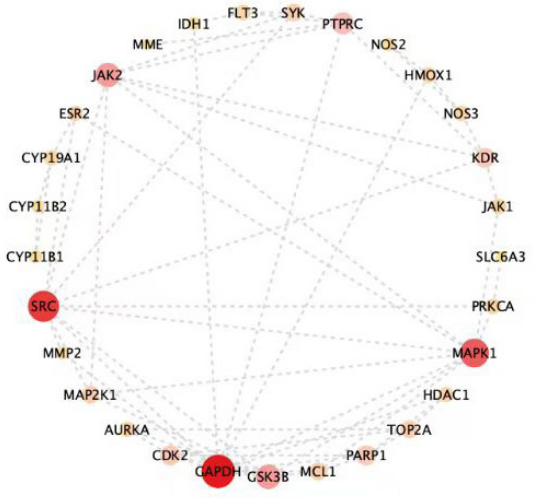

Figure 2. PPI network for 43 common targets between sulforaphane and colorectal cancer

Table 1. Top ten genes in PPI network

name	Degree number	The Shortest Path Length	Betweenness Centrality	Closeness Centrality	Clustering Coefficient
GAPDH	11	1.821428571	0.379987374	0.549019608	0.181818182
SRC	10	1.821428571	0.281189875	0.549019608	0.266666667
MAPK1	9	1.928571429	0.197613035	0.518518519	0.333333333
GSK3B	7	2.107142857	0.090714687	0.474576271	0.428571429
JAK2	7	2.285714286	0.043209877	0.4375	0.428571429
PTPRC	6	2.321428571	0.119235009	0.430769231	0.2
CDK2	5	2.535714286	0.035555956	0.394366197	0.5
PARP1	5	2.571428571	0.022274331	0.388888889	0.5
KDR	5	2.321428571	0.074294533	0.430769231	0.3
MCL1	4	2.285714286	0.012851732	0.4375	0.666666667

2.3 Enrichment Analysis

2.3.1 GO analysis

In order to know the deeper insight into the GO-terms through which sulforaphane may exert its effects against colorectal cancer, we employed Metascape to conduct a GO enrichment analysis for 43 identified targets. Based on the adjusted p-values, the GO-term implicated include response to hormone, heme binding and peptidyl-amino acid modification.

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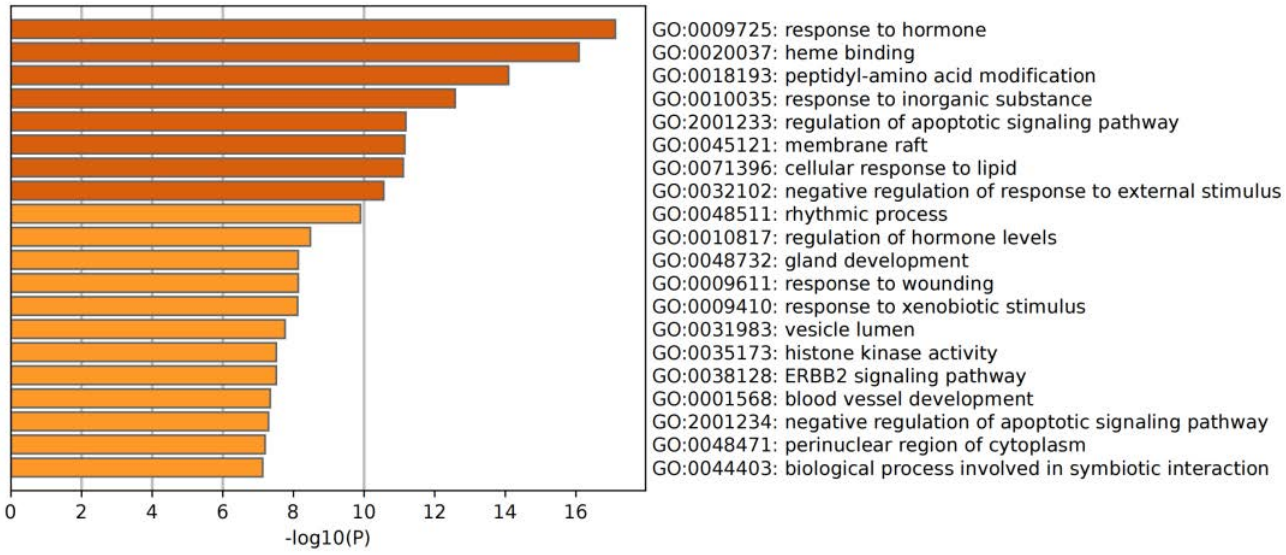

Figure 3. GO enrichment for 43 common targets between sulforaphane and colorectal cancer

2.3.2 KEGG enrichment analysis

In order to know the deeper insight into the potential mechanisms through which sulforaphane may exert its effects against colorectal cancer, we employed Metascape to conduct a KEGG pathway enrichment analysis for 43 identified targets. Based on the adjusted p-values, the KEGG pathways predominantly implicated include those related to cancer, resistance to EGFR tyrosine kinase inhibitors, formation of neutrophil extracellular traps, the AGW pathway associated with diabetic complications, and viral carcinogenesis.

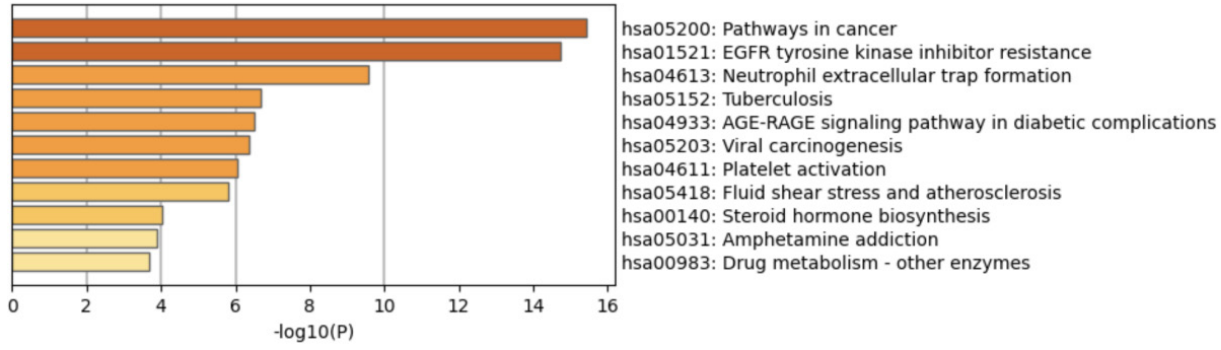

Figure 4. KEGG enrichment for 43 common targets between sulforaphane and colorectal cancer

2.4 Compound-gene-pathway

We developed a sophisticated network, informed by principal signaling pathways and prospective targets, to investigate its anticancer mechanisms. This network comprises 53 nodes and 177 edges, with red nodes denoting various cancers, green nodes also representing cancers, and pink oval nodes indicating nodes (Figure 5). The associated pathways are illustrated by green diamond nodes, while the connections between the nodes are depicted by edges (Figure 5).

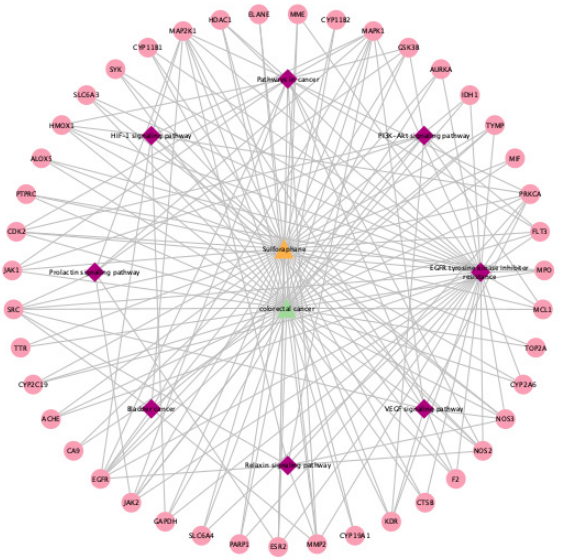

Figure 5. Network for 43 common targets

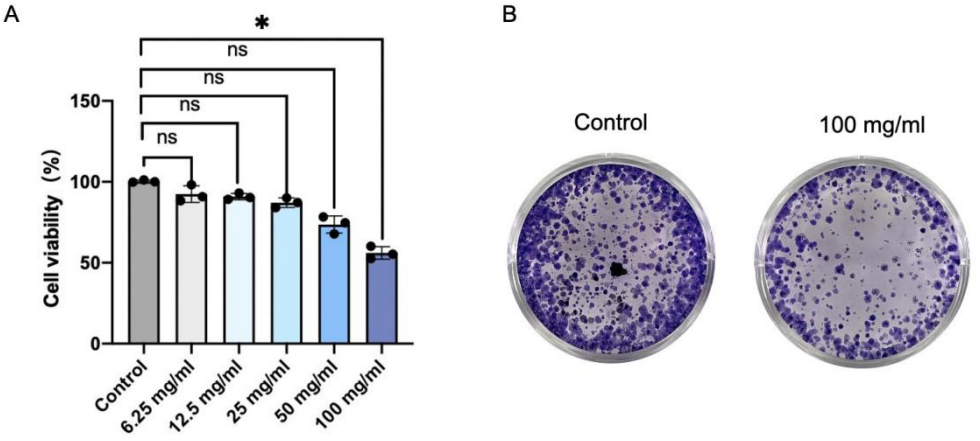

Figure 6. Sulforaphane inhibits the cell proliferation of SW-116

(A) Sulforaphane decreases the viability of SW-116 measured by the CCK-8 after 24h after treatment with different concentration of sulforaphane. *P<0.05 (B). Representative images showing the number of colony formation by SW-116 treated with 100 mg/ml of sulforaphane for 48h

Discussion

As economy continues to develop, the incidence rates of colorectal cancer were rising annually. Currently, there is no effective treatment available for recurrent colorectal cancer. Traditional Chinese Medicine (TCM) is recognized as the standard adjunctive therapy in this context. Recent studies have indicated that TCM and its constituents exhibit notable anti-tumor properties, which encompass the inhibition of tumor proliferation and migration, as well as the reversal of drug resistance. (Hsu et al., 2021; Liu et al., 2020). This study aimed to identify the targets

between sulforaphane and colorectal cancer

2.5 Sulforaphane has been shown to impede cell proliferation and the formation of colonies in HCT-116 cells.

The influence of sulforaphane on the cell viability of HCT-116 cells was assessed using the CCK8. HCT-116 cells were exposed to varying concentrations of sulforaphane for 24 h. The inhibition rate of cell proliferation was notably elevated at a high concentration of 100 mg/ml ($P < 0.05$) (Figure 6A). Additionally, colony formation assays corroborated the anti-proliferative effects of sulforaphane, revealing a significant reduction in the number of colonies formed by SW-116 cells following treatment with sulforaphane, compare to the control (Figure 6B). These findings indicate that sulforaphane effectively inhibits both the cell viability and colony formation of HCT-116 cells.

and pathways associated with sulforaphane's effects on colorectal cancer through network pharmacology, which were subsequently validated through in cell experiments. Utilizing this methodology, we identified 43 potential targets for sulforaphane in the context of colorectal cancer. The PPI network showed that *GAPDH, MAPK1, JAK2, GSK3B* might be potential genes of sulforaphane in colorectal cancer.

The cytoplasmic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) has various functions, including its conventional role in the glycolytic pathway (Butera et al., 2019). The translocation of GAPDH to the nucleus is modulated by factors associated with cancer, and GAPDH serves as a fundamental mechanism for the regulation of autophagy and cell death processes (Zhang et al., 2015). The stimulation of autophagy by nuclear GAPDH may

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have a significant role in cancer cells by functioning as a pro-survival factor and facilitating the energy demands associated with rapid cellular proliferation, even in the presence of adverse conditions. Furthermore, the translocation of GAPDH to the nucleus is a critical phenomenon in the context of neurodegenerative diseases, as an increase in intranuclear GAPDH levels has been linked to neuronal cell death (Berry, 2004). SRC-3 has been recognized as a transcriptional coactivator for nuclear receptors, particularly the ER, and plays a significant role in the proliferation of hormone-dependent malignancies. Recent research has indicated that SRC-3 may influence various cellular processes in cancer independently of nuclear receptor signaling pathways. Furthermore, transgenic mouse models expressing SRC-3 have demonstrated its capacity to induce tumorigenesis across multiple tissue types in mice (Ma, Ren, Wang, & He, 2011). *MAPK1* is a kinase that phosphorylates histones and modulates gene expression by interacting with the promoter regions of genes as a transcription factor. Prior research has indicated that the expression levels of *MAPK1* are significantly increased in human gastric cancer, implying that *MAPK1* may act as a kinase that facilitates the migration and invasion of gastric cancer cells (Wang et al., 2023). Janus kinase 2 (JAK2) is classified as a non-receptor tyrosine kinase and plays a crucial role in numerous cellular processes, including cell cycle progression, apoptosis, and genetic stability. The equilibrium among these functions is a significant determinant in assessing whether a cell exhibits benign or malignant characteristics. (Qian, Yao, & Si, 2011). GSK3B is a critical factor in the process of tumorigenesis. It modulates the proliferation and migration of cervical cancer cells through its influence on the PI3K/Akt signaling pathway and EMT (Zheng et al., 2024). The findings indicate that sulforaphane possesses various anti-colorectal cancer properties through the inhibition of specific targets and pathways.

Consequently, additional experiments are required to elucidate the specific molecular mechanisms involved. Ultimately, further experimental investigations and clinical trials are essential to validate the aforementioned findings and to investigate the specific effects of sulforaphane on colorectal cancer, thereby establishing a basis for the development of novel therapeutic agents.

Conclusion

This investigation examined the anti-colorectal cancer properties of sulforaphane utilizing a multi-target and multi-channel network pharmacology approach. Subsequent in vitro experiments corroborated that sulforaphane can enhance the viability of colorectal cancer cells. This research offers a foundational and innovative strategy for the treatment of colorectal cancer.

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