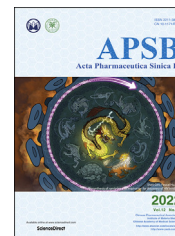




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REVIEW

New opportunities and challenges of natural products research: When target identification meets single-cell multiomics



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Abstract Natural products, and especially the active ingredients found in traditional Chinese medicine (TCM), have a thousand-year-long history of clinical use and a strong theoretical basis in TCM. As such, traditional remedies provide shortcuts for the development of original new drugs in China, and increasing numbers of natural products are showing great therapeutic potential in various diseases. This paper reviews the molecular mechanisms of action of natural products from different sources used in the treatment of inflammatory diseases and cancer, introduces the methods and newly emerging technologies used to identify and validate the targets of natural active ingredients, enumerates the expansive list of TCM used to treat inflammatory diseases and cancer, and summarizes the patterns of action of emerging technologies such as single-cell multiomics, network pharmacology, and artificial intelligence in the pharmacological studies of natural products to provide insights for the development of innovative natural product-based drugs. Our hope is that we can make use of advances in target identification and single-cell multiomics to obtain a deeper understanding of actions of mechanisms of natural products that will allow innovation and revitalization of TCM and its swift industrialization and internationalization.

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1. Introduction

Natural medicines are defined as chemical substances with pharmacological or biological activities that are produced in nature by living organisms, such as plants, animals, insects, marine organisms, and microorganisms. These natural products are precious treasures gifted to us by nature and serve as key sources of substances for the prevention and treatment of human diseases. As such, they also have an important role and irreplaceable status in drug development and design. More than 10,000 species of medicinal plants are used in China, and their use in traditional Chinese medicine (TCM) is guided by a complex theoretical system with a history of clinical use of more than 2000 years. The identification of the active ingredients in these medicines is an important component of China's drug research, as natural products provide useful shortcuts in new drug development.

New drugs are needed to cope with the changes in the modern human population, in which accelerating aging and altered dietary habits and lifestyles are now increasing the incidence of inflammatory diseases, making these disorders one of the major threats to human health worldwide. Inflammation is associated with many infections, autoimmune diseases, malignant tumors, neurodegenerative diseases, cardiovascular diseases, diabetes, and other major chronic non-communicable diseases, and these disorders are spreading globally and becoming important public health problems that seriously endanger both human health and sustainable socioeconomic development. Natural products have made great contributions to the prevention and treatment of inflammatory diseases and cancer, and scientists have been greatly encouraged to invest in the development of natural product-based drugs by the discovery of the potent antimalarial natural product artemisinin by Youyou Tu, a Chinese scientist who won the 2015 Nobel Prize in Physiology or Medicine. This paper reviews the important research progress made in the use of natural products for the treatment of inflammatory diseases and cancer, summarizes methods for target identification and validation, and reports on the application of frontier technologies in natural product research aimed at elucidating the targets and mechanisms of natural product action and promoting the development of new drugs to combat major diseases.

2. Natural products in the treatment of inflammatory diseases and cancer

Natural products, especially of plants, animals, marine and mineral origins, play an important role in inflammatory diseases and cancer, as outlined in Fig. 1.

2.1. Plant natural products

Plants are vital components of TCM, as they serve as important guarantees of the quality of the natural product library. Herbal

remedies have played a major role in the treatment of various diseases since Shennong first tasted all kinds of herbs. Plant-derived natural products are characterized by diverse chemical structures and activities, wide ranges of action, and a low occurrence of toxic side effects.

2.1.1. Berberine

Berberine is an isoquinoline alkaloid extracted from *Coptis chinensis* Franch., *Phellodendron chinense* Schneid. and other plants of the *C. Salisb.* group. *C. chinensis* Franch. was first recorded in the “*Shennong Ben Cao Jing*” as having roots that taste extremely bitter, and impart a cold sensation, with clearing heat, drying dampness, and firing detoxification. It is commonly used in the treatment of dysentery, thirst, carbuncle swelling, and poisoning. Berberine is a broad-spectrum antibacterial drug that is clinically used for the treatment of gastrointestinal diseases caused by bacterial infections. It is also approved for the treatment of hyperlipidemia in several countries.

Jiang and colleagues^{1,2} found that berberine has hypolipidemic atherosclerosis-improving effects and can be used as a complement to statins, an undervalued attribute of this drug in the prevention and treatment of hyperlipidemia, diabetes, and cardiovascular diseases. Berberine can inhibit the action of the potassium voltage-gated channel subfamily H member 6 (KCNH6) potassium channel, which has a newly determined high glucose-dependent pro-insulin secretion effect, so berberine can greatly reduce the risk of hypoglycemia³ and may be used to develop a whole new class of hypoglycemic drugs in the future. Oral administration of berberine activates the gut–brain axis and enhances tyrosine hydroxylase activity by triggering the biosynthesis of tetrahydrobiopterin in the gut microbiota. This, in turn, elevates blood and brain dopamine concentrations to produce L-dopa, thereby ultimately improving Parkinson's disease progression⁴. Berberine can also promote osteoblast proliferation and differentiation⁵, as well as inhibit osteoclast differentiation⁶, to improve osteoporosis. Berberine administration can also improve the effects of organ ischemia–reperfusion injury, ischemic stroke⁷, peritoneal adhesions⁸, nonalcoholic fatty liver⁹, and oral diseases¹⁰.

Berberine has significant effects on many common cancers¹¹ (e.g., lung, breast, colon, and liver cancer). Fang and colleagues¹² have shown that berberine could also reduce cancer recurrence after endoscopic resection of adenoma, a precancerous colorectal disease.

2.1.2. Capsaicin

Capsaicin is the main pungent secondary metabolite component of the fruit of *Capsicum annuum* L. and is known for its analgesic effects. According to the “*Yao Jian*” *C. annuum* L. can “dispel wind and blood, dispel cold and relieve depression, induce stagnation, stop diarrhea, and wipe out ringworm”. Modern medicine proves that *C. annuum* L. has pharmacological effects, such as

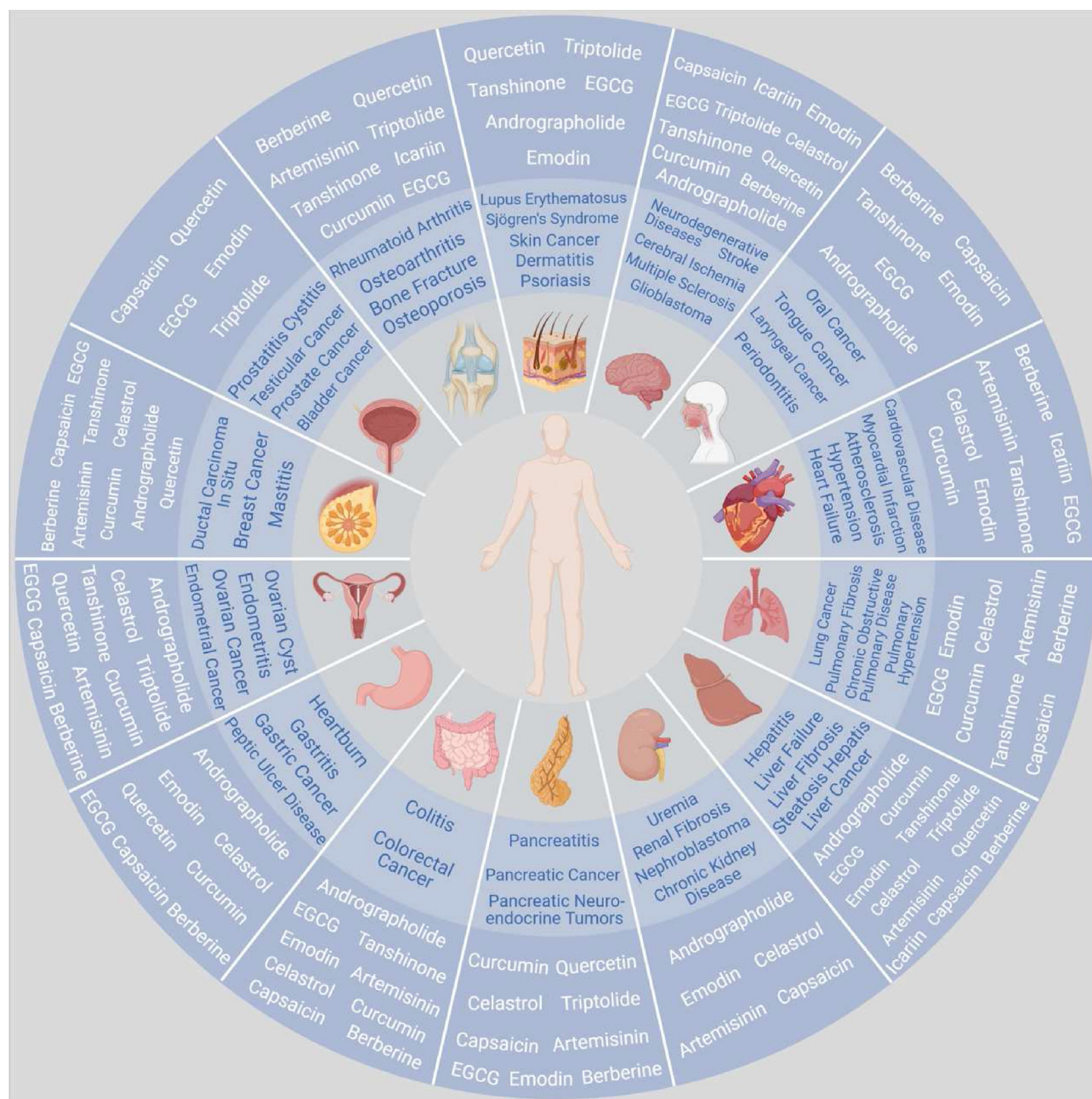


Figure 1 Schematic representation of natural products (in white) improving inflammatory diseases and cancer (in blue). This figure is created with biorender.com.

analgesic, antipruritic, hypolipidemic, hypoglycemic, antibacterial, and antitumor activities.

The United States Food and Drug Administration (FDA) has approved the Qutenza (capsaicin) 8% patch for the treatment of neuropathic pain caused by postherpetic neuralgia¹³ and neuropathic foot pain associated with diabetic peripheral neuropathy (DPN) in adult patients¹⁴. Capsaicin reversibly desensitizes and defunctionalizes the TRPV1 receptor, which plays a key role in pain signaling¹⁵. Years of research of Zhu's team on capsaicin systems^{16–21} have provided an important scientific basis for the prevention of cardiovascular metabolic diseases by spicy diets. Capsaicin also reportedly exerts anti-obesity effects by altering gut microbial composition, reducing intestinal permeability, and

regulating the gut microbe–brain axis²². Capsaicin also activates TRPV1 ion channels to improve glucose homeostasis in the body, thereby preventing and improving prediabetic insulin resistance, diabetes, and its complications²³. Capsaicin also has a therapeutic effect on allergic rhinitis by disrupting the TRPV1–substance P nociceptive signaling pathway in the nasal mucosa²⁴. Oral administration of capsaicin was also shown to activate somatosensory nerves by binding to TRPV1, thereby greatly alleviating the acute vaso-occlusive episodes in sickle cell disease mice and significantly preventing chronic liver and kidney damage²⁵.

Capsaicin also has significant antitumor activity. Han's group²⁶ used an ingenious combination of natural capsaicin and CaCO_3 as a nanocarrier to exploit this activity. The nanocarrier undergoes

rapid cleavage in the acidic tumor microenvironment and releases capsaicin, which then specifically activates TRPV1 channels and increases the calcium ion concentration within the tumor. This causes mitochondrial damage and increases the intracellular levels of reactive oxygen species, ultimately leading to apoptosis of tumor cells and inhibition of tumor growth²⁶. Capsaicin can increase the level of calcitonin gene-related peptide (CGRP) in bone marrow extracellular fluid and promote the movement of hematopoietic stem cells from bone marrow to blood vessels, indicating its promise as a therapy for hematologic tumors²⁷.

2.1.3. Quercetin

Quercetin is a plant secondary metabolite widely found in the bark, flowers, leaves, buds, seeds, and fruits of many plants. Quercetin is a dietary polyphenol that has protective effects when consumed in the diet or as a food supplement.

Senolytics is a drug combination composed of dasatinib and quercetin, which can selectively kill senescent cells²⁸ in a variety of affected organs, repair physiological functions, and delay aging^{29–34}. Liu et al.³⁵ showed that quercetin not only delayed the aging of UV-irradiated human primary dermal fibroblasts, but it also delayed the aging of HES1-deficient human primary dermal fibroblasts. Quercetin regulates the gut microbiota and affects the progression of non-alcoholic fatty liver disease (NAFLD) in mice via the intestinal–hepatic axis³⁶.

Quercetin can also enhance the anticancer effects of adriamycin chemotherapy in hepatocellular carcinoma cells while protecting normal hepatocytes³⁷. Quercetin combined with cisplatin nanoparticles significantly inhibited the progression of bladder cancer³⁸. Quercetin activated gastric cancer cell autophagy through regulating Akt–mTOR signaling and hypoxia-inducible factor 1 α (HIF-1 α) signaling, thereby inhibiting gastric cancer progression³⁹. The synergistic effect induced by the co-administration of quercetin and alantolactone as a colorectal cancer treatment was able to reactivate antitumor immunity by inducing immunogenic cell death (ICD), causing cytotoxicity, and modulating the immunosuppressive tumor microenvironment⁴⁰.

2.1.4. Icariin

Icariin, an isopentenyl flavonoid glycoside compound from *Epi-medium* Linn., shows antioxidant, anti-inflammatory, and antitumor pharmacological effects and has extensive therapeutic capabilities for the treatment of cardiovascular diseases⁴¹, neurodegenerative diseases⁴², and tumors. Icariin can also reduce morbidity in MRL/Lpr mice⁴³.

Icariin Softgel, an original new drug in TCM, was approved for marketing by the State Drug Administration in China on January 10, 2022, for unresectable hepatocellular carcinoma in patients who are unsuitable for or have refused standard treatment and have not previously received systemic therapy. Mechanistically, icariin inhibits the IKK–NF- κ B inflammatory pathway by directly binding to MyD88/IKK α , thereby reducing the production of inflammatory factors, such as TNF- α and IL-6, inhibiting the effects of PD-L1 expression and MDSC, and activating IFN- γ positive CD8⁺ T cells to exert antitumor effects. Icariin can also induce mitochondrial autophagy and synergize with adriamycin to induce immunogenic cell death in hepatocellular carcinoma⁴⁴.

2.1.5. Artemisinin

Artemisinin, a sesquiterpene lactone containing a peroxy-bridge structure, was first discovered by Chinese scientist Youyou Tu in the 1970s, who then pioneered the design of antimalarial drugs with

peroxy-bridges as the active group. In addition to its use as a first-line antimalarial drug, artemisinin has received increasing attention for its other potential pharmacological effects, including antiviral, antifungal, anti-inflammatory, and anticancer activities.

Artemisinin and its derivatives exhibit cytotoxic effects against a variety of cancers, both *in vivo* and *in vitro*, and also play roles in the prevention and treatment of carcinogenesis and tumor metastasis^{45,46}. Wang's team^{47–49} has been researching the antitumor effects of artemisinin and its derivatives for more than ten years, and has elucidated that artemisinin and its derivatives can exert anticancer effects by inhibiting tumor growth and cycle progression, promoting apoptosis of tumor cells, and sensitizing tumor cells to the therapeutic effects of clinical chemotherapeutic and target drugs. Dihydroartemisinin, the main *in vivo* metabolite of artemisinin drugs, is the active form, and it exerts antitumor effects by targeting autophagy and ferroptosis in tumor cells⁵⁰. Wang's team⁵¹ was the first to discover the molecular mechanism by showing that dihydroartemisinin selectively inhibits PDGFR α -positive tumor cell growth, metastasis, and the epithelial–mesenchymal transition by targeting PDGFR α and promoting its ubiquitous degradation, as well as having a co-sensitizing effect on clinical PDGFR α inhibitors.

Youyou Tu's group⁵² also found that dihydroartemisinin is uniquely effective in treating lupus erythematosus. In 2016, Kubicek and colleagues⁵³ demonstrated the efficacy of artemisinin as a diabetes treatment. The ability of artemisinin to achieve α -cell to β -cell transition has identified a new and surprising treatment for type I diabetes. Artemisinin can exert anti-atherosclerosis effects by inhibiting inflammatory responses in macrophages through the AMPK/NF- κ B/NLRP3 signaling pathway⁵⁴. Artemisinin has also displayed great potential in the fight against fibrosis⁵⁵.

2.1.6. Triptolide

Triptolide, an epoxide diterpene lactone compound, is one of the main active ingredients of *Tripterygium wilfordii* Hook. f. (family Euonymusaceae). Triptolide shows the anti-inflammatory and immunosuppressive effects. Tripterygium tablets and tripterygium glycoside tablets are mainly used in the treatment of rheumatoid arthritis.

Triptolide can covalently bind XPB and inhibit its DNA-dependent adenosine triphosphatase (ATPase) activity, thereby inhibiting RNA polymerase II-mediated transcription. This, in turn, leads to the inhibition of cell activation and proliferation and explains its pharmacological activity as well as its extreme cytotoxicity⁵⁶. Shen and colleagues⁵⁷ found that triptolide can target TAB1 in macrophages and modulate inflammatory diseases by regulating the MAPK signaling pathway.

As part of its wide range of antitumor effects, triptolide inhibits the activity of genes such as SLC7A11, inhibits the Nrf2-associated glutathione synthesis pathway, and allows IDH1-mutated cancer cells to die from oxidative stress, thereby offering hope for the treatment of IDH1-mutated cancers, such as glioma, acute myeloid leukemia, and pancreatic cancer⁵⁸. However, its serious systemic toxicity and poor water solubility greatly hinder its clinical application; therefore, improving its solubility and bioavailability and reducing adverse effects are the focus of current research related to its antitumor effects. Hui et al.⁵⁹ developed a pH-sensitive folic acid-coated triptolide nanoform designed to release the drug in the acidic microenvironment of cancer cells. The drug promoted overexpression of the folic acid receptor in some hepatocellular carcinoma cells, which not only significantly inhibited the growth of hepatocellular carcinoma but also

significantly reduced the cytotoxicity to normal hepatocytes. Liu's group⁶⁰ found that glutriptolide 2, a glucose–triptolide conjugate, achieved targeted delivery and showed durable anti-prostate cancer activity. Tan's group⁶¹ coupled triptolide with the amino-modified aptamer AS1411 to obtain an aptamer triptolide conjugate that significantly inhibited the progression of triple negative breast cancer *in vitro* and *in vivo*. This conjugate is expected to become a broad-spectrum and efficient antitumor targeting drug.

2.1.7. Celastrol

Celastrol, another major active ingredient in *T. wilfordii* Hook. F., originates from the root bark and has remarkable anti-rheumatoid, anti-oxidant, analgesic, and anti-tumor effects.

In 2015, Ozcan and colleagues⁶² provided the first demonstration that celastrol is a potentially effective herbal medicine for weight loss. They found that the celastrol-enhanced leptin sensitivity and weight loss effects required IL1R1 mediation, as the absence of IL1R1 completely eliminated the weight loss and antidiabetic effect of celastrol on obese mice⁶³. Zhang's team⁶⁴ found that celastrol was effective in inhibiting high-fat diet-induced weight gain in mice and that the mechanism of action involved binding of celastrol to the orphan nuclear receptor Nur77 in the nucleus and promotion of the selective clearance of damaged mitochondria, with a resulting inhibition of the inflammatory response and obesity. This team subsequently found that celastrol promoted ubiquitination of the LBD structural domain at the C-terminus of Nur77 to interact with the UBA structural domain of the autophagy receptor P62, promoted the size and mobility of the P62 phase-separated particles, and completed the clearance of damaged mitochondria in the lysosome⁶⁵. Celastrol also activates the heat-stimulating factor HSF1, which increases energy expenditure and helps mice resist high-fat diet-induced obesity⁶⁶. Celastrol can antagonize obesity by altering the distribution of intestinal microbiota under a high-fat diet and reducing lipid absorption in the intestine⁶⁷. In addition, celastrol improved metabolism, which may help improve glucose metabolism in obese patients, such as diabetics⁶⁸. Our group⁶⁹ found that celastrol targeted adenyl cyclase associated protein 1 (CAP1) to inhibit macrophage inflammation and improve metabolic syndrome in mice. Liang's team⁷⁰ revealed that celastrol inhibits angiotensin (Ang II)-induced hypertensive heart injury by binding to STAT3, thus exerting a protective effect on heart function. They also found that celastrol could directly bind to and inhibit PRDX2 protein activity, which in turn upregulated oxidative stress levels and finally achieved the effects of killing gastric cancer cells⁷¹. In addition, celastrol also improves liver fibrosis⁷². Celastrol has some limitations in its clinical translational application, such as poor water solubility, short biological half-life, and unknown optimal dose interval; therefore, high-technology research and development is needed on molecular modifications and new dosage forms.

2.1.8. Tanshinone

Tanshinone is a lipophilic diterpenoid isolated from the rhizome of *Salvia miltiorrhiza*. Tanshinone is used in TCM to treat heart disease, stroke, and vascular diseases. Tanshinone IIA, the most studied active component of tanshinone, has anti-inflammatory and antioxidant activities^{73,74}. Clinically, tanshinone IIA sodium sulfonate injection is mainly used for the treatment of coronary heart disease, angina pectoris, myocardial infarction, and premature ventricular contractions.

Renshaw's group⁷⁵ found that tanshinone IIA reduces inflammation by inducing neutrophil apoptosis and promoting the reverse migration of neutrophils, offering hope for inflammatory diseases such as chronic obstructive pulmonary disease. Fan's group⁷⁶ conducted scRNA-seq assays of the hearts of myocardial infarction model mice and found that the administration of tanshinone IIA attenuated myocardial infarction progression by inhibiting early infiltrating macrophage subpopulations. Tanshinone IIA also protected neurons and improved cognitive impairment in patients with Alzheimer's disease^{77,78} and Parkinson's disease^{79,80}. Tanshinone IIA also shows considerable antitumor activity^{81,82}.

2.1.9. Andrographolide

Andrographolide is a well-known natural lactone with a range of pharmacological actions in TCM. Andrographolide is reported to improve radiation-induced pneumonia and pulmonary fibrosis by inhibiting the activation of the AIM2 inflammasome⁸³. Andrographolide improves lipopolysaccharide-induced acute lung injury in mice by inhibiting the NF- κ B pathway⁸⁴. Andrographolide reversed the colitis–colon cancer transformation by inducing mitophagy in macrophages⁸⁵. Andrographolide ameliorated TNBS-induced colitis in mice by inhibiting Th1/Th17-mediated immune response, and it could downregulate P38, MAPK, STAT3, and NF- κ B to improve mouse sepsis⁸⁶. Andrographolide mediated apoptosis by binding Bax to restore the sensitivity of drug-resistant colon cancer cells to 5-fluorouracil⁸⁷. Andrographolide alleviated the symptoms of an *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced mouse Parkinson's model by targeting the mitochondrial division protein DRP1⁸⁸. Andrographolide improved the symptoms of Alzheimer's disease in mice by regulating mitochondrial homeostasis⁸⁹. Andrographolide improved imiquimod-induced psoriasis-like skin inflammation in mice by inducing the autophagic degradation of MyD88⁹⁰. These series of studies provide a theoretical basis for the new use of old drugs in clinical preparations containing andrographolide as the main component. A recent review has systematically summarized the structure–activity relationship analysis, pharmacokinetics, new andrographolide delivery systems, and the protective functions of andrographolide against inflammatory diseases and cancer⁹¹.

2.1.10. Emodin

Emodin is an anthraquinone natural compound extracted from the rhizomes of several Chinese herbs, including *Rheum palmatum* L., *Fallopia multiflora* (Thunb.) Harald., and *Reynoutria japonica* Houtt. In traditional studies, emodin is mostly considered an active ingredient of laxatives, but modern pharmacological studies have found that emodin has significant anti-inflammatory, anti-oxidant, antibacterial, and antitumor effects.

Song et al.⁹² developed ferromagnetic responsive rhodopsin-loaded micelles to realize magnetic resonance imaging (MRI)-guided magnetothermal–chemotherapy combination therapy for malignant tumors. The micelles could target tumor sites under external magnetic field guidance and release emodin to kill significant numbers of tumor cells at very low doses. Emodin can selectively inhibit 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1), which can effectively limit the effect of glucocorticoids and improve diabetes and insulin resistance^{93,94}. Emodin nanoparticles can specifically release emodin in the diseased colon and effectively enhance the anti-colitis effect of emodin related to improving the intestinal wall barrier. Improvements in the therapeutic efficacy and reduction of side effects will make emodin a novel alternative to oral

colon-targeted ulcerative colitis therapy⁹⁵. In addition, emodin could alleviate the progression of cardiac fibrosis⁹⁶.

Emodin also had a highly significant ameliorative effect on neurodegenerative changes in Alzheimer's disease mice, including inhibition of Alzheimer's disease pathology and enhancement of learning memory capacity⁹⁷. An AI-based drug discovery scheme for Alzheimer's disease treatment, which is efficient and feasible, has been proposed to provide rapid development of anti-Alzheimer's disease drugs and represents a major breakthrough in the modernization of TCM research.

2.1.11. Curcumin

Curcumin is an acidic polyphenolic compound extracted from *Curcuma longa* L. and used in Chinese medicine to treat bruises and injuries, canker sores, and pain. Curcumin is the main component of *C. longa* L. and is widely used as a natural pigment in the food industry, TCM, and Indian medicine. Curcumin has antibacterial, antioxidant, anti-inflammatory, analgesic, antitumor, antidiabetic, and antihyperlipidemic activities and is traditionally used in the treatment of liver diseases.

Curcumin can function as an anti-inflammatory agent by blocking NF- κ B and STAT3-mediated inflammatory response signaling pathways, and preventing the development of inflammation-related chronic diseases^{98,99}. Curcumin significantly improves atherosclerosis¹⁰⁰. Curcumin and its metabolites extend the lifespans of nematodes, drosophila, and mice. Curcumin also plays an important role in the prevention and treatment of aging-related diseases¹⁰¹ and it regulates the abundance and composition of gut microbiota and improves intestinal barrier function¹⁰². Banerjee et al.¹⁰³ used X-ray crystallography and a specificity analysis technique with a kinase inhibitor to successfully reveal that curcumin binds and inhibits the kinase dual specific tyrosine regulated kinase 2 (DYRK2), thereby impeding the function of the cellular proteasome, impairing tumor cell proliferation, and inhibiting cancer progression. In addition, curcumin also showed quite good effect in anti-fibrosis¹⁰⁴.

However, the low bioavailability and poor stability of curcumin due to its low solubility have affected its application in the pharmaceutical field. Therefore, in recent years, many studies have combined nanomedicine with curcumin to improve its pharmacological activity. Bao et al.¹⁰⁵ established α -lactalbumin nanotubes that successfully overcame intestinal mucus and cellular barriers to improve the bioavailability of lipid-soluble curcumin and effectively promoted the colonic anti-inflammatory effect of curcumin. Yu's team¹⁰⁶ fabricated nano-inducers composed of curcumin, iron oxide nanoparticles, and organic silica nanoparticles that significantly enhanced intracellular oxidative stress and endoplasmic reticulum stress to induce ICD and systemic antitumor immunity. In addition, gut microbiota can biotransform curcumin by demethylation and hydroxylation to produce derivatives that show improved bioavailability and bioactivity¹⁰².

2.1.12. Epigallocatechin gallate (EGCG)

EGCG is a catechin-like monomer isolated from tea leaves. It is the main component of tea polyphenols and is a well-known antioxidant. EGCG can prolong the lifespan by inducing mitochondrial reactions¹⁰⁷. EGCG cross-linked in chitosan hydrogels has been shown to promote proliferation and remodeling processes, such as regeneration of the epidermis, dermis, and skin attachments, to accelerate skin wound healing¹⁰⁸. EGCG shows neuroprotective effects in Alzheimer's disease and other neurodegenerative disorders by reducing A β its expression and inflammatory

responses^{109,110}. Oral administration of EGCG attenuated ulcerative colitis in mice by regulating gut microbiota¹¹¹. EGCG-targeted action of TAK1 effectively alleviated joint swelling due to rheumatoid arthritis¹¹². Liu et al.¹¹³ discovered a new regulator of cGAS, G3BP1, and showed that EGCG can ultimately inhibit the activation of cGAS by inhibiting the binding of G3BP1 to cGAS. They confirmed its effectiveness in autoimmune animal models and cells of AGS patients and suggested the use of EGCG as a therapeutic strategy option for autoimmune diseases, such as AGS syndrome, which currently lack therapeutic remedies¹¹³.

EGCG has inhibitory effects on a variety of tumors. EGCG reduces the risk of pancreatic cancer by inhibiting the activity of LDHA, thereby altering the metabolism of pancreatic cancer cells¹¹⁴. EGCG can inhibit tongue cancer progression in K-Ras transgenic mice by targeting the Notch pathway¹¹⁵. EGCG also acts as a drug delivery system to protect protein drugs from degradation while synergistically exerting its own antitumor effects¹¹⁶. Wang's group¹¹⁷ was the first to report that EGCG can directly interact with and cause conformational changes in P53 protein that disrupt its interaction with P53-MDM2. This promotes the apoptosis of cancer cells and provides a new concept of nutritional intervention in disease using dietary polyphenols¹¹⁷.

In addition, Wang et al.¹¹⁸ found that the natural glycoside product ginsenoside Rh2 could activate the pentose phosphate pathway after cancer pretreatment discontinuation to improve redox disorders in tumor cells. It also enhanced the antitumor effects of adriamycin by further inhibiting the growth of ovarian cancer spheroids¹¹⁸. Cheng's team¹¹⁹ found that the antitumor activity was much stronger for the rare ginsenoside protopanaxadiol (PPD) than for other common ginsenosides, making this a potentially more potent anticancer drug component. Ginseng polysaccharides enhanced the effect of anti-PD-1 monoclonal antibodies in improving lung cancer and modulated immunity by regulating the composition of gut microbiota and metabolites, which in turn enhanced responses to immunotherapy¹²⁰. Ginseng extract enriched the intestinal microbiota with *Escherichia faecalis*, stimulated the thermogenic activity of brown adipose tissue, and induced the formation of beige adipose tissue to reduce fat accumulation and obesity¹²¹. The role of natural products in tumor, represented by paclitaxel and camptothecin, has been described in detail in the review by Huang et al.¹²². We will not reiterate them here. The discovery time and main efficacy of typical natural products from plants are shown in Fig. 2. The relationship between natural products and disease treatment mentioned in above are shown in Table 1.

2.2. Animal natural products

Animal medicinal compounds are indispensable and important components of TCM. According to Chinese medicine, animal medicine is a "flesh and blood sentient product, which is able to run and pass, attack poison and dispel evil". Animal-derived medicines have unique therapeutic effects on many difficult and miscellaneous diseases, and their clinical value is irreplaceable. The following is an example of the current research progress in animal medicines as treatments for inflammatory diseases and cancer.

Toad venom is the dried secretion from the skin glands of *Bufo bufo gargarizans* Cantor or *Bufo melanostictus* Schneider. In TCM practice, toad venom is considered an anti-infectious agent for the treatment of pyogenic infection-induced unconsciousness and is prescribed to patients with "heat and toxins" syndrome, which has

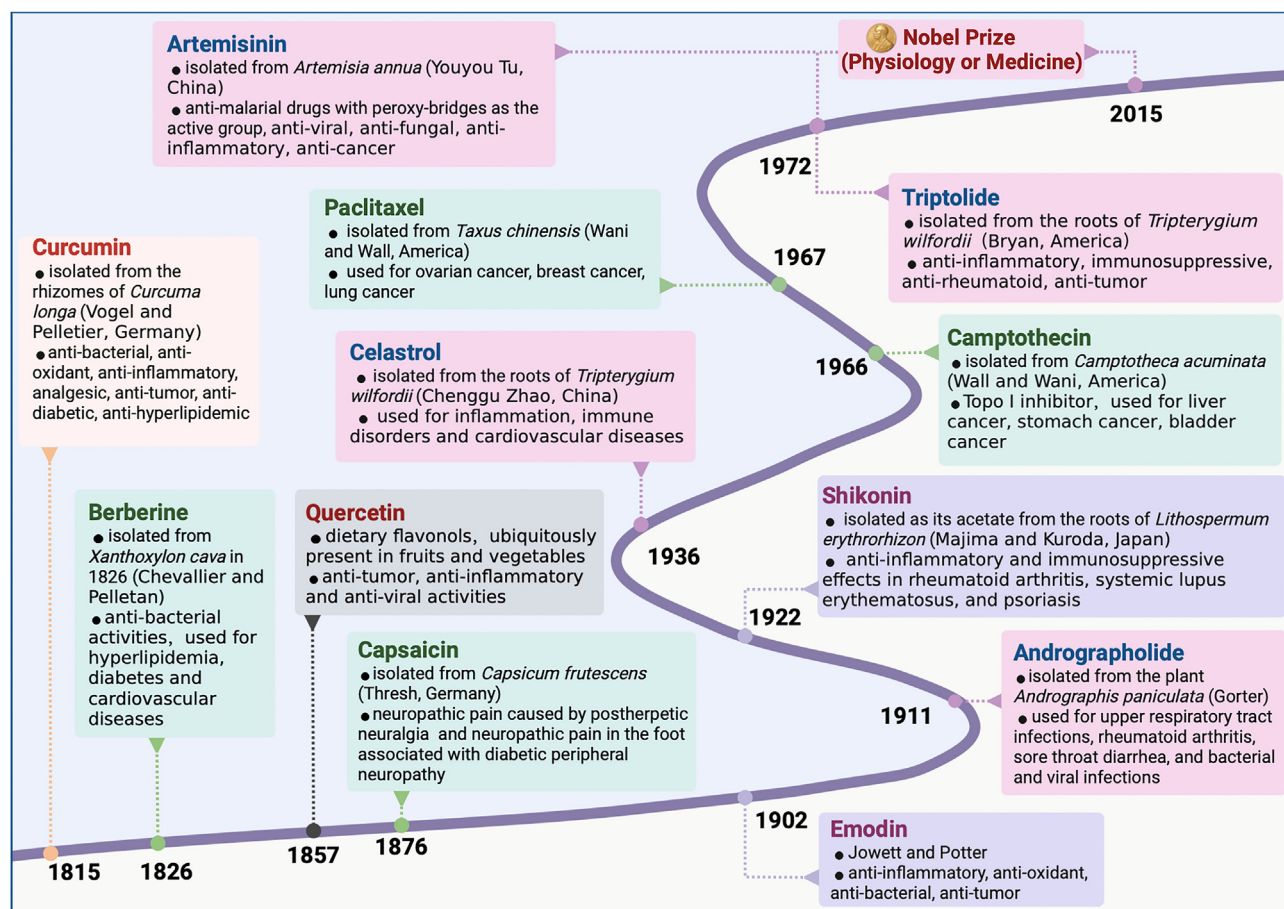


Figure 2 Milestones in the discovery of typical natural products for the treatment of inflammatory diseases and cancer. This figure is created with biorender.com.

Table 1 Types of diseases treated by typical natural products.

Natural product	Disease type
Berberine	Gastrointestinal diseases ¹²³ , hypoglycemia ³ , atherosclerosis ¹²⁴ , diabetes ¹²⁵ , cardiovascular diseases ² , Parkinson's disease ⁴ , ischemic stroke ⁷ , peritoneal adhesions ⁸ , nonalcoholic fatty liver ⁹ , oral diseases ¹⁰ , lung cancer ¹²⁶ , breast cancer ¹²⁶ , liver cancer ¹²⁶ , colon cancer ¹²⁷
Capsaicin	Neuropathic pain ¹⁴ , cardiovascular diseases ¹⁸ , obesity ²² , diabetes ²³ , nasal mucosa ²⁴ , hematologic tumors ²⁷ , Lassa hemorrhagic fever ¹²⁸
Quercetin	Aging ³⁴ , non-alcoholic fatty liver disease ³⁶ , hepatocellular carcinoma ³⁷ , bladder cancer ³⁸ , gastric cancer ³⁹ , colorectal cancer ⁴⁰
Icariin	Cardiovascular diseases ⁴¹ , neurodegenerative diseases ⁴² , hepatocellular carcinoma ⁴⁴
Artemisinin	Lupus erythematosus ⁵² , diabetes ⁵³ , atherosclerosis ⁵⁴ , fibrosis ⁵⁵ , breast cancer ¹²⁹
Triptolide	Rheumatoid arthritis ¹³⁰ , IDH1-mutated cancers ⁵⁸ , hepatocellular carcinoma ¹³¹ , prostate cancer ⁶⁰ , breast cancer ⁶¹
Celastrol	Rheumatoid arthritis ¹³² , obesity ⁶² , diabetes ⁶⁸ , metabolic syndrome ⁶⁹ , hypertensive heart injury ⁷⁰ , gastric cancer ⁷¹ , liver fibrosis ⁷² , psoriasis ¹³³
Tanshinone	Coronary heart disease ¹³⁴ , angina pectoris ¹³⁴ , myocardial infarction ⁷⁶ , chronic obstructive pulmonary disease ⁷⁵ , myocardial infarction ⁷⁶ , Alzheimer's disease ⁷⁷ , Parkinson's disease ⁷⁹
Andrographolide	Pulmonary fibrosis ⁸³ , acute lung injury ⁸⁴ , colitis ⁸⁴ , colon cancer ⁸⁷ , Parkinson's disease ⁸⁸ , Alzheimer's disease ⁸⁹ , psoriasis ⁹⁰
Emodin	Malignant tumor ⁹² , diabetes ⁹³ , ulcerative colitis ⁹⁵ , cardiac fibrosis ⁹⁶ , Alzheimer's disease ⁹⁷
Curcumin	Atherosclerosis ¹⁰⁰ , aging-related diseases ¹⁰¹ , cancer progression ¹⁰³ , fibrosis ¹⁰⁴ , colitis ¹⁰⁵
EGCG	Alzheimer's disease ¹⁰⁹ , ulcerative colitis ¹¹¹ , rheumatoid arthritis ¹¹² , pancreatic cancer ¹¹⁴ , tongue cancer ¹¹⁵
Shikonin	Rheumatoid arthritis ¹³⁵ , psoriasis ¹³⁶ , bladder cancer ¹³⁷ , pancreatic cancer ¹³⁸ , pulmonary hypertension ¹³⁹ , hypertrophic scars ¹⁴⁰
Campthothecin	Liver cancer ¹⁴¹ , breast cancer ¹⁴² , bladder cancer ¹⁴³
Paclitaxel	Ovarian cancer ¹⁴⁴ , breast cancer ¹⁴⁵ , lung cancer ¹⁴⁶

cancer-like symptoms. Numerous pharmacological studies have revealed that the anti-inflammatory and antineoplastic effects of toad venom were due to its content of bioactive steroidal cardiac glycosides, called bufadienolides, including bufalin, cinobufagin, and gamabufotalin¹⁴⁷.

The typical antitumor mechanisms included induction of apoptosis and proliferation through targeting of the IKK β /NF- κ B/COX-2 signaling pathway¹⁴⁸, the AKT/mTOR pathway¹⁴⁹ or Notch signaling pathway¹⁵⁰, induction of G0/G1 or G2/M cell cycle arrest through HIF-1 α - and NF- κ B-mediated Plk1^{151,152}, inhibition of invasion and metastasis through cortactin expression and nuclear translocation¹⁵³ or RIP3-mediated necroptosis¹⁵⁴, and reversal of multi-drug resistance by regulation of P-glycoprotein (P-gp)¹⁵⁵, as demonstrated *in vitro* or *in vivo* in homograft/xenograft tumor models in mice. Several findings have also provided evidence that cardiac glycosides, including bufalin, can exert potent antineoplastic effects by targeting the Na⁺/K⁺-ATPase, hence the intracellular accumulation of Ca²⁺ ions¹⁵⁶, and they appear to increase the immunogenicity of dying cancer cells¹⁵⁷. Recent studies have indicated that AHSA1, identified as the targeted protein of bufalin, acts as a co-chaperone of HSP90A to activate CDK6 and PSMD2, thereby regulating multiple myeloma proliferation and proteasome inhibitor resistance, respectively¹⁵⁸. Yang et al.¹⁵⁹ found that bufalin directly targeted Syndecan-4 and increased its interaction with substrate protein DEAD-box helicase 23 to inhibit the progression of hepatocellular carcinoma. Other mechanisms associated with the anti-inflammation action of bufadienolides have included modulation of NF- κ B signaling¹⁶⁰ and enhancement of immune responses¹⁶¹.

2.3. Marine natural products

Oceans cover more than 70% of the earth's surface and were the origin of life on the planet. Since 2008, more than 1000 new marine natural products (MNPs) had been found annually, and approximately 30% of them have bioactive properties¹⁶². Marine microorganisms have evolved special metabolic pathways due to long-term adaptation to special living environments, resulting in a large number of active substances with novel structures and unique functions, making natural products derived from marine microorganisms a hot spot in the development of new marine drugs. However, a relatively low number of MNPs have been approved in the clinical setting. Here, we provide an overview of the currently approved MNPs.

The marine natural nucleosides cytarabine (Ara-C; originally isolated from the Caribbean sponge *Cryptotheca crypta*) and vidarabine (Ara-A; originally isolated from the Caribbean sponge *Tethya crypta*) are well known as the first FDA-approved MNPs for anticancer treatment (in 1969) and antiviral treatment (in 1976), respectively^{163–166}. Ara-C and Ara-A work as inhibitors of tumor cell or viral DNA synthesis and replication¹⁶⁷. Ziconotide (originally isolated from the venom of a marine snail) is a novel powerful antinociceptive drug that acts as a specific calcium channel blocker for the treatment of severe chronic pain, especially in patients refractory to opioids, but it still has the potential for systemic and central nervous system side effects^{168,169}. Eribulin (originally isolated from the natural Japanese marine sponge *Halichondria okada*) is a macrocyclic ketone analog that acts as an anticancer drug by inducing irreversible mitotic blockade and is now used to treat people with locally advanced or metastatic breast cancer or unresectable liposarcoma¹⁷⁰. Dolastatins (originally isolated from the sea hare *Dolabella uricularia*) are broad-

spectrum cytotoxic anticancer pentapeptides that can impede tubulin assembly and induce cell apoptosis and are widely used in the treatment of lymphoma, and other carcinomas^{171,172}. However, according to the strong adverse reactions observed in preclinical toxicology research, dolastatins are now used as payloads for antibody–drug conjugates (ADCs)^{173,174}. ADC technology is based on the idea that the linking of a cytotoxic drug to a monoclonal antibody specific for antigens of cancer cells can deliver high doses of the cytotoxic drug specifically to cancer cells while sparing normal tissues¹⁷⁵. Brentuximab vedotin is a CD30-directed ADC that consists of a human-specific CD30 antibody and the microtubule-disrupting agent monomethyl auristatin E (MMAE; a synthetic analog of the naturally occurring dolastatin 10) and is now used as a lymphoma treatment^{176,177}. Cephalosporin C, the best known MNP, is a β -lactam type natural antibiotic derived from marine fungi and is widely used to treat bacterial infections by disrupting the synthesis of the peptidoglycan layer that forms the bacterial cell wall¹⁷⁸. Omega-3 fatty acid, another well-known MNP, was originally derived from fish and fish oils in 1929. Dietary consumption of omega-3 fatty acids reduces the incidence of cardiovascular disease, osteoarthritis, and rheumatoid arthritis. Dietary supplementation with omega-3 fatty acids provides antioxidant activity by regulating the antioxidant signaling pathway and may modulate inflammatory processes¹⁷⁹.

2.4. Mineral natural products

As one of the important components of TCM, mineral medicine has a long history and abundant resources. The earliest extant pharmacological work in China, the *Shennong Ben Cao Jing*, contains a total of 41 mineral drugs. Here, we use arsenic as an example to introduce the pharmacological effects of mineral drugs in diseases.

The arsenic-containing compound realgar (mainly As₄S₄) is a highly recognized and widely used TCM. Although realgar minerals contain large amounts of arsenic, the toxicity related to their structures is far less than that observed for other compounds, such as arsenolite (which contains arsenic trioxide, As₂O₃) and arsenite (NaAsO₂)^{180,181}. Realgar is widely used in prescriptions for treating infectious inflammation symptoms, ranging from tonsillitis to delirium and allergy¹⁸². Several recent clinical studies have revealed that realgar provided therapeutic benefits as a cancer treatment, especially for adult and pediatric acute promyelocytic leukemia (APL), where encouraging responses were obtained, including a high complete remission rate, long disease-free survival period, and tolerable side effects^{183–185}.

Numerous *in vitro* and *in vivo* pharmacological studies have demonstrated the anti-inflammation and antitumor mechanisms of realgar and realgar-containing preparations. Realgar has been reported to induce G2/M phase arrest, apoptosis, and autophagy in osteosarcoma through mechanisms related to the activation of the ROS/JNK and suppression of the Akt/mTOR signaling pathways¹⁸⁶. Other studies have shown that realgar preparations inhibited breast cancer through downregulation of HIF-1 α expression *via* the PI3K/Akt/mTOR pathway¹⁸⁷ and reversed drug resistance by degrading the BCR–ABL fusion oncoprotein¹⁸⁸. Several studies^{189–191} identified potential binding proteins of realgar in rat metabolism through interactions with sulfhydryl groups in specific proteins, such as pyruvate dehydrogenase, thioredoxin, DNA repair enzymes, and metallothionein, and suggested a high relationship with realgar anti-inflammation activity.

3. Mechanisms of natural products in inflammatory diseases

Exploration of the recent research progress in the use of natural products in inflammatory diseases has revealed three main categories of common mechanisms: inflammation-related signaling pathways, programmed cell death, and gut microbiota (Fig. 3).

The inflammation-related signaling pathways, such as MAPK, PI3K/AKT, NF- κ B, and JAK/STAT, can transduce extracellular signals into cells and conduct cellular signals through cascade reactions to regulate cell proliferation, differentiation, and migration as well as inflammatory responses and vascular development¹⁹².

Programmed cell death can occur by apoptosis, ferroptosis, pyroptosis, or autophagy¹⁹³ and describes the active extinction of cell responses to stimulation by certain signals or factors to maintain the stability of the internal environment. Programmed cell death removes unwanted cells, as well as infected or potentially tumorigenic cells, so it plays an important role in homeostasis, host defenses against pathogens, cancer, and a range of other pathologies. Apoptosis is a relatively “mild” form of cell death and generally does not elicit an immune or inflammatory response. Many natural products exert their antitumor effects by promoting apoptosis, mainly mediated by apoptotic caspases (caspase-2, 3, 6, 7, 8, 9, and

10). Pyroptosis is involved in the body’s defense against pathogenic bacteria, occurs more rapidly, is accompanied by the release of large amounts of pro-inflammatory factors, and is mainly induced by inflammatory caspases (caspase-1, 4, 5, and 11). Ferroptosis is an iron-dependent, non-apoptotic, oxidative form of cell death caused by the failure or blockage of cellular glutathione-dependent anti-oxidant defenses. This leads to uninhibited lipid peroxidation and ultimately kills cells; consequently, inhibition of iron-related death by natural products has great potential in the treatment of tumors, diabetes, ischemic organ damage, and degenerative diseases associated with lipid peroxidation. Autophagy, the process by which cellular cargoes are transported to lysosomes and degraded, removes functionally abnormal intracellular proteins, organs, and microorganisms under normal conditions and is essential for maintaining cellular, tissue, and organ homeostasis. Autophagy is tightly regulated by autophagy-related genes, and mutations in these genes can induce a range of diseases, including neurodegenerative diseases, inflammation, and even cancer.

The gut microbiota represents the second largest genome in the body and is involved in a variety of physiological functions in the liver, intestines, brain, and other organs. Imbalance of the gut microbiota is associated with most diseases in the body, so the

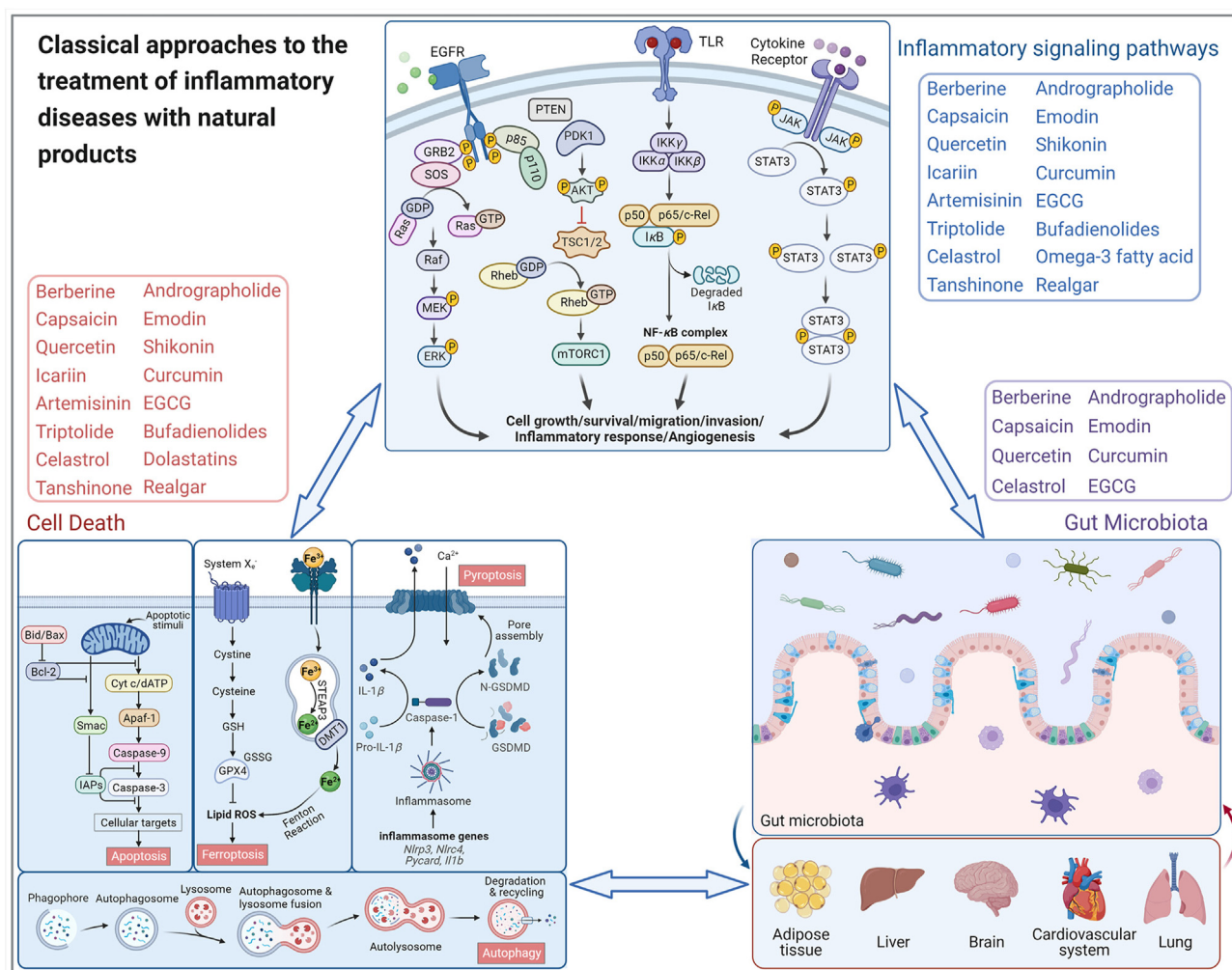


Figure 3 Molecular mechanisms of natural products ameliorating inflammatory diseases and cancer through inducing cell death, inhibiting inflammatory signaling and affecting gut microbiota. This figure is created with [biorender.com](https://www.biorender.com).

study of the gut microbiota has become a hot research topic in the field of Chinese medicine in recent years. Gut microbe interactions fit with the theory of TCM, but they also represent one of the important ways by which orally administered TCM can exert its medicinal effects¹⁹⁴. A variety of gut microbes, especially *Bacillus* spp., *Bifidobacterium* spp., and *Lactobacillus* spp., can biotransform herbal components to improve the bioavailability and bioactivity of some difficult-to-absorb natural drug components and provide a theoretical basis for their remarkable therapeutic effects. The combination of gut microbiota with TCM can help modernize TCM and rejuvenate traditional medicine.

Active TCM ingredients show significant anti-inflammation and anti-tumor activities *in vitro*, but in clinical practice, TCM is usually supplied as compound prescriptions. We also systemically retrieved literature for 106 Chinese compound prescription preparations (Table 2) using preparation names as keywords and searching different electronic databases, including PubMed (<https://pubmed.ncbi.nlm.nih.gov>), Web of Science (<https://www.webofscience.com/wos>), Chinese National Knowledge Infrastructure (CNKI, <https://www.cnki.net>), the Wanfang Database (<https://new.wanfangdata.com.cn>), and Pharmacopoeia of China. Here, we reorganized and evaluated the literature on 40 inflammatory diseases and cancer from five aspects: animal experiments, retrospective or real-

world experiments, clinical randomized controlled trials, historical human usage, and treatment guideline inclusions or recommendations (Table 3). We graded the retrieved articles for quality and quantity, guideline inclusions or recommendation levels, and history of human use, and drew heatmaps according to the disease scores (Fig. 4). Generally, the preparations, and especially medicines for cardiovascular and cerebrovascular diseases, have been widely studied in inflammatory diseases. In the clinically related literature, retrospective or real-world experiments have been common, whereas few randomized controlled trials were conducted for TCM preparations. The combination of multiple ingredients in prescribed herbal medicinal compounds is consistent with the TCM concept and occurs widely in numerous preparations. In actual use, the key ingredients are likely to provide therapeutic effects, while the ancillary constituents might assist in dissolution or absorption.

4. Target identification and validation of natural products

Natural products are an important source of new compounds for drug research and development³⁰⁰. At present, a considerable proportion of clinical drugs are directly or indirectly derived from natural drugs. A drug target is defined as a specific molecule in the human body that interacts with a given drug and confers its

Table 2 Large varieties of TCM for treatment of inflammatory diseases and cancer.

Category	TCM
Medicines for cardiovascular and cerebrovascular diseases	Danhong Injection ¹³⁴ , Danshen Injection ¹⁹⁵ , Shenfu Injection ¹⁹⁶ , Shenmai Injection ¹⁹⁷ , Pulse-activating Injection ¹⁹⁸ , Panax Notoginseng Saponins ¹⁹⁹ , Breviscapine Injection ²⁰⁰ , Erigeron asarum Injection ²⁰¹ , Safflower Injection ²⁰² , Ginkgo Biloba ²⁰³ , Bitter Dish Injection ²⁰⁴ , Shuxuetong Injection ²⁰⁵ , Mailuoning Injection ²⁰⁶ , Xingnaojing Injection ²⁰⁷ , Compound Danshen Dropping Pill ²⁰⁸ , Shensong Yangxin Capsule ²⁰⁹ , Naointong Capsule ²¹⁰ , Xueshuoxinmaining Tablet ²¹¹ , Yixinshu Capsule ²¹² , Yangxin's Tablet ²¹³ , Heart Comfort Tablet ²¹⁴ , Xinyuan Capsule ²¹⁵ , Shexiang Baoxin Pill ²¹⁶ , Qi-Shen-Yi-Qi Dripping Pill ²¹⁷ , Sanqi Tongshu Capsule ²¹⁸ , Yindanxin Naotong Soft Capsule ²¹⁹ , Xinnaoning Capsule ²²⁰ , Suxiao Jiuxin Pill ²²¹ , Huatuo Zaizao Pill ²²² , Zhenyuan Capsule ²²³ , Xuefuzhuyu Capsule ²²⁴
Medicines for cardiac and metabolic diseases	Wenxin Granule ²²⁵ , Songlingxuemaikang Capsule ²²⁶ , Hedan Tablet ²²⁷ , Qiliqiangxin Capsule ²²⁸ , Qizhijiangtang Capsule ²²⁹
Medicines for digestive system diseases	Hugan Tablet (HGP) ²³⁰ , Xiaochaihu Granule ²³¹ , weifuchun Tablet ²³² , Simotang Oral Liquid ²³³ , Weisu Granule ²³⁴ , Jianweixiaoshi Tablet ²³⁵ , Huoxiangzhengqi Liquid ²³⁶ , Sanjiuweitai Granule ²³⁷
Medicines for nervous system diseases	Angong Niu Huang Pill ²³⁸ , Yangxueqingnao Granule ²³⁹ , Tongtian Oral Liquid ²⁴⁰ , Zhengtian Pill ²⁴¹ , Tianshu Capsule ²⁴² , Gastrodin Injection ²⁴³ , Zhennaoning Capsule ²⁴⁴ , Jiyeu Pill ²⁴⁵
Medicines for respiratory system diseases	Qingkailing Injection ²⁴⁶ , Reduning Injection ²⁴⁷ , Lianhua Qingwen Capsule ²⁴⁸ , Shuanghuanglian Injection ²⁴⁹ , Tanreqing Injection ²⁵⁰ , Xiyanping Injection ²⁵¹ , Antiviral Oral Liquid ²⁵² , Shufengjiedu Capsule ²⁵³ , Suhuangzhike Capsule ²⁵⁴
Medicines for urinary system diseases	Shenkang Injection ²⁵⁵ , NiaoDuQing Granule/Uremic Clearance Granule ²⁵⁶ , Shenyan Kangfu Tablet ²⁵⁷ , Qianliexin Capsule ²⁵⁸ , Yinhua Miyanning Tablet ²⁵⁹
Tonic medicines	Fufang E'jiao Syrup ²⁶⁰ , Yixuesheng Capsule ²⁶¹ , Liuwei Dihuang Pill ²⁶² , Huangqijing Oral Liquid ²⁶³ , Shenqifuzheng Injection ²⁶⁴
Medicines for tumor	Compound Kushen Injection ²⁶⁵ , Aidi Injection ²⁶⁶ , Shenyi Capsule ²⁶⁷ , Kang-ai Injection ²⁶⁸ , Brucea Javanica Oil Emulsion ²⁶⁹ , Cinobufacini Injection ²⁷⁰ , Compound Huangdai Tablet ²⁷¹ , Pingxiao Capsule ²⁷²
Medicines for skeletal musculature diseases	Duyiwei Capsule ²⁷³ , Xiaotong Plaster ²⁷⁴ , Tongluo Qutong Plaster ²⁷⁵ , Shangke Jiegu Tablet ²⁷⁶ , Huoxue Zhitong Capsule ²⁷⁷ , Wangbi Tablet ²⁷⁸ , Xianling Gubao Capsule ²⁷⁹ , Yunnan Baiyao Capsule ²⁸⁰
Medicines for anorectal and dermatologic diseases	Musk Hemorrhoids Ointment ²⁸¹ , Shexiang Hemorrhoid Suppository ²⁸² , Menthacamphorate Emulsion ²⁸³ , Compound Cortex Phellodendri Fluid ²⁸⁴
Medicines for gynecology diseases	Guizhifuling Capsule ²⁸⁵ , Baofukang Suppository ²⁸⁶ , Kangfute Suppository ²⁸⁷ , Fukeqianjin Tablet ²⁸⁸ , Bujibai Feng Pill ²⁸⁹
Medicines for ophthalmology and otorhinolaryngology diseases	Qingyan Pill ²⁹⁰ , Fufang Caoshanhu Tablet ²⁹¹ , Biyan Kang Tablet ²⁹² , Liushen Pill ²⁹³
Clearing heat and detoxication medicines	Xinhuang Tablet ²⁹⁴ , Pien Tze Huang ²⁹⁵ , Yiqing Capsule ²⁹⁶ , Niu Huang Qingxin Pill ²⁹⁷ , Xuebijing Injection ²⁹⁸ , Dihydroartemisinin Preparations ²⁹⁹

Table 3 Descriptions of score calculated formula.

Category	Aspects	Grade	Score
Evaluation aspect	(a) Animal experiment	—	15
	(b) Retrospective or real-word experiment	—	25
	(c) Clinical randomized controlled trial	—	35
	(d) Human using history	—	15
	(e) Treatment guideline inclusions or recommendation	—	10
Evaluation index	(m) Article quantity	(1) Very few (1–2 articles)	0.2
		(2) Few (3–4 articles)	0.4
		(3) General (5–6 articles)	0.6
		(4) Much (7–8 articles)	0.8
		(5) Very much (more than 8 articles)	1.0
	(n) Article quality	(1) Chinese general periodical	0.4
		(2) Chinese core periodical	0.6
		(3) Science citation index (SCI) included journal	0.8
		(4) Very famous work (including impact factor > 7)	1.0
	(x) Human using history	(1) Modern preparation (<15 years)	0.5
		(2) Shorter history preparation (15–50 years)	0.8
		(3) Long history preparation (>100 years)	1.0
	(y) Guideline inclusion or recommendation level	(1) Recommendation	1.0
		(2) No recommendation	0.0
Computational formula	$(a*m + a*n + b*m + b*n + c*m + c*n)/2 + d*x + c*y$		

effects. Natural products with clear targets are not only conducive to clinical observation of drug metabolism but also further the exploration of mechanisms in related basic research fields. In addition, for natural small-molecule drugs with clear targets, the *in vivo* signal response pathways can be predicted. Therefore, by using appropriate antagonists and adjuvants, the associated pathways that induce adverse reactions can be inhibited, thereby enhancing the pharmacodynamic pathways and reducing the drug's side effects.

The identification of drug targets and related research has important theoretical significance and practical value in the field of pharmaceutical research. The discovery of new targets of natural active small molecules will also open up a broad research space for the treatment of related diseases. At present, the identification of natural product targets is becoming increasingly important in the biomedical field, and the identification methods are also diverse, each with its own advantages and disadvantages³⁰¹. The current identification methods can be used both independently and complementarily, and their combined use may be more conducive to the identification of natural product targets.

4.1. Methods for target identification of natural products

4.1.1. Natural product-centric target identification strategies

The method of coupling molecules with affinity probes is one of the main methods for discovering and identifying drug targets. The small molecule probe is mainly composed of a reporter group, a linking group, and an active group. The principle of action is that the active group part of the small molecule will tightly bind to its target, while the reporter group part can effectively label the target biological macromolecules. The targets can then be confirmed by a number of methods, such as chromatography, gel electrophoresis, and mass spectrometry (Fig. 5).

4.1.1.1. Covalent binding to affinity magnetic beads. Li et al.⁵³ coupled artemisinin to a solid support and performed pull-down experiments in the presence and absence of a competing free artemether and identified gephyrin as the most significantly

enriched specific interacting protein by mass spectrometry. They showed that gephyrin is the mammalian target of this antimalarial drug and that the mechanism depends on the enhancement of GABAA receptor signaling. Zhang's group³⁰² studied the anti-inflammatory effect and target protein of the natural sesquiterpene lactone IJ-5, which reacts with epoxy-activated Sepharose 6 B beads. They used the active hydroxyl group in the molecular structure of IJ-5 to form a covalent bond, and then bonded the IJ-5 molecule to the surface of a solid support. The interaction between IJ-5 and the target protein then identified the target protein as the ubiquitin ligase Ubch5. Further research determined that IJ-5 preferentially binds to its target protein through the active site cysteine by forming a covalent adduct. This prevents ubiquitin molecules from binding to Ubch5, thereby inhibiting the activation of NF-κB inflammatory signaling pathway and resulting in the observed anti-inflammatory effects³⁰².

4.1.1.2. Biotin modification. The biotin affinity purification system is one of the most commonly used schemes, mainly because the avidin protein has a high ability to recognize its substrate biotin, and the binding energy of the two is almost as close as that of a covalent bond. Labeling a small molecule with biotin as a purification tag and then immobilizing avidin on a matrix as a purification matrix allows isolation of the probe-bound protein complex from a complex mixture for analysis and identification. Lei's group³⁰³ modified the structure of the natural anti-inflammatory active molecule ainsliadimer A by introducing biotin long-chain molecules to its hydroxyl group. They used the resulting biotin-ainsliadimer A and identified the target protein of its anti-inflammatory activity as IκB kinase (IKKα/β). Ainsliadimer A can selectively form a covalent bond with cysteine 46 of IKKα/β, thereby inhibiting the activity of IKKα/β and down-regulating the NF-κB inflammatory signaling pathway to achieve anti-inflammatory effects. Liu et al.³⁰⁴ reported that adenanthin, a diterpenoid isolated from the leaves of *Rabdosia adenantha*, induces the differentiation of acute promyelocytic leukemia (APL) cells. Using biotin-tagged adenanthin, they found that adenanthin directly binds to the conserved cysteines of Prx I and Prx II and



transformed the key active component of TCM sappanone A into a chemical probe, and used the reverse drug targeting strategy to “target fishing” drug target IMPDH2 in cells, which explained the molecular mechanism of its anti-inflammatory effect from the

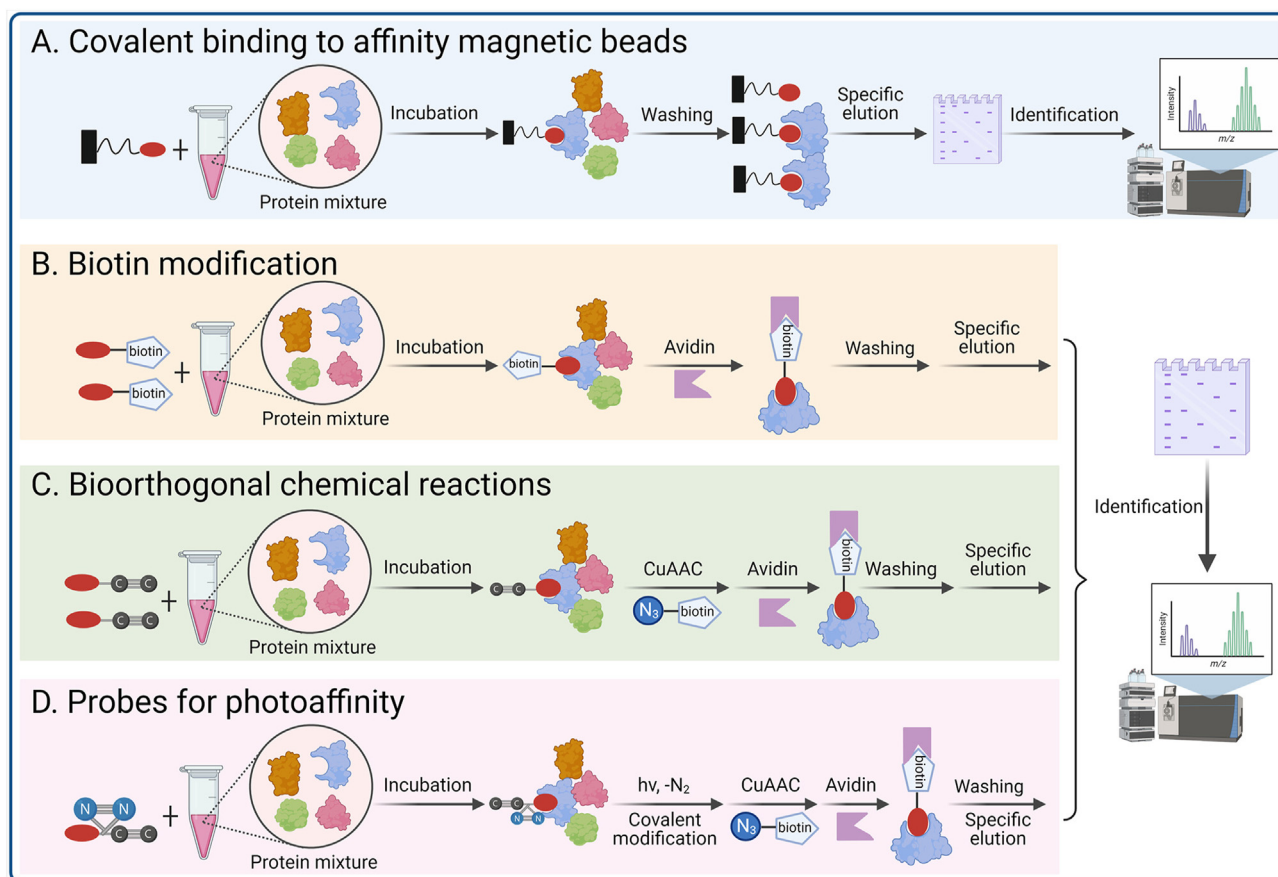


Figure 5 Natural product-centric target identification strategies. (A) Covalent binding to affinity magnetic beads; (B) Biotin modification; (C) Bioorthogonal chemical reactions; (D) Probes for photoaffinity groups. This figure is created with [biorender.com](https://www.biorender.com).

cellular and molecular level, and laid a theoretical foundation for the international promotion of TCM.

4.1.1.3. Bioorthogonal chemical reactions. Introducing affinity tags onto small-molecule compounds by derivatization is a very challenging task. For some compounds, the introduction of sterically hindered affinity tags easily leads to a loss of activity of the compounds. Fortunately, these problems can be resolved by bioorthogonal chemistry, such as Cu-catalyzed click reactions. Wang et al.³⁰⁶ described a novel approach that combined isobaric tags for the relative and absolute quantitation with clickable activity-based protein profiling to identify the targets of andrographolide, a natural product with known anti-inflammation and anticancer effects. They identified a series of specific targets for andrographolide, further deepening the understanding of the drug's mechanism of action.

4.1.1.4. Probes for photoaffinity groups. Most biologically active compounds usually bind with their respective target proteins through non-covalent interactions, which have a certain instability and are inconvenient to study. The introduction of photoactive groups enables the complex that forms based on a non-covalent interaction to undergo covalent cross-linking under light excitation and be transformed into a strong covalent conjugate, thereby improving the detection limit of the target protein.

Dai et al.³⁰⁷ discovered that baicalin acts as a natural allosteric activator of carnitine palmitoyltransferase 1 (CPT1), the rate-limiting enzyme in the fatty acid β -oxidation (FAO) pathway. They designed

and synthesized a photoaffinity probe for baicalin and found that baicalin directly binds to CPT1 and activates it to accelerate fatty acid degradation. Their study provided a mechanism that would explain the biological activity of baicalin, namely, its ability to reduce lipid accumulation. Matrine is a plant alkaloid that has shown potent anticancer activity, but with unknown molecular targets Wang et al.³⁰⁸, using a photoaffinity labeling approach, have recently identified annexin A2 as a direct binding target of matrine in cancer cells.

Despite the feasibility and effectiveness of the classical compound-centric design of molecular probes, the probes have certain limitations: (1) proteins with low intracellular abundance or that bind only weakly to the probes are difficult to identify; (2) nonspecific binding of the probes to protein impurities has a certain interference effect; (3) the structure-activity analyses of the compound are required, as the synthesized probe molecule should maintain its original biological activity and mechanism of action; and (4) connection of the compound to the linking chain and the reporter group usually requires the introduction of functional groups, such as amino, hydroxyl and carboxyl groups, to the core of the compound, and this may affect the activity of the compound³⁰⁹.

4.1.2. Label-free proteomics to identify the targets of natural products

Identifying the target proteins of small-molecule drugs is crucial to understanding the mechanism of action of drugs. Methods based on chemical modifications have certain limitations, all of which require

labeling or derivatization of small-molecule drugs, which may lack sites for covalent cross-linking, or chemical modifications. Therefore, the establishment of screening technology for small molecule drug target proteins without the need for chemical modifications is very important. At present, a variety of label-free methods have been developed to identify drug targets (Fig. 6).

4.1.2.1. Drug affinity responsive target stability (DARTS). In 2009, Lomenick et al.^{310–312} established the DARTS technology based on the principle that proteins are protected from protease degradation after binding to their ligands. The main strategy involves incubation of the small molecule drug with the sample protein for a certain time, and then adding protease for digestion. Because the small molecule drug can protect its target protein after binding to its target, the sensitivity of the target protein to proteases is reduced.

Therefore, after electrophoresis gel staining, a comparison of the digested proteins in the absence and presence of the drug allows identification of the protected band, and the target protein can then be further identified by mass spectrometry.

The advantage of the DARTS method is that it does not require any chemical modification during the experiment, and it can theoretically be used for the interaction screening of any small molecule and its target protein. Using DARTS, Lomenick's group³¹² successfully identified the eIF4A protein as the target of resveratrol, a common plant natural product. Their findings pointed to eIF4A as a previously uncharacterized drug target for antiaging treatments. Zeng's group³¹³ used Pull-down assay and DARTS assay to identify the ATP6V0D1 subunit in V-ATPase as the direct cellular target of natural small-molecule schisandrol A (SolA). SolA is significantly protective against AGEs-induced neuronal apoptosis by allosterically mediating

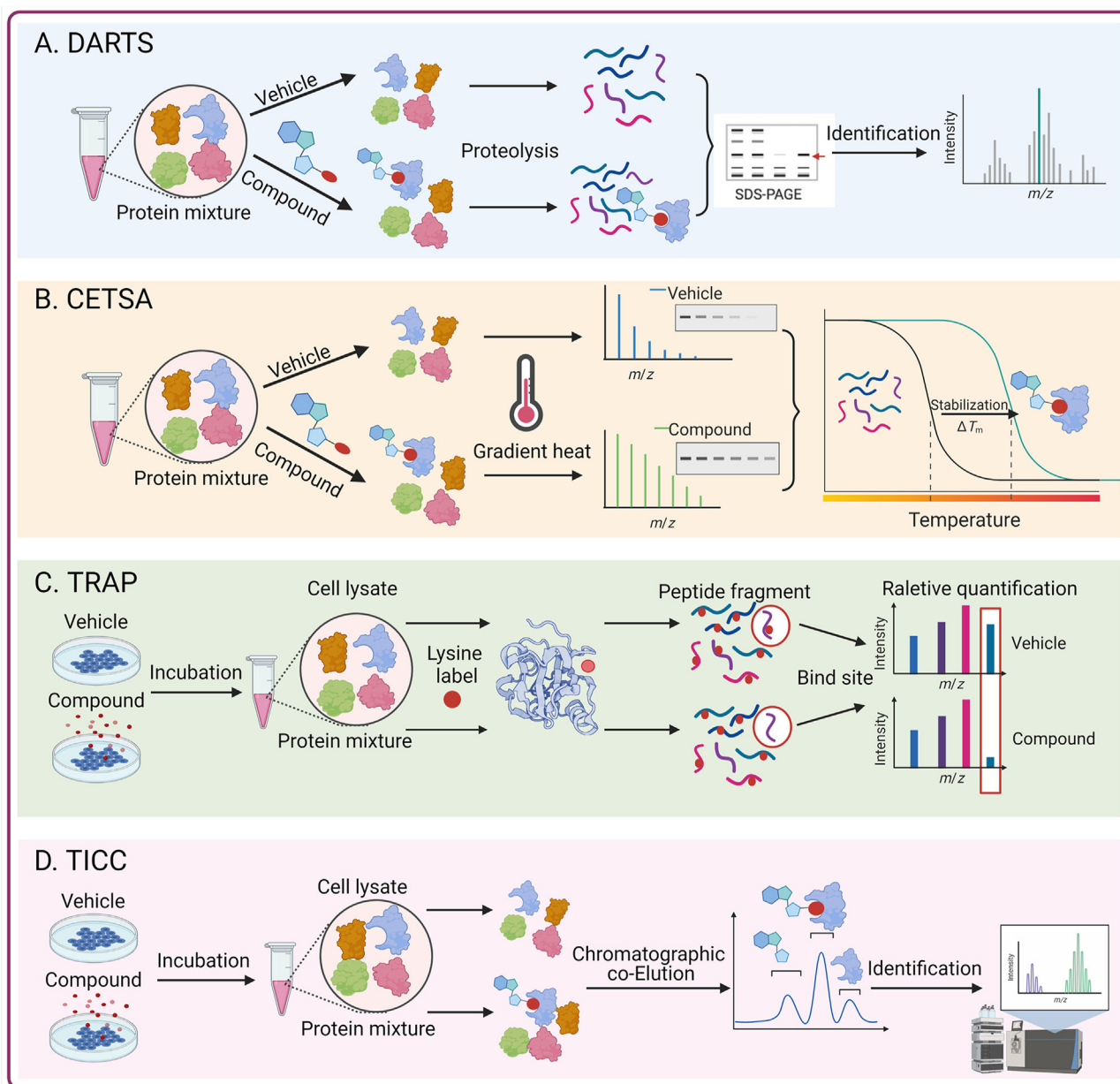


Figure 6 Method for target identification of natural products with Label-free. (A) Drug Affinity Responsive Target Stability (DARTS); (B) Cellular Thermal Shift Assay (CETSA); (C) Target-Responsive Accessibility Profiling (TRAP); (D) Target Identification by Chromatographic Co-Elution (TICC). This figure is created with [biorender.com](https://www.biorender.com).

ATP6V0D1 conformation targeting the unique cysteine 335 residue to activate V-ATPase-dependent lysosomal acidification. Similarly, Geng et al.⁸⁸ used a DARTS method to identify Dynamin-related protein 1 (DRP1) as the target protein of andrographolide, and further study found that DRP1 is a key effector mediating mitochondrial fission. Andrographolide binds to DRP1 and inhibits its GTPase activity, thereby preventing the excessive mitochondrial fission and neuronal damage associated with Parkinson's disease. The protein samples used in DARTS technology can be purified proteins or whole cell lysates and can be used for low-affinity target screening because no washing is required during the experimental procedure. However, because this technique requires the use of gel staining for visual comparison, it has certain limitations when attempting to identify low-abundance target proteins³¹⁰.

4.1.2.2. Cellular thermal shift assay (CETSA). Martinez Molina et al.^{314,315} applied clinical drugs to four different target proteins in cell lysates and found that each of the four target proteins had its own unique melting curve. When drugs known to bind to these proteins were added to cell lysates, significant melting curve shifts were observed. This principle led to the development of the CETSA method, which can directly measure the extent of a drug that reaches target cells and allows the detection of the effect of small molecules in cell lysates and even in intact cell tissues. CETSA is based on the principle of ligand-induced changes in the thermodynamic stability of target proteins. This technique mainly identifies the possible targets of natural products based on the principle that drugs that combine with target proteins change the protein stability³¹⁶.

Wang et al.³¹⁷ used molecular docking and CETSA to demonstrate that two ginsenosides, Rg5 and Rk1, bind directly to annexin A2. This study was the first to demonstrate that G-Rg5 and G-Rk1 inhibit tumor cell growth by targeting annexin A2 and the NF- κ B pathways. During a screening of natural products to identify STAT3 inhibitors, Jin et al.³¹⁸ found a dose-dependent reduction in STAT3 enzymatic activity by geranylarnigenin (CG902). The interaction of CG902 with STAT3 was further verified by DARTS and CETSA, indicating that CG902 is a novel STAT3 pathway inhibitor. CETSA has a wide range of applications and can directly measure whether drug molecules reach their target at the cellular and whole-animal levels, thereby validating important clinical drug targets. However, the heat treatment during CETSA may affect the permeability of the cell membrane and could allow entry of drugs that normally would not enter the cells at physiological temperature, leading to false positives. Therefore, the conditions for heat treatment, including the shortest time and the most effective heating method, need improvement.

4.1.2.3. Target-responsive accessibility profiling (TRAP). The DARTS and CETSA methods use the *in vivo* changes in the dynamic balance of the target proteins due to binding with the drug compounds and ligand-induced protein stability enhancement to identify drug targets. By contrast, TRAP identifies binding proteins for drug molecules in the cellular environment by monitoring the ligand-induced changes in lysine accessibility at the proteomic level. This method measures the steric hindrance induced in the protein targets due to ligand binding by global analysis of the accessibility changes to reactive lysine. Briefly, peptides that contain TRAP-induced and exhibit significant abundance changes in the presence of drug molecules are designated as target-responsive peptides³¹⁹. Our research group used TRAP technology to identify the target of celastrol as adenylyl cyclase associated protein 1 (CAP1). Mechanistically, we

found that celastrol interacts with CAP1 and resists to inhibit the cAMP–PKA–NF- κ B signaling pathway and ameliorate high-fat diet-induced metabolic syndrome in mice. Our study showed that celastrol binds to CAP1, inhibits the interaction between resistin and CAP1, effectively attenuates the subsequent inflammatory response, and ultimately improves metabolic syndrome⁶⁹.

4.1.2.4. Target identification by chromatographic co-elution (TICC). TICC is a co-fractionation based on the formation of stable ligand–target complexes during native HPLC. The main premise is that binding to one or more target proteins changes the chromatographic properties of the compounds so that the ligand–target complexes exhibit different characteristic elution profiles relative to the free (unbound) drug. That is, the retention time of a compound is “transferred” to the retention time of its interacting protein partner. Binding proteins are then identified by high performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS). Chan et al.³²⁰ have used TICC to reveal the sterol biosynthesis enzyme Erg6p as a novel putative antifungal target. The TICC target identification method is more suitable for TCM because it can identify multiple component targets at the same time. This method is also suitable for detecting low-abundance target proteins and low-affinity (micromolar) interactions. One limitation is the need to separate the unbound compounds from protein-bound compounds without using covalent bonding agents. The co-elution of proteins with similar retention properties may also complicate the identification of true targets^{190,321}.

At present, the concept of a drug acting on multiple proteins has gradually been accepted. Drug development and synthesis are not conducted only for pathogenic genes and proteins; they are necessary to study the entire network of drug and pathogenic effects. This increases the need for the identification of drug targets. The continuous development of many disciplines, such as genomics, proteomics, bioinformatics, genetics, and biotechnology, will continue to improve existing methods, and new methods and strategies will continue to emerge. Currently, there are various methods for the identification of natural product targets, each with advantages and disadvantages (Table 4). It can be used independently or complementary, and the combination of the two may be more conducive to the identification of natural product targets. Many drug target proteins with unique structures and functions can be explored and discovered for natural products and will provide key theoretical information for subsequent innovative drug design.

4.2. Natural product target validation

The target identification methods used with a natural drug molecule usually deliver a series of target proteins. If several proteins are candidate targets, they need to be prioritized based on their known function and relevance to the phenotype induced by the drug molecule. For this prioritization, designing and implementing appropriate control experiments are essential to differentiate nonspecific binding. A further complexity that should be taken into account is that the identified protein may not be the direct target, but merely part of a protein complex. Therefore, confirmation of drug molecule targets is also crucial.

4.2.1. Binding experiments to validate target proteins

Determining the binding affinity of small molecules to their putative targets provides strong evidence for target validation. Several methods used to examine protein–protein interactions, such as

Table 4 Advantages and disadvantages of various target identification methods.

Method	Principle	Advantage	Disadvantage	Ref.
Coupling molecules with affinity probes	Active group part of the natural products will tightly bind to its target, while the reporter group part can effectively label the target biological macromolecules	(1) Compounds directly bind to their target proteins in lysates or live cells; (2) Biotin–avidin purification system with high specificity; (3) Experimental process is relatively simple.	(1) Difficulty identifying proteins that are low in intracellular abundance or bind weakly; (2) Introduced reporter groups may affect compound biological activity and mechanism of action.	53,302–304,306–308
Drug Affinity Responsive Target Stability (DARTS)	Compounds that bind to their target proteins reduce the sensitivity of the target protein to proteases	(1) Compounds do not require any chemical modification during the experiment; (2) Lower affinity interacting proteins can be identified.	(1) Difficulty identifying low abundance interacting proteins; (2) Few proteins have different susceptibility to proteolysis	88,310–313,318
Cellular Thermal Shift Assay (CETSA)	Compounds that change protein stability after binding to target proteins, and the bound target protein has a unique melting curve	Wide range of applications and can directly measure whether drug molecules reach their target at the cellular and whole-animal levels	Heat treatment may affect the permeability of cell membranes and may allow the entry of drugs that would not normally enter cells at physiological temperatures, resulting in false positives	314–318
Target–Responsive Accessibility Profiling (TRAP)	TRAP identifies binding proteins for compounds in the cellular environment by monitoring the ligand-induced changes in lysine accessibility at the proteomic level	(1) Compounds do not require any chemical modification during the experiment; (2) Compounds directly bind to their target proteins in lysates or live cells.	No difference in lysine abundance at sites where compounds bind to target proteins	69,319
Target Identification by Chromatographic Co–Elution (TICC)	Compounds bind to the target protein alters the chromatographic properties of the compound so that the ligand–target complex exhibits a different characteristic elution profile relative to the free (unbound) compound	(1) More suitable for traditional Chinese medicine because it can identify multiple component targets at the same time; (2) Suitable for detecting low-abundance target proteins and low-affinity (micromolar) interactions.	(1) Need to separate the unbound compounds from protein-bound compounds without using covalent bonding agents; (2) Suitable for soluble proteins, not for membrane proteins.	190,320,321

surface plasmon resonance (SPR), isothermal titration calorimetry (ITC)³²², fluorescence polarization (FP)³²³, homogeneous time-resolved fluorescence (HTRF)³²⁴ and microthermophoresis (MST)³²⁵, have been successfully used in small molecule–protein interaction studies. Most of these methods require purified target proteins, and some require fluorescent labeling³²⁶. If the drug molecule has a strong affinity for the target protein, the specific conformation and position of the drug molecule and the target protein can be further obtained through nuclear magnetic resonance, small angle scattering, and co-crystallization experiments to provide a structural basis for the development of new drugs that target specific diseases.

4.2.2. Biological function verification of target proteins

The binding of a small molecule to a protein does not necessarily modulate its function. For this reason, functional experiments are also required to confirm a protein target³²⁷. When the target has enzymatic activity, the modulation of this activity should be assessed with an enzymatic assay³²⁸. For confirmed targets, *in vitro*, RNA interference (siRNA/shRNA), and/or cDNA overexpression experiments should be performed, and both positive and negative aspects should be analyzed to verify whether the confirmed targets might affect the biological activity of the drugs. *In vivo* target validation can be achieved by breeding different Flox and Cre mice to achieve tissue/cell-specific knockout/knockin of specific genes. This type of analysis can determine the role of the target protein in the disease phenotype and establish whether the protein is the primary target that dictates the function of the drug molecule.

In conclusion, target validation of drug molecules is as important as target recognition. Validation of a target should not be limited to determining the binding affinity of the target to the ligand, but should also confirm the cellular context indicated by phenotypic screening. A combination of biophysical, biochemical, cell biology, and structural biology approaches will help to identify the final target protein. Broadly speaking, drug targets include proteins that directly interact with drugs, but they can also be intracellular signal-responsive molecules that are triggered by drug molecules. The discovery and research of these signal-responsive molecules have very important theoretical and practical value for understanding the mechanism of action of existing drugs and improving their clinical efficacy. Therefore, the identification of biologically active natural products is of great significance for advancing biomedical research. Identification of target proteins will aid in elucidating the mechanism of drug action, establishing the potential therapeutic value of the drug, and understanding its off-target-related side effects.

5. New techniques and strategies for researching the mechanism of natural small molecular compounds

5.1. Single-cell omics

Single-cell omics, as a rapidly developing frontier technology in life science, describes the genome sequencing, transcriptome sequencing, proteome detection, and metabolome detection in a single individual isolated cell from a sample³²⁹.

5.1.1. Single-cell transcriptome

The fundamental principle of single-cell sequencing (scRNA-seq) is similar to that of bulk RNA-seq, except that scRNA-seq is aimed at a single cell rather than a group of tissues. This imparts some particularity with respect to single cell isolation and capture,

as well as trace RNA amplification. The main methods for single cell separation include fluorescence-activated cell sorting (FACS)³³⁰, microwell³³¹, and microfluidic technology³³². Recently, with the widespread use of SPLit-seq, the separation of individual cells has become a well-established method³³³. The appearance of the terminal tail method, Smart-seq³³⁴, Cel-seq³³⁵, and unique molecular identifiers (UMIs)³³⁶ has further improved the accuracy of single cell transcriptome quantification. The emergence of a variety of high-throughput, low-cost, automated commercial sequencing platforms, such as BD RhapsodyTM, 10 × Genomics Chromium, and IlluminaBio-Rad, has greatly promoted the application of scRNA-seq.

The advent of scRNA-seq has opened up new avenues for studies on human physiology and disease pathologies, such as tumorigenicity³³⁷, inflammation, and immunity³³⁸, and now allows identification of cell subclusters³³⁹, heterogeneity of gene expression, cell development trajectories³⁴⁰, and cell–cell interactions³⁴¹. It is also widely used in drug screening, efficacy evaluation, and pharmacological research. This technology was selected as “Method of the Year 2019” by NatureMethods³⁴². In the study of the mechanisms of natural products, scRNA-seq can comprehensively and accurately describe the differences in cell types and molecular states between physiologically normal and pathological tissue prior to or following drug treatment to provide more information for the discovery of drug targets and pathways. For example, Fan’s group⁷⁶ investigated the systematic post-infarction dynamics of cardiac immune cells in the progression of myocardial infarction and found that macrophages Mø-5 and Mø-6, which express chemokines CCL7, CCL2, and PF4, were crucial for disease progression. Trajectory analysis revealed that the Mø-5 and Mø-6 macrophages were mainly derived from monocytic progenitors. The natural product tanshinone IIA significantly inhibited the expression of Mø-5 and Mø-6 and their chemokines.

5.1.2. Single-cell multiomics

Recent advances in molecular biology and systems biology have given rise to a multitude of multiomics technologies, which integrates different levels of information such as genes, mRNA, regulatory factors, proteins and metabolites, and then constructs gene regulatory networks and reveals the regulation and causal relationship between various molecules.

Bai’s group³⁴³ integrated transcriptome, proteome and metabolome data and confirmed that P53 was a key target of ginsenoside, and 20(S)-protopanaxatriol directly targeted adjacent regions of the P53 DNA-binding pocket and promoted the stability of P53–DNA interactions with the application of affinity mass spectrometry (MS) screening and SPR. Liu’s group³⁴⁴ integrated omics data including gene expression, DNA methylation and copy number alterations from TCGA, combined with bioinformatics including the similarity network fusion (SNF) method and the LASSO algorithm, to identify that SIRT3 and SF3B3 are potential autophagic regulators in invasive breast carcinoma. Li et al.³⁴⁵ integrated lipidomic and transcriptomic analysis revealed that SSa and SSd regulated TF-dependent gene expression to ameliorate non-alcoholic fatty liver disease.

For single cell level, multiomics enable a more comprehensive delineation of the state of single cells than is provided by single omics data based on multichannel molecular readouts³⁴⁶, and greatly promote the development of exploring rare cell types, identifying accurate cell types, and improving cell annotation information^{347,348}.

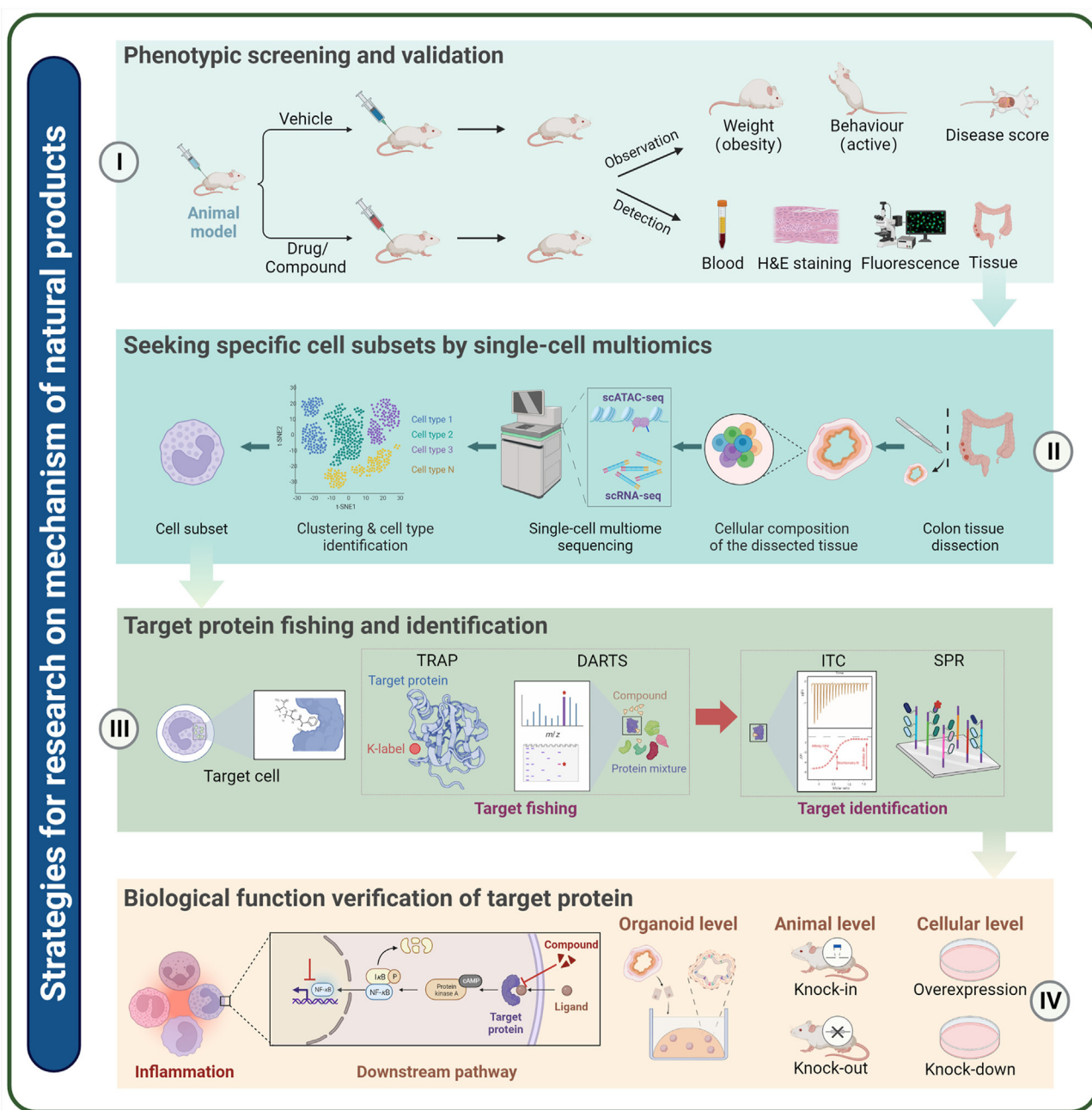


Figure 7 Strategies for research on mechanism of natural products based on deciphering target protein. This figure is created with [biorender.com](#).

Zhang et al.³⁴⁹ combined scRNA-seq, TCR-seq, and ATAC-seq to investigate immune cell dynamics of patients with triple-negative breast cancer (TNBC) treated with paclitaxel or paclitaxel plus atezolizumab, and found that CD8–CXCL13, CD4–CXCL13, Tregs, and Bfoc cells decreased in responders treated with paclitaxel, whereas they increased in those treated with paclitaxel plus atezolizumab. This difference in response indicated that the paclitaxel regimen could selectively reduce key antitumor immune cells while elevating immunosuppressive macrophages in TNBC, thereby providing novel insights into the treatment of TNBC by the combination of paclitaxel and atezolizumab.

Single-cell proteomics and single-cell metabolomics have also been used in pathological research. However, the technological

development of both is still in the early stages, and their application is lagging, with no mature commercial solution at present³⁵⁰. In view of the similarities in the detection principles and analysis strategies, subsequent studies should use single-cell proteomics to analyze the protein expression profiles of single cells in the context of drug intervention to render comprehensive and accurate reflections of drug targets in different types of cells.

In view of the widely used single-cell sequencing technologies, we proposed new strategies that focused on elucidating the mechanisms of natural products and their target proteins (Fig. 7). Firstly, an appropriate animal model of disease was constructed to verify the therapeutic effect of natural products. Secondly, tissues from the animal models were selected as samples for single-cell multiome

sequencing (including sRNA-seq and scATAC-seq) to identify the different cell subclusters that showed significant changes in response to drug treatment, which we defined as target cells (cell lines or primary cell cultures). Thirdly, approaches such as TRAP and DARTS were used to find the binding protein for a natural product in a target cell, with isothermal titration calorimetry (ITC), surface plasmon resonance (SPR), and microscale thermophoresis (MST) then used to confirm the binding of the target protein. Finally, the biological function of the target protein was verified by combining gene knockout/knockin at the animal level and knock-down/overexpression at the cellular level, followed by analysis of downstream pathways to determine the effects of natural product binding to the target protein on the progression of the disease. These new strategies are also applicable to research on the mechanisms of small molecules produced by chemical syntheses.

5.2. Network pharmacology and artificial intelligence

Big data and artificial intelligence technologies will drive the rapid development of modernization of TCM. Integrative pharmacology-based traditional Chinese medicine (TCMIP) is a database- and algorithm-dependent research strategy, which form multidimensional association network with complex interaction types including constituent–target interactions, constituent–gut microbiota interactions, constituent–constituent interactions, gut microbiota–target interactions, and target–target interactions³⁵¹. Protein three-dimensional structure analysis and computer virtual screening based on bioinformatics also provide directions for drug design for a variety of diseases including SARS-CoV-2³⁵².

5.2.1. Network pharmacology

Network pharmacology is based on database data and builds a component–target–disease interaction network to predict drug substance efficacy, targets, and pathways. It breaks the model of “one disease–one target–one drug,” which is the main reason for the failure of 70% of new drugs in clinical trials. A variety of chronic diseases, such as tumors, cardio-cerebrovascular disease, and diabetes, are multi-gene and multi-factor diseases, so achieving a good therapeutic effect based on only a single target is difficult³⁵³. The technology of network pharmacology plays an important role in research into the prescription of TCM and natural products. For example, Wang’s group³⁵⁴ used an integrated strategy combining metabolomics and network pharmacology to research the mechanism of hydroxysafflor yellow A (HSYA) in counteracting acute brain injury. Metabolomics of the brain tissues identified differentially expressed metabolites, while network pharmacology was applied to unearth potential targets against traumatic brain injury (TBI). Their findings revealed that HSYA alleviated the neurological deficits of TBI and that NOS1, AChE, and Ptgs2 were the key regulatory genes.

5.2.2. Artificial intelligence (AI)

Recent improvements in computer data processing ability and the rapid accumulation of multi-group data have laid the foundation for the development of more accurate drug target reasoning algorithms, making the combination of AI and new experimental technologies an efficient method for drug discovery. AI plays an important role in all preclinical stages of drug development, including cell sorting, cell classification, quantum mechanics calculation of compound properties, computer-aided organic synthesis, designing new molecules, developing assays, predicting the 3D structures of target proteins, and many others³⁵⁵. For

example, Jiang’s group³⁵⁶ proposed a novel Siamese spectral-based graph convolutional network (SSGCN) model based on gene transcriptional profiles. The model successfully predicted and verified the potential host targets of nelfinavir (NFV)-cyclophile A, and revealed the possible mechanism of interaction with SARS-CoV-2. The AI technology represented by a SSGCN model can significantly improve the prediction accuracy of drug targets, and therefore represents a powerful tool for the study of drug mechanisms and confirmation of targets. This method is also suitable for the prediction of the molecular targets of natural products.

5.3. Biosynthesis of natural products

In the past 20 years, advances in synthetic biology and the emergence of microbial cell factories have provided a novel idea for efficient and sustainable production of natural products. Through the metabolic transformation of microbial cells, the relevant metabolic enzyme components were transferred into microbial cells, so as to obtain the improved genetic engineering bacteria, and then the engineering bacteria were used to transform cheap carbon and nitrogen sources into natural products³⁵⁷.

Triterpenes are a large class of natural products with a wide range of bioactivities. More than 20,000 kinds of triterpenes have been identified and over 400 kinds of proprietary medicines, including many important steroid drugs and ginsenosides widely used in clinic. Their biosynthesis begins when squalene synthase catalyzes dimerization of farnesyl pyrophosphate (FPP) to squalene, which is further oxidized to epoxy squalene, and then triterpene synthase (TrTSs) catalyzes the cyclization of squalene or epoxy squalene to holene or lanosterol. The huge structural diversity is catalyzed by many different modifying enzymes³⁵⁸. Recently, Tao et al.³⁵⁹ found that two fungal chimeric class I TrTSs-TvTS and MpMS can directly synthesize triterpene core skeletons from IPP and DMAPP or HexPP, which broke the inherent understanding that the triterpene skeleton can only be synthesized with squalene as the starting unit.

6. Conclusions and perspectives

Natural products are components or metabolites of living organisms that have evolved over a long period in nature, and they are often structurally diverse and have unique pharmacological and biological activities. Currently, more than 60% of the pharmaceuticals on the market are related to the structure and information derived from natural products, and natural product-based drug development strategies still dominate in modern new drug development. The Nobel Prize awarded to Youyou Tu for her research on artemisinin set off a wave of interest in TCM and natural products among scientists. Liu and Shen’s group³⁶⁰ led the research and development of “*Moringa alba* tablets” for the treatment of type 2 diabetes, and this drug has been approved for marketing by the State Drug Administration. It represents the first original natural drug for lowering blood sugar in China, and the first new TCM in this field approved in China in the past ten years. Icariin Softgels have also been approved for marketing in China as a first-line treatment for hepatocellular carcinoma, and are original innovative TCM drugs in China. The use of TCM active ingredients to develop new drugs is a special path that China can follow for a long time.

Natural products have some problems, such as poor water solubility, low bioavailability, and poor stability, and the development of new dosage forms using nanotechnology is a hot

research area at present. Many difficulties still remain to be overcome before clinical application. Among them, TCM and natural products are still quite good choices for ADC drugs.

Knowledge of drug targets is a prerequisite for the identification of innovative drugs, and the targets have a source innovation significance for new drug development. An urgent need exists to establish an *in vivo* method for finding drug targets directly in the real physiological environment, as this is important for promoting biomedical research by elucidating the mechanism of action of drugs and discovering their potential therapeutic value, as well as for further discovery of the side effects unrelated to the targets. The present use of TCM is mostly in the form of compounding, and the identification and characterization of the targets of the chemicals that are combined in TCM compounding is also one of the difficulties. The identification of the targets and the interpretation of the clinical efficacy of the complex system of TCM prescriptions will help to explain the profound laws of TCM in a more scientific way and will play an important role in the development of TCM. Natural products, especially TCM products with a long history of use, have a rich foundation of clinical applications, which should be bestowed and continuously innovated with the help of advanced science and technology to boost the development of innovative drugs in China and to realize TCM industrialization and internationalization in the near future.

Single-cell multiomics technology has a profound impact on the development of life science. The research paradigm of “based on advanced science and technology, driven by data and knowledge” is of great significance for the modernization of TCM. Single-cell technology makes the precision medicine from tissue and organ to a single cell, revealing the dynamic regulation of natural products on different cell types. The application of single-cell multiomics also provides insights into the molecular mechanisms underlying the precise regulation of key molecules at various levels during disease onset and drug intervention. Drug target hooking and validation at the single cell level is of great significance for identifying sensitive cell types and studying the mechanism of drug resistance, especially for the research on tumor microenvironment, and provides insights for the clinical usage of anti-tumor drugs and the mechanism of drug resistance. The application of single-cell multiomics analysis is still in its early stage, with the great breakthroughs of experimental techniques of target identification and the improvement of multiomics analysis methods, it will drive a new era of disease intervention and drug discovery.

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Author contributions

Yang Sun, Qiang Xu, and Hongyue Ma conceived the manuscript, Yuyu Zhu, Zijun Ouyang, Haojie Du, Meijing Wang and Jiaojiao

Wang wrote the manuscript, Haiyan Sun and Lingdong Kong gave advice and suggestion.

Conflicts of interest

The authors have no conflicts of interest to declare.

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