Natural products as anticancer agents and enhancing their efficacy by a mechanism-based precision approach

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Abstract

Traditional medicines and their active ingredients and some natural products and derived analogs have been used for treating multiple diseases including cancer. Among these compounds cytotoxic agents such as bleomycin, paclitaxel and vincristine block essential pathways and genes required for cancer cell growth and these agents have diverse clinical applications. Dietary phenolics including flavonoids and related compounds are associated with multiple health benefits however most individual dietary compounds and other natural products that show promising anticancer activity in preclinical studies exhibit minimal clinical effectiveness and this is particularly true for cancer. Many of the compounds perform poorly in clinical trials due to pharmacokinetic consideration and limited uptake (e.g., curcumin) and these are issues that can be addressed. The clinical effectiveness of flavonoids and many other natural product-derived anticancer compounds can also be enhanced by a more targeted approach. This would include identifying a significant response/gene or target in a specific cancer and then identifying the optimal compound. In this review, I have discussed a limited number of targets including non-oncogene addiction genes such as Sp transcription factors, reactive oxygen species (ROS) or the orphan nuclear receptor 4A (NR4A) sub-family. Thus, the most active compound for these responses could be used only for treating patients that are ROS-inducible or highly express targets such as Sp1 or NR4A sub-family members. A mechanism-based precision medicine approach should enhance the clinical efficacy of dietary and related natural products as anticancer agents and decrease toxic side effects for some combination therapies.

Keywords

Phenolics, flavonoids, cancer, precision medicine

Introduction

Several anticancer drugs have been isolated from natural products and play an important role in cancer therapy. Many of these compounds such as paclitaxel and doxorubicin are plant or microbial-derived

chemicals that inhibit cancer due to their mechanisms of cytotoxicity. There is also evidence that the diet can play a significant role in disease prevention and in some cases specific diets may exhibit chemotherapeutic activity. However, many dietary compounds such as polyphenolics, flavonoids and related phytochemicals exhibit minimal to modest effects in clinical trials despite their promising preclinical anticancer activities suggesting that studies on the mechanisms of action of these compounds may enhance their efficacy as precision medicines. This review discusses examples of this approach with dietary compounds and natural products that act through targeting specificity protein (Sp) transcription factors (TFs), inducing reactive oxygen species (ROS) and binding the orphan nuclear receptor 4A1 (NR4A1).

# Phytochemicals and derived compounds: health benefits and anticancer activities

Background

Traditional medicines have been a major form of health care for several different societies, and they include Chinese medicines, Indian medicines (Ayurveda, Unani), Japanese (Kampo), traditional Korean (SCM, TKM), traditional aboriginal (Australia), African and Russian medicines. Most of these medicines are from herbal or plant extracts, and other natural product medicines and drugs are also derived from microbial, marine and other sources [1–9]. These medicines usually contain complex mixtures of individual compounds and in many cases, a single or only a few constituents have been associated with the activity of the mixture. The effects of traditional medicines on multiple diseases are being extensively investigated and a PubMed research (Feb, 2024) under "Traditional medicines" lists over 290,000 manuscripts including approximately 3,000 publications that appeared on-line during the January-February period of 2024. The focus of these articles is variable but includes evidence for the efficacy of traditional medicines in treating multiple diseases. A recent report summarized results of several studies on the treatment of gastric ulcers with traditional Chinese medicines (TCM) and other drugs [2]. Most of the referenced studies showed that both individual drugs and traditional medicines improve health conditions in rodent model studies. However, direct comparisons of the efficacy of TCM vs individual drugs were not clear; nevertheless, it was concluded that the multiple activities exhibited by the chemical ingredients of herbal medicines contributed to their effectiveness in treating gastric ulcers and comparison of "active" drugs vs mixtures present in traditional medicines need to be further investigated. It is evident that many natural products are highly effective drugs for clinical applications however, for both herbal medicines and health promoting diets, the individual compounds associated with the activity have had only limited clinical applications.

Clinically active natural product-derived anticancer agents

Clinically used agents

Traditional medicines and some of their major active component(s) have had a highly significant impact on treatment of several diseases including cancer where natural products and their derivatives play an important role in cancer chemotherapy [10–17]. For example, several key plant-based anticancer agents that are used in the clinic are illustrated in Figure 1. These included paclitaxel (*Taxus brevifolia* bark), camptothecin (*Camptotheca acuminata* Decne), vinblastine and vincristine (*Vinca Rosea* L.) and podophyllotoxin (*Podophyllum*) [14]. These phytochemicals and their synthetic analogs have well defined mechanisms of action that are usually linked to inhibition of important pathways enabling cancer cell proliferation and viability. Paclitaxel targets microtubules which leads to mitotic arrest; camptothecin is a topoisomerase I inhibitor that enhances DNA damage and apoptosis in cancer cells; vinblastine acts as a mitosis inhibitor that binds microtubular proteins and podophyllotoxin also binds tubulin and inhibits topoisomerase II resulting in mitotic arrest. These phytochemicals and other synthetic analogs are highly cytotoxic and are extensively used alone and in combination therapies for treating multiple cancers.

Several other anticancer agents are microbial products (Figure 2) including bleomycin, actinomycin D, mitomycin C, doxorubicin, pentostatin and daunorubicin (*Streptomyces*), carfilzomib (*Actinomyces*) [12] and drugs such as bleomycin bind DNA resulting in subsequent DNA damage. Actinomycin D intercalates
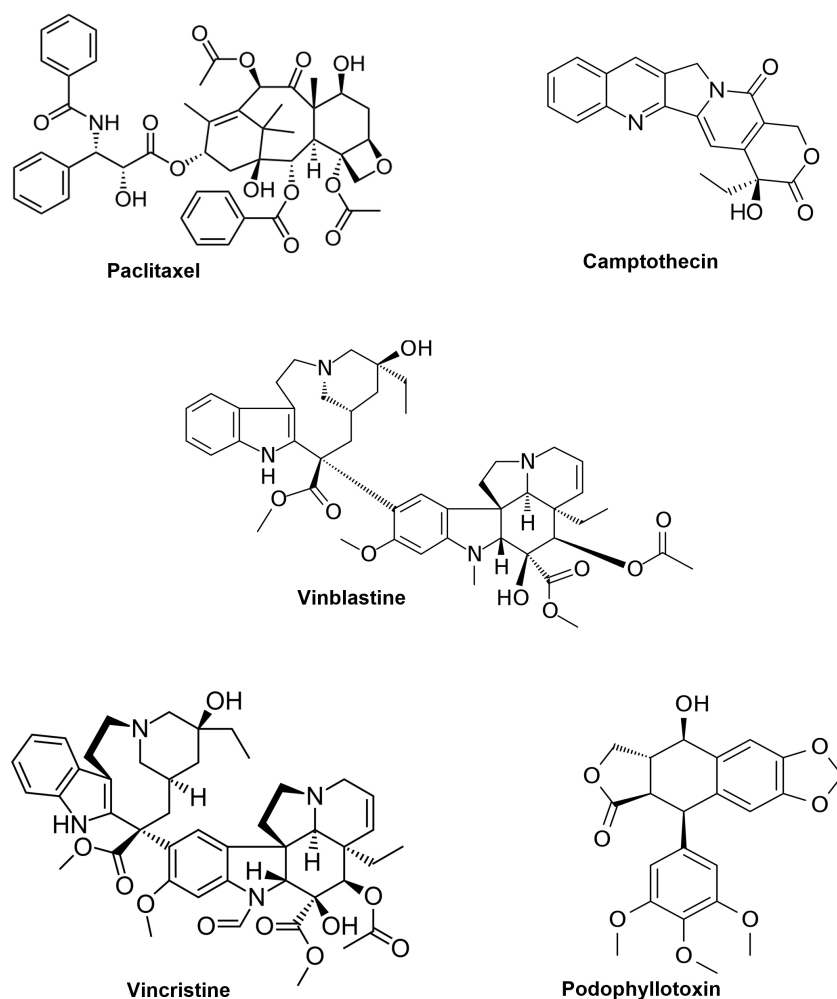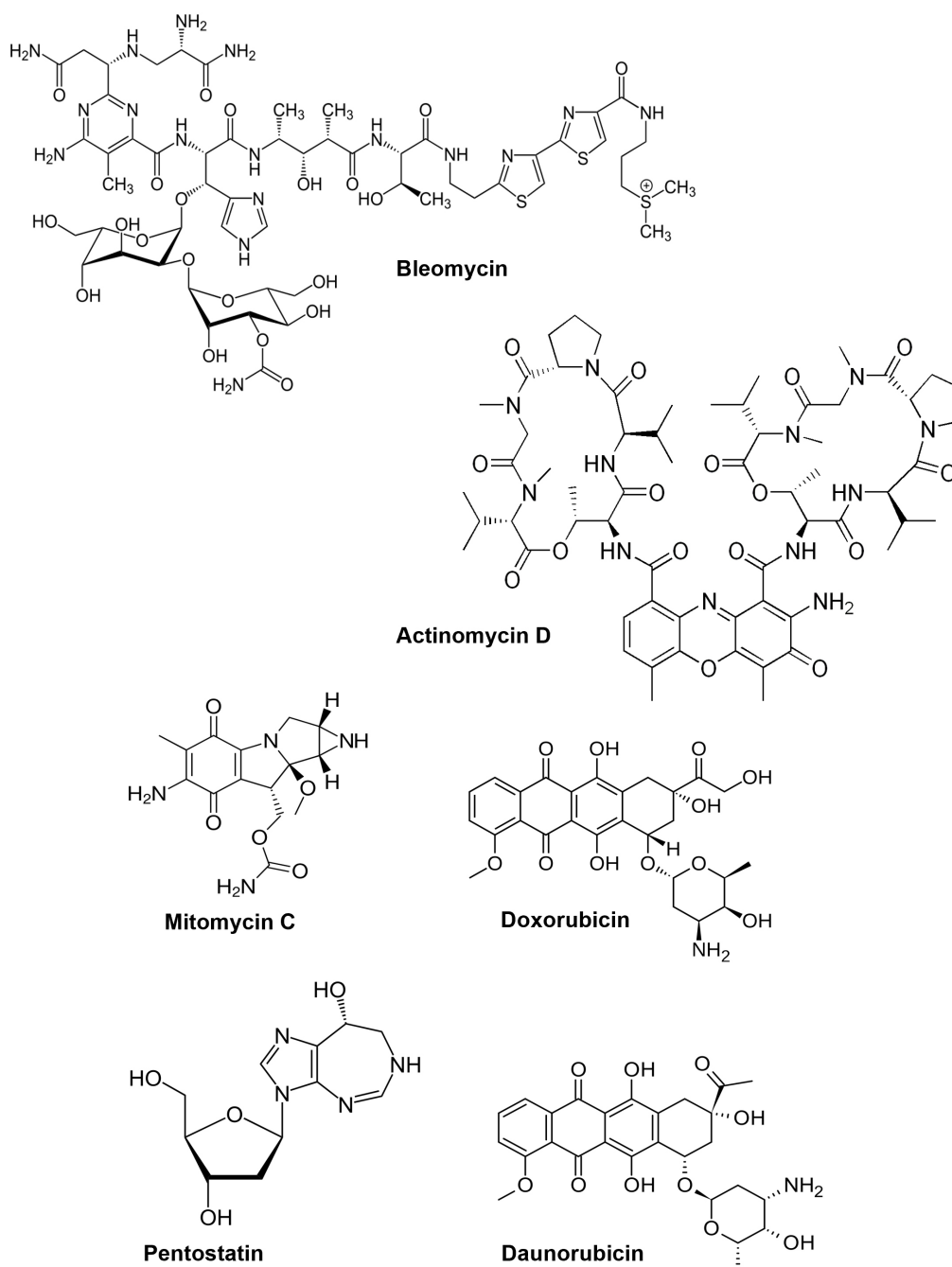
Figure 1. Clinically used anticancer agents derived from plant sources. Structures of 5 key plant derived compounds that are clinically used for cancer chemotherapy. Synthetic analogs of these compounds including other taxanes are also extensively needed

DNA and inhibits cell proliferation; mitomycin C also alkylates DNA to inhibit cell growth; doxorubicin also intercalates DNA and induces cell death; pentostatin is an antimetabolite that binds and inhibits adenosine deaminase; daunorubicin also intercalates DNA and inhibits topoisomerase II activity and carfilzomib is a proteosome inhibitor. This group of microbial-derived anticancer agents and/or their derivatives are clinically proven drugs and there is an ever-expanding list of microbial metabolites that are currently being investigated for their potential clinical applications as cancer chemotherapeutics. It should be noted that activities of these compounds are related to their well defined effects on "critical/susceptible" pathways in cancer cells that include DNA damage/inhibition of repair, targeting microtubules and mitosis and inhibition of proteasomes. In addition, there is also evidence that some natural products from marine sources show promise as anticancer agents [12, 18]. It is anticipated that additional new and promising natural product-derived anticancer agents will also be approved for clinical applications in which they retain the efficacy of their precursors but with decreased toxic side-effects [12].

Herbal/traditional medicine-derived natural products with limited activity

Yuan and coworkers [9] summarized a number of natural products derived from Chinese herbal medicines in which the individual compound and the herbal medicine are used for treating the same disease. These structurally-diverse herbal medicine-derived drugs and their targeted disease include; puerarin (diabetes), salvianolic acid B (cardiovascular and cerebrovascular diseases), rhynchophylline (antihypertensive), costunolide (anti-gastric ulcer, antispasmodic), gastrodin (anti-convulsion, analgesic), artemisinin (anti-malarial), tetrahydropalmatine (analgesic), tetramethylpyrazine (myocardial ischemia-reperfusion injury), paeoniflorin (analgesic), icariin (osteoporosis). However, even though their preclinical effects (in vitro/in

Figure 2. Microbial derived anticancer agents used for clinical application. Structures of microbial metabolites that are clinically used for cancer chemotherapy

vivo) look promising the clinical applications and effectiveness of these "active" of components of TCM is limited. For example, puerin is poorly absorbed with low bioavailability which "limits its application in the clinic" [19]; for salvianolic acid B the "low bioavailability is a serious obstacle" [20]; for gastrodin "more detailed clinical trials are still in need" [21]; costunolide exhibits activities but limited clinical data [22]; few clinically relevant studies have been determined for rhynchophylline [23]. For paeoniflorin the anti-depressive activity is promising but "more high quality pre-clinical and clinical studies are expected" [24]; tetramethylpyrazine is "a promising drug with great research potential" [25]. Thus, many of these compounds display promising preclinical activities for treating one or more diseases including cancer, but their clinical applications are limited or non-existent. They exhibit highly diverse structures and their mechanisms of action are not well defined. This does not necessarily mean that these compounds and other natural products will not be clinically useful however extensive studies are required to identify their mechanisms of action and specific disease targets as previously illustrated in the discovery of taxol derivatives [13].

Health benefits and anticancer activities of dietary-derived phytochemical mixtures

As indicated above, many important chemotherapeutic agents for single and combined treatments have been discovered from plant-derived traditional medicines, microbial metabolites and marine sources. Another significant source of human exposure to potential therapeutic agents are dietary compounds, which have been associated with health promoting activities (e.g., chemoprevention) and with their activities as therapeutic agents [26]. Population studies have identified specific diets and dietary components that have significant health impacts and the Mediterranean diet stands out as an example [27–36]. The enhanced health of individuals on this diet is similar to that observed for other diets that are high in fruits, vegetables and nuts, whole grains, olive oil, moderate amounts of yogurt, cheese, fish and eggs, smaller amounts of red and white meat and low amounts of alcohol [27]. For example, comparisons between individuals on the Mediterranean diet and on so-called "Western" diets show that the former diet is associated with increased longevity, decreased cardiovascular diseases, lower rates of some cancers and lower rates of neuronal dysfunction including cognitive decline, Alzheimer's disease and other neurotoxicities [26–40]. Coffee is another dietary component and is the most widely consumed beverage; increased consumption of up to 5 cups of coffee per day correlated with many of the same health benefits observed for the Mediterranean diet [41]. Only a few studies have investigated interactions of a Mediterranean diet and coffee consumption: In a case control study, effects of coffee and diet on colorectal cancer gave equivocal results [42] whereas in a randomized trial moderate coffee consumption and a Mediterranean diet were associated with decreased body fat in 1,483 participants with metabolic syndrome and on a Mediterranean diet [43]. In addition to the health benefits associated with some diets and coffee consumption [44–51] these health effects have also been correlated with relative serum levels of individual classes of dietary components. For example, a study in a Spanish population showed an inverse association between dietary phenolics and cardiovascular disease [49]. This association was also observed for specific phenolic sub-groups including dihydroflavonols, flavonols, alkyl methoxyphenols, hydroxy-cinnamic acids, hydroxyphenyl acetic acid (Figure 3). A dose-response meta-analysis of several prospective cohort studies also showed an inverse association between polyphenolic subgroups and the risk of type 2 diabetes [52]. Inverse associations were observed for flavonoids, flavanols, flavan-3-ols, catechins, anthocyanidins, isoflavones, daidzein, genistein, and stilbenes. Analysis of two other prospective studies showed that higher consumption of phytoestrogens was also associated with decreased mortality from cardiovascular disease and lower risks also correlated with higher intakes of isoflavones, lignans and coumarins [53].

The association of individual and sub-classes of polyphenolic and other phytochemicals on risk for various cancers has been extensively investigated and potential mechanisms have been proposed [54–66]. In most of these studies the chemopreventive effects are linked to the antioxidant and anti-inflammatory activities of polyphenolics (Figure 3) and many other pathways may also be important. An extensive meta-analysis of case-control and prospective studies indicates that consumption of dietary flavonoids is inversely related to risks of lung and stomach cancers and decreased risk for breast and colorectal cancer were observed but not significant [64]. Studies on clinical applications of dietary-derived phenolics show that these mixtures and individual compounds can be used alone or with others and provide health benefits. For example, a combination of luteolin, quercetin and rutin decreased some behavioral symptoms in children autism spectrum disorder [67]; higher tea intake and some flavonoids were inversely related to hospitalization of elderly women at a high risk for fracture [68]. These studies confirm that both diets and sub-classes of dietary phytochemicals are associated with some health benefits. Many individual dietary-derived natural products and related compounds show promising preclinical activity whereas their clinical effects are primarily modest or not observed.

Dietary-derived natural products and related compounds as anticancer agents

While it is clear that the Mediterranean and other diets enriched in fruits, nuts, vegetables, grains and polyunsaturated fatty acids are chemopreventive in terms of both non-cancer and cancer endpoints, their cancer chemotherapeutic activities are much less impressive. For example, a comprehensive review of
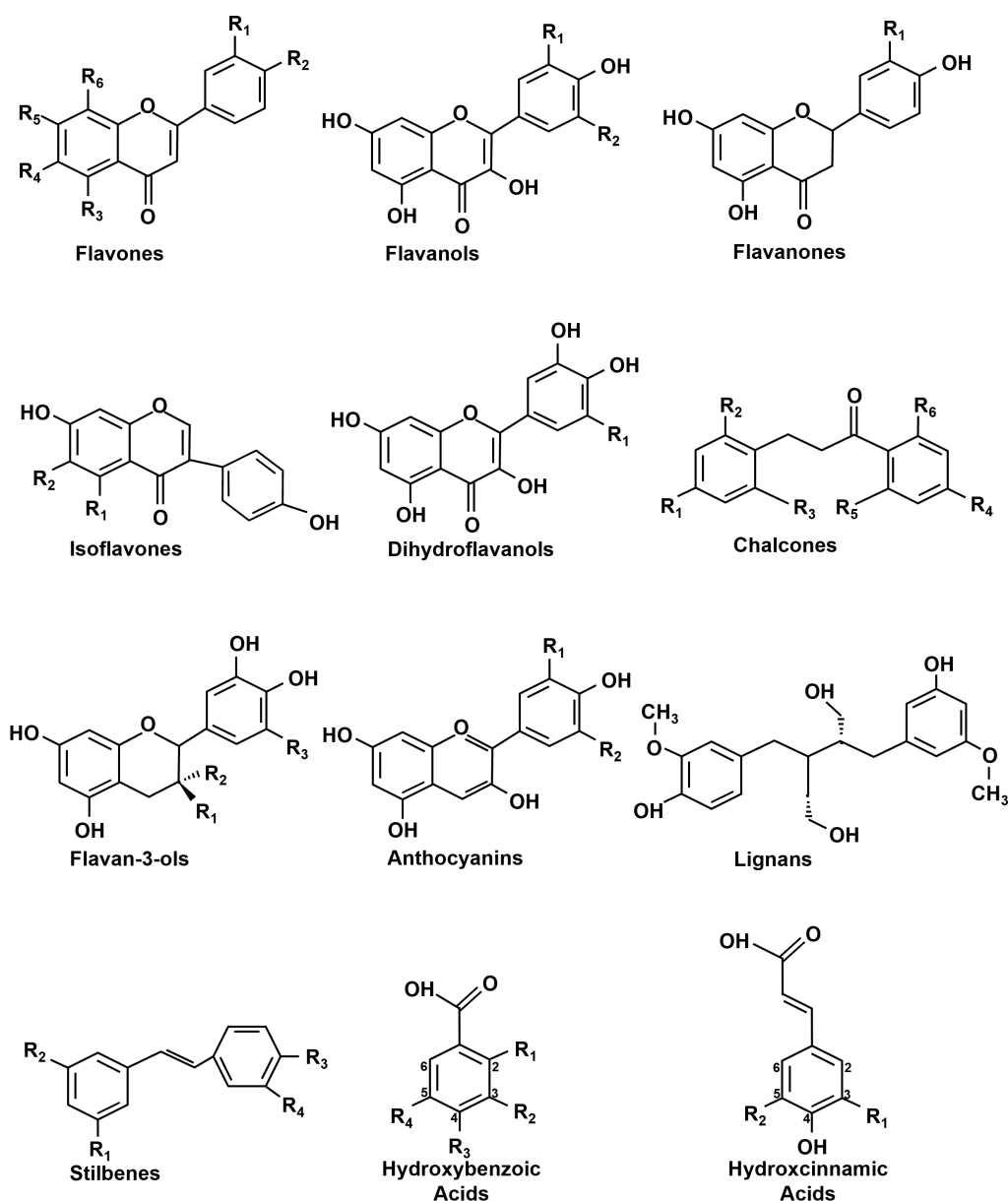
Figure 3. Structures of polyphenolic compounds present in various foods and beverages

dietary interventions in the treatment of cancer patients concluded that "Evidence demonstrated little effects of dietary interventions on overall mortality and secondary cancers" [69]. A phase II study reported that soy isoflavones did not increased survival of patients with advanced pancreatic cancer being treated with gemcitabine and erlotinib [70]. Thus, evidence for dietary polyphenolics as enhancers of anti-tumor activity in patients being treated with standard-of-care anticancer agents is lacking, however, success might be increased using a more mechanism-based approach where the polyphenolics are targeted to specific pathways. Another study systematically reviewed phase I and phase II clinical trials of individual flavonoids as anticancer agents [60]. Most of the flavonoids exhibit promising preclinical activity in cell culture and animal models but exhibit minimal clinical activity although some synthetic flavonoids such as flavopiridol gave promising results for blood-derived but not solid tumors. The overall impacts of dietary flavonoids and other polyphenolics as cancer chemotherapeutics in clinical studies are minimal but can be enhanced with improved formulations and drug delivery. In contrast to the less than favorable clinical results obtained for flavonoids, there is extensive ongoing research on flavonoids and other natural products as anticancer agents and under the heading "Flavonoids as Anticancer Agents" in PubMed over 31,000 papers are listed and approximately 200 have been published in January–March, 2024. Quercetin alone (on PubMed) has over 3,300 papers listed. There is concern that continued preclinical testing with these compounds will not improve clinical outcomes until their mechanisms of action are determined.

Curcumin, a polyphenolic stilbene compound is an important traditional Indian medicine that is being developed for treating both non-cancer and cancer endpoints [71–73]. Under a search for "Curcumin an Anticancer Agent" over 16,000 papers are listed on PubMed for this compound alone. Problems associated with the therapeutic effects of curcumin include poor bioavailability and this is being addressed in ongoing studies by improving formulations and delivery systems for this agent [71]. A recent review paper on clinical trials of curcumin for cancer therapy concluded that "available clinic evidence is not strong enough to support the therapeutic use for curcumin in cancer" [73]. However, it is conceivable that with the discovery of more effective delivery of curcumin or a synthetic analog that activities may be sufficiently increased for clinical efficacy. It is also possible that the efficacy of curcumin and related natural products can be improved in cancer chemotherapy by more precision targeting and this will be discussed in the following section.

Phytochemicals and natural products as anticancer agents—examples of mechanism-based precision targeting of Sp transcription factors, reactive oxygen species and orphan nuclear receptor 4A1

Background

While it is clear that some natural products such as the taxanes and microbial metabolites are mainstays as cancer chemotherapeutics (Figures 1 and 2) many other natural products that exhibit promising preclinical anticancer activity in cell culture and in vivo are not effective in human clinical studies. Most of the clinically used natural products target critical genes and pathways required for DNA integrity and tumor growth and this includes effects on microtubules, DNA damage and DNA repair enzymes. In contrast, many other natural products such as dietary and other polyphenolics tend to target cell context-dependent multiple proteins/pathways in cancer cells but do not exhibit therapeutic efficacy in human clinical trials. This problem will be difficult to resolve for natural products, but one possible solution is to uncover additional critical targets in cancer cells and focus on patients that highly express these targetable genes and pathways. This will be discussed with examples in the following section.

Natural product anticancer agents that target Sp transcription factors

Sp TFs Sp1, Sp2, Sp3 and Sp4 are members of the Sp—Kruppel-like factor family which exhibit many common structural features [74–79]. These TFs are particularly important in early development and there is some evidence for a decrease in Sp1 with age [80, 81]. In contrast, Sp1, Sp3 and Sp4 (and possibly Sp2) are highly expressed in many tumors and cancer cells. Most reports on Sp TFs have focused primarily on Sp1 and it is possible that Sp3 and Sp4 may have similar effects but have not been as extensively investigated and there are even fewer studies on expression of Sp2 [81]. A focus on Sp TFs as an important druggable target in cancer is due to several factors including evidence that cellular transformation of a normal cell to a cancer cell is accompanied by increased expression of one or more Sp TFs [81–89]. For example, carcinogen or oncogene-induced transformation of human fibroblasts into fibrosarcoma cells was accompanied by an 8- to 18-fold increase in Sp1 protein expression. Moreover, knockdown of Sp1 in the fibrosarcoma cells abrogated their ability to form tumors in an athymic nude mouse model [82, 83]. Sp1 (primarily) or Sp3 and Sp4 are overexpressed in many tumors and are the negative prognostic factor for patients with multiple cancers including; liver, ovarian, lung, breast, colon, gastric, pancreatic, head and neck, bladder, prostate and esophageal cancers, glioblastoma and astrocytoma [75]. Sp TFs are associated with many other proteins, long non-coding RNAs and microRNAs that often play pro-oncogenic roles in cancers [78]. Moreover, based on results of knockdown studies in pancreatic cancer cells Sp1, Sp3 and Sp4 regulate genes/pathways that are responsible for cancer cell growth, survival and migration/invasion and Sp TFs have been referred to as non-oncogene addiction genes [90].

These properties suggest that drugs targeting Sp TFs in tumors would potentially be effective. Figure 4 illustrates structures of several compounds that inhibit tumor growth and exhibit functional characteristics similar to those observed after Sp knockdown. Interestingly some of these compounds such as celecoxib, panobinostat, metformin and bortezomib [91–94] already have other clinical applications and it can be
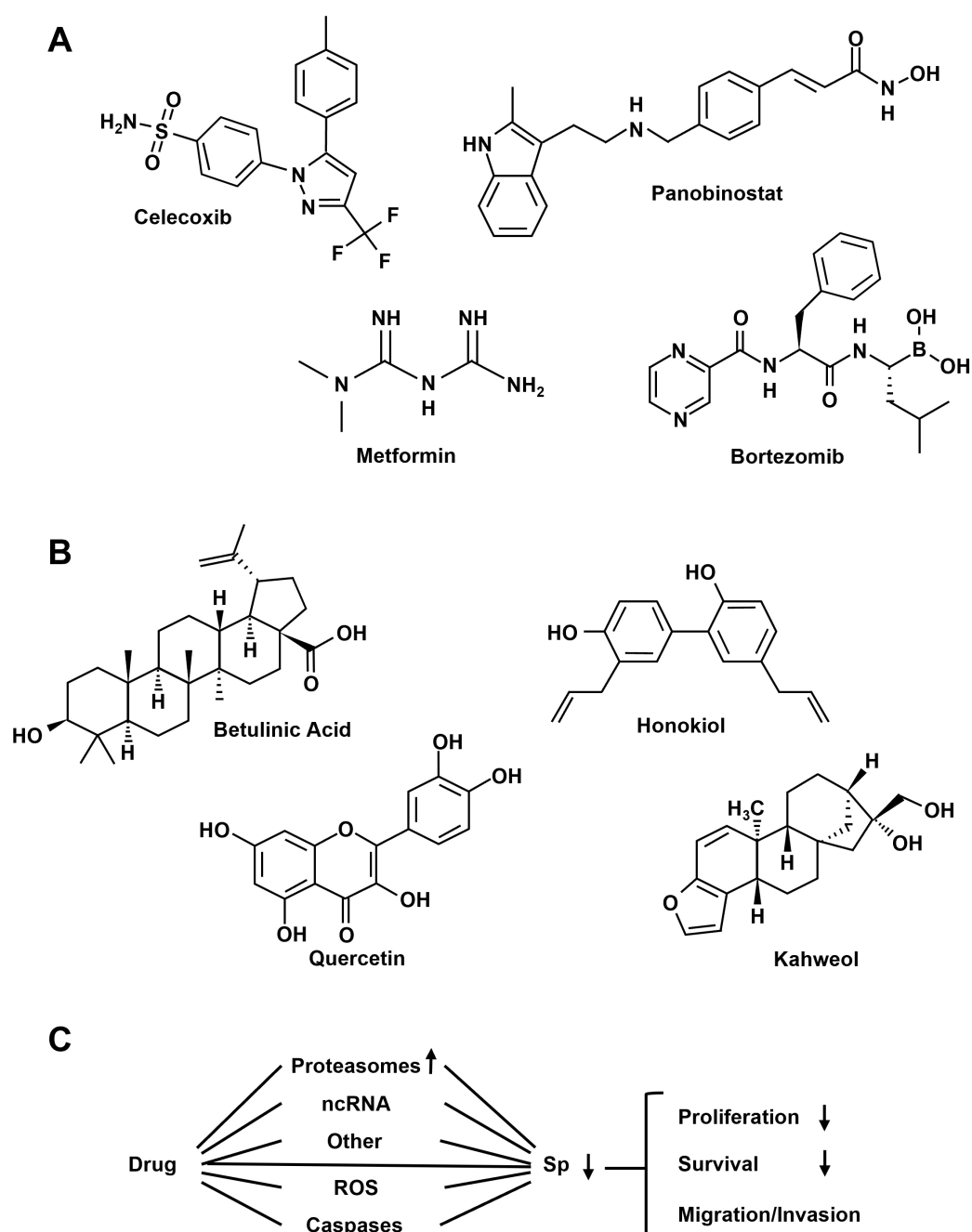
Figure 4. Drugs that target downregulation of Sp TFs. Structures of clinically used anticancer agents (A) and phytochemicals (B) that induce Sp downregulation and pathway associated with this response (C) [75]

assumed that their downregulation of Sp TFs contributes to the effectiveness of these compounds as anticancer agents. Moreover, many other natural products including betulinic acid, honokiol, quercetin and phenethyl isothiocyanate [95–103] which downregulate Sp TFs exhibit promising preclinical activity in cancer cells and rodent models but in clinical trials their effects are modest. The differences in lack of potency of natural product-derived compounds vs clinically used drugs (Figure 4A and B) could be due to several factors including pharmacokinetics and different mechanisms of drug-induced Sp-downregulation (Figure 4C). A summary of these mechanisms has previously been reported and discussed and this includes examples of where the phytochemical (e.g., murrayafoline [104] and quercetin [105]) directly bind Sp1 and decrease its activity. However, it is possible that the efficacy of these compounds may be enhanced by treating only patients that overexpress Sp1, Sp3 and Sp4. The clinical challenge for these natural products will be to identify optimal compounds for each tumor type and target the susceptible patient group overexpressing Sp TFs.

Natural product anticancer agents that activate reactive oxygen species

The chemopreventive activities of dietary polyphenolics have been associated, in part, with their activity as antioxidants in normal cells that maintain low levels of ROS. In contrast, many of these some molecules that exhibit antioxidant activity in normal cells induce ROS in a cancer cell context and this contributes to their anticancer activity [60–62, 106–109]. The inverse activities of ROS and many natural phenolics as chemopreventive and chemotherapeutic agents also reflect the difference between normal tissue vs tumors where the high requirements for energy by rapidly proliferating tumors invariably are accompanied by higher levels of ROS. The flavonoids are examples of major dietary chemicals that may protect cells during growth as normal cells by their activities as antioxidants however many studies show that flavonoids such as phloretin, kaempferol luteolin, 3,5,7-trihydroxyflavone, quercetin, apigenin, acacetin, genistein, and isorhamnetin induce ROS in cancer cells [110–122]. Other studies do not report induction of ROS by flavonoids, and this may be due to lack of measurement of this response. However, it was reported that inhibition of melanoma cell growth by luteolin via effects on extracellular matrix and not by induction of ROS [123] whereas luteolin induced ROS and decreased mitochondrial membrane potential in rat liver tumor hepatocytes [112]. Moreover, some cytotoxic anticancer agents such as bleomycin and daunomycin induce ROS in non-tumor tissue as a toxic side-effect and this is in contrast to the effects of flavonoids. It is possible that the activities of flavonoids as ROS inducers may be both structure-dependent and tumor specific and these relationships have not been determined. Thus, precision targeting of tumors by ROS-inducing natural products must take into account several factors required for selection of optimal agents for a specific tumor.

A report by O'Hagan and coworkers [124] showed that after treatment of SW480 colon cancer cells with hydrogen peroxide, there was a chromatin-wide relocation of DNA methyltransferases from non-GC rich to GC-rich areas. This resulted in significant changes in gene expression and the cMyc gene which contains a GC-rich promoter was one of the genes downregulated by hydrogen peroxide. The oncogenic activity of cMyc has been well described and studies in this laboratory showed that a number of ROS inducing natural products including benzyl and phenethyl isothiocyanate also decrease cMyc expression in cancer cells [124, 125]. Figure 5 illustrates the critical role of cMyc in the ROS-induced response where downregulation of cMyc resulted in decreased expression of microRNA-27a and other microRNAs in the miR-17-92 complex. Decreased miRs resulted in the induction of at least 3 Sp-repressor proteins ZBTB4, ZBTB10 and ZBTB39 [103, 125, 126]. The ZBTB repressors competitively displace Sp TFs from GC-rich sites in Sp-regulated pro-oncogenic genes (including Sp TFs) and this is accompanied by Sp protein downregulation. Mechanistic studies show that in cancer cells knockdown of cMyc or cMyc-regulated miRs or overexpression of ZBTBs also results in downregulation of Sp TFs in the absence of ROS. These results suggest that the effectiveness of ROS-inducing natural products that trigger the cMyc-Sp TF pathway (Figure 5) could be enhanced in tumors overexpressing Sp1, Sp3 or Sp4. This drug-induced ROS-dependent downregulation of Myc by natural products has been observed for many compounds [126], however methods for selecting optimal ROS-inducing agents for targeting this response have not been determined and based on mechanism studies these compounds may also have increased clinical efficacy for treating patients that overexpress Sp TFs.

ROS induction of other "death" pathways in cancer

Natural products that induce ROS are cytotoxic to cancer cells and tumors however, this may be accompanied by several independent and overlapping pathways [106–109]. This may include induction of intrinsic and extrinsic apoptosis, DNA damage, endoplasmic reticulum (ER) stress, necroptosis, autophagy and ferroptosis. Ferroptosis is an iron-dependent form of cell death that is emerging as a therapeutic pathway for treating various cancers [127–131]. Ferroptosis-induced cell damage is characterized by enhanced lipoperoxidation and the subsequent cytotoxicity mediated by intracellular lipid hydroperoxides. Several genes are important for induction/repression of ferroptosis and these include: (I) glutathione peroxidase 4 (GPX4) which helps to maintain cellular glutathione levels and inhibits ferroptosis; (II) stearoyl coenzyme A (CoA) desaturase 1 (SCD1) which catalyzes formation of monounsaturated fatty acids
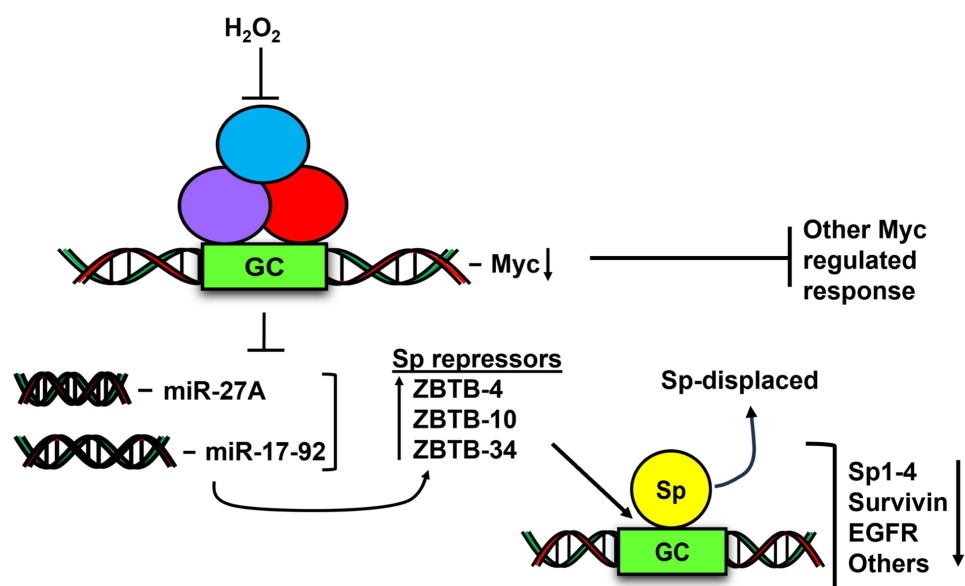
Figure 5. Hydrogen peroxide and ROS decrease Myc and Myc-regulate miRNA and this results in the induction of Sp repressors (ZBTB-10, 4, 34) which in turn competitively displace Sp transcription factors from GC-rich gene promoter sites

that inhibit ferroptosis; (III) transferrin and the transferrin receptor (TFR) which facilitate iron import into the cell and enhance ferroptosis. There is evidence that structurally diverse natural products may be significant players in development of ferroptosis-based anticancer agents. Compounds that induce ferroptosis include curcumin, glycyrrhetinic acid, gallic acid, quercetin and other flavonoids, artesunate, piperlongumine, ursolic acid, capsaicin, honokiol, withaferin A and many other compounds and plant extracts [108, 132–135]. The mechanisms of action of these compounds are variable and include ROS induction and inhibition or activation of key ferroptosis-related gene products including TFR, SCD1 and GPX4. However, development of clinically effective ferroptosis inducers will require cancer type specific optimization of individual natural products and their preferred targets.

Phytochemicals that target nuclear receptor 4A

The NR superfamily of receptors play a major role in maintaining cellular homeostasis and in pathophysiology and these receptors are a major drug target for treating both cancer and non-cancer endpoints. The orphan NR4A family includes NR4A1 (Nur77, TRB), NR4A2 (Nurr1) and NR4A3 (Nor1) and endogenous ligands for these receptors have not yet been identified. NR4A play an important role for multiple functions including innate and adaptive immunity, immune cell differentiation, steroidogenesis, response to inflammation and stress, atherogenesis and central nervous system functions [136–138]. NR4A1 and NR4A2 exhibit pro-oncogenic activities in solid tumors and their derived cell lines and results of knockdown studies show that NR4A1 and NR4A2 regulate cell proliferation survival, migration and invasion (Figure 6). Studies in our laboratory have identified synthetic bis-indole derived compounds (CDIMs) that bind NR4A1, NR4A2 or both receptors and inhibit cancer cell proliferation, survival, migration and invasion [139, 140] (Figure 6). The effects of these compounds are comparable to effects observed after knockdown of NR4A1 or NR4A2 and they exhibit inverse NR4A1/NR4A2 agonist activity based on their functional effects. There are multiple reports on phytochemical anti-cancer agents that exhibit activities similar to that observed for the bis-indole derived NR4A1/NR4A2 ligands and this is particularly true for flavonoids. Based on their functional similarities it was hypothesized that the anticancer activities of the flavonoids quercetin and kaempferol might be due to interactions with NR4A1 and NR4A2 to block receptor-mediated pro-oncogenic activities. It was recently reported that both quercetin and kaempferol directly bound NR4A1 and acted as inverse NR4A1 agonists in rhabdomyosarcoma cells [141]. A subsequent study also showed that like the CDIM inverse NR4A1 agonists, both quercetin and kaempferol inhibited pro-endometriotic genes/pathways including fibrosis [142]. Subsequent studies show that over 20 structurally diverse hydroxyflavones also bind NR4A1 and other natural products including, broussochalcone, fangchinoline, tetrandrine, resveratrol, and piperlongumine are also NR4A1 ligands [143–

146] that exhibit anticancer activities as illustrated in Figure 6. Previous clinical studies on flavonoids and other phytochemical NR4A1 ligands showed minimal effectiveness as anticancer agents. However, using a precision medicine approach, future studies on NR4A1-active flavonoids and related phytochemicals should first identify the optimal compound for a specific tumor type and then target cancer patients with tumors that overexpress NR4A1. This approach would also be relevant for phytochemicals that interact with other NRs. Moreover, several apoptosis inducing agents such as retinoids and phorbol esters induce nuclear export of NR4A1 which in turn activates pro-apoptotic pathways that also kill cancer cells [139].
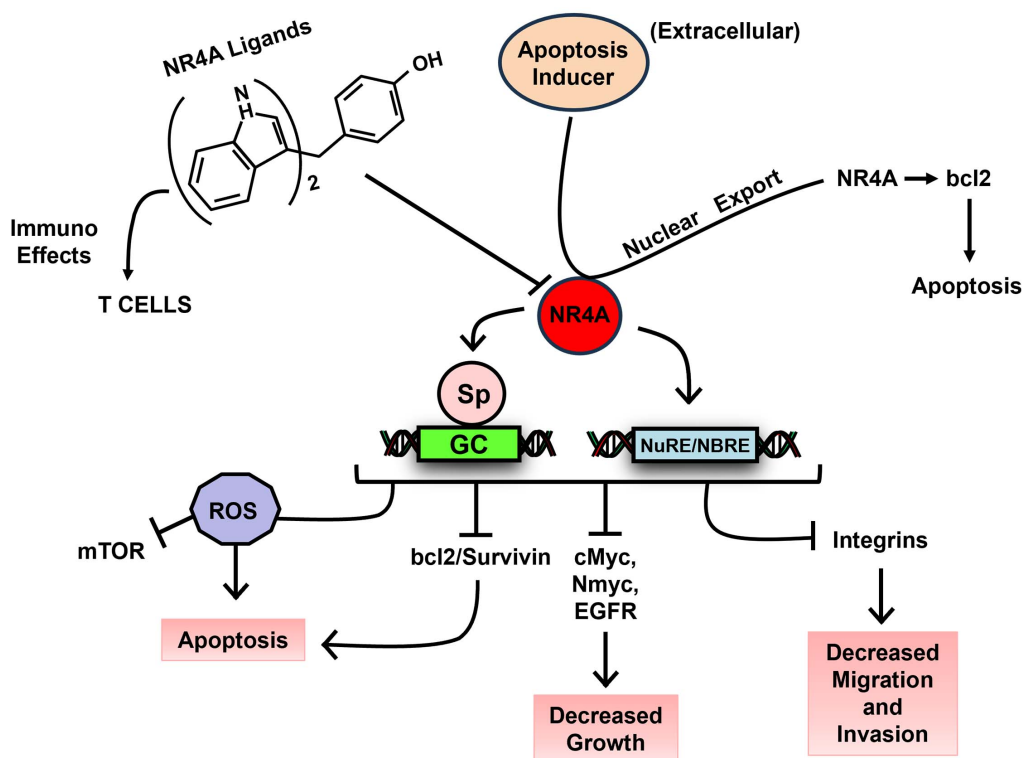
Figure 6. Mechanism of action of NR4A1/2 ligands. Prototypical NR4A1/2 ligands (e.g., CDIM, flavonoids and other polyphenolics) inhibit NR4A1/2-dependent pro-oncogenic pathways in tumor cells and reverse T cell exhaustion; apoptosis inducers activate nuclear export of NR4A1 to induce apoptosis [139]

Conclusions

Natural products and their derivatives have been an important source of several clinically effective agents for treating multiple cancers. However, many polyphenolics and other natural products, including phytochemicals in the diet, that exhibit promising preclinical anticancer activities are minimally effective in human clinical trials. These compounds need to be re-examined to determine their major mechanisms of action that can be used in a mechanism-based targeted approach that will more effectively inhibit tumorigenesis. In this review 3 major targetable pathways by phytochemicals have been discussed, namely downregulation of Sp TFs, induction of ROS and related death pathways, and binding to NR4A1. Other druggable targets also need to be determined. Having identified the most effective tumor cell type-specific target, compound screening should be carried out to identify the optimal compound and clinical studies should select a patient group that overexpresses or is responsive to the target gene/pathway. In the case of NR4A1 this would include patients with tumors overexpressing the receptor. A precision medicine approach using these natural products should significantly enhance the clinical outcomes of a more defined group of cancer patients and identify specific natural products suited for stand-alone or combination cancer therapies. The increased incorporation of natural products into cancer chemotherapies will also facilitate decreased toxic side-effects for some drug combinations.

Abbreviations

NR4A: nuclear receptor 4A

ROS: reactive oxygen species

Sp: specificity protein

TCM: traditional Chinese medicines

TFs: transcription factors

Declarations

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Author contributions

SS: Conceptualization, Investigation, Writing—original draft, Writing—review & editing.

Conflicts of interest

The author declares no conflicts of interest.

Ethical approval

Not applicable.

Consent to participate

Not applicable.

Consent to publication

Not applicable.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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References

1. Wang Z, Guo S, Cai Y, Yang Q, Wang Y, Yu X, et al. Decoding active compounds and molecular targets of herbal medicine by high-throughput metabolomics technology: A systematic review. Bioorg Chem. 2024;144:107090. [DOI] [PubMed]
2. Gong H, Zhao N, Zhu C, Luo L, Liu S. Treatment of gastric ulcer, traditional Chinese medicine may be a better choice. J Ethnopharmacol. 2024;324:117793. [DOI] [PubMed]
3. Pirintsos S, Panagiotopoulos A, Bariotakis M, Daskalakis V, Lionis C, Sourvinos G, et al. From Traditional Ethnopharmacology to Modern Natural Drug Discovery: A Methodology Discussion and Specific Examples. Molecules. 2022;27:4060. [DOI] [PubMed] [PMC]

4.   Nasim N, Sandeep IS, Mohanty S. Plant-derived natural products for drug discovery: current approaches and prospects. Nucleus (Calcutta). 2022;65:399–411. [DOI] [PubMed] [PMC]

5.   Christensen SB. Natural Products That Changed Society. Biomedicines. 2021;9:472. [DOI] [PubMed] [PMC]

6.   Tian Y, Shi Y, Zhu Y, Li H, Shen J, Gao X, et al. The modern scientific mystery of traditional Chinese medicine processing--take some common traditional Chinese medicine as examples. Heliyon. 2024; 10:e25091. [DOI] [PubMed] [PMC]

7.   Arji G, Safdari R, Rezaeizadeh H, Abbassian A, Mokhtaran M, Ayati MH. A systematic literature review and classification of knowledge discovery in traditional medicine. Comput Methods Programs Biomed. 2019;168:39–57. [DOI] [PubMed]

8.   Shen S, Zhong H, Zhou X, Li G, Zhang C, Zhu Y, et al. Advances in Traditional Chinese Medicine research in diabetic kidney disease treatment. Pharm Biol. 2024;62:222–32. [DOI] [PubMed] [PMC]

9.   Yuan H, Ma Q, Ye L, Piao G. The Traditional Medicine and Modern Medicine from Natural Products. Molecules. 2016;21:559. [DOI] [PubMed] [PMC]

10.   Hui Z, Wen H, Zhu J, Deng H, Jiang X, Ye X, et al. Discovery of plant-derived anti-tumor natural products: Potential leads for anti-tumor drug discovery. Bioorg Chem. 2024;142:106957. [DOI] [PubMed]

11.   Al-Antary ET, Gupte A, Carter J, Kaafarani M, Howard M, Edwards H, et al. Curing childhood cancer the "Natural" Way: Nature as the source of chemotherapy agents. Biochem Pharmacol. 2023;213: 115630. [DOI] [PubMed]

12.   Giurini EF, Godla A, Gupta KH. Redefining bioactive small molecules from microbial metabolites as revolutionary anticancer agents. Cancer Gene Ther. 2024;31:187–206. [DOI] [PubMed] [PMC]

13.   Cech NB, Oberlies NH. From plant to cancer drug: lessons learned from the discovery of taxol. Nat Prod Rep. 2023;40:1153–7. [DOI] [PubMed] [PMC]

14.   Shaik BB, Katari NK, Jonnalagadda SB. Role of Natural Products in Developing Novel Anticancer Agents: A Perspective. Chem Biodivers. 2022;19:e202200535. [DOI] [PubMed]

15.   Naeem A, Hu P, Yang M, Zhang J, Liu Y, Zhu W, et al. Natural Products as Anticancer Agents: Current Status and Future Perspectives. Molecules. 2022;27:8367. [DOI] [PubMed] [PMC]

16.   Chunarkar-Patil P, Kaleem M, Mishra R, Ray S, Ahmad A, Verma D, et al. Anticancer Drug Discovery Based on Natural Products: From Computational Approaches to Clinical Studies. Biomedicines. 2024; 12:201. [DOI] [PubMed] [PMC]

17.   Guo M, Jin J, Zhao D, Rong Z, Cao L, Li A, et al. Research Advances on Anti-Cancer Natural Products. Front Oncol. 2022;12:866154. [DOI] [PubMed] [PMC]

18.   Molinski TF, Dalisay DS, Lievens SL, Saludes JP. Drug development from marine natural products. Nat Rev Drug Discov. 2009;8:69–85. [DOI] [PubMed]

19.   Zhou Y, Zhang H, Peng C. Effects of Puerarin on the Prevention and Treatment of Cardiovascular Diseases. Front Pharmacol. 2021;12:771793. [DOI] [PubMed] [PMC]

20.   Xiao Z, Liu W, Mu Y, Zhang H, Wang X, Zhao C, et al. Pharmacological Effects of Salvianolic Acid B Against Oxidative Damage. Front Pharmacol. 2020;11:572373. [DOI] [PubMed] [PMC]

21.   Liu Y, Gao J, Peng M, Meng H, Ma H, Cai P, et al. A Review on Central Nervous System Effects of Gastrodin. Front Pharmacol. 2018;9:24. [DOI] [PubMed] [PMC]

22.   Huang H, Park S, Zhang H, Park S, Kwon W, Kim E, et al. Targeting AKT with costunolide suppresses the growth of colorectal cancer cells and induces apoptosis in vitro and in vivo. J Exp Clin Cancer Res. 2021;40:114. [DOI] [PubMed] [PMC]

23.   Zhou J, Zhou S. Antihypertensive and neuroprotective activities of rhynchophylline: the role of rhynchophylline in neurotransmission and ion channel activity. J Ethnopharmacol. 2010;132:15–27. [DOI] [PubMed]

24. Wang X, Feng S, Wang Y, Chen N, Wang Z, Zhang Y. Paeoniflorin: A neuroprotective monoterpenoid glycoside with promising anti-depressive properties. Phytomedicine. 2021;90:153669. [DOI] [PubMed]

25. Lin J, Wang Q, Zhou S, Xu S, Yao K. Tetramethylpyrazine: A review on its mechanisms and functions. Biomed Pharmacother. 2022;150:113005. [DOI] [PubMed]

26. Kaiser J. A plateful of medicine. Science. 2021;372:20–3. [DOI] [PubMed]

27. Giuffrè D, Giuffrè AM. Mediterranean diet and health in the elderly. AIMS Public Health. 2023;10: 568–76. [DOI] [PubMed] [PMC]

28. Keys A, Menotti A, Karvonen MJ, Aravanis C, Blackburn H, Buzina R, et al. The diet and 15-year death rate in the seven countries study. Am J Epidemiol. 1986;124:903–15. [DOI] [PubMed]

29. Knoops KTB, de Groot LCPGM, Kromhout D, Perrin AE, Moreiras-Varela O, Menotti A, et al. Mediterranean diet, lifestyle factors, and 10-year mortality in elderly European men and women: the HALE project. JAMA. 2004;292:1433–9. [DOI] [PubMed]

30. Castelló A, Rodríguez-Barranco M, de Larrea NF, Jakszyn P, Dorronsoro A, Amiano P, et al. Adherence to the Western, Prudent and Mediterranean Dietary Patterns and Colorectal Cancer Risk: Findings from the Spanish Cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC-Spain). Nutrients. 2022;14:3085. [DOI] [PubMed] [PMC]

31. van den Hoogen PCW, Feskens EJ, Nagelkerke NJ, Menotti A, Nissinen A, Kromhout D. The relation between blood pressure and mortality due to coronary heart disease among men in different parts of the world. N Engl J Med. 2000;342:1–8. [DOI] [PubMed]

32. Willett WC, Sacks F, Trichopoulou A, Drescher G, Ferro-Luzzi A, Helsing E, et al. Mediterranean diet pyramid: a cultural model for healthy eating. Am J Clin Nutr. 1995;61:1402S–6S. [DOI] [PubMed]

33. Shannon OM, Ranson JM, Gregory S, Macpherson H, Milte C, Lentjes M, et al. Mediterranean diet adherence is associated with lower dementia risk, independent of genetic predisposition: findings from the UK Biobank prospective cohort study. BMC Med. 2023;21:81. [DOI] [PubMed] [PMC]

34. Hertog MG, Feskens EJ, Hollman PC, Katan MB, Kromhout D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. Lancet. 1993;342:1007–11. [DOI] [PubMed]

35. Kromhout D, Keys A, Aravanis C, Buzina R, Fidanza F, Giampaoli S, et al. Food consumption patterns in the 1960s in seven countries. Am J Clin Nutr. 1989;49:889–94. [DOI] [PubMed]

36. Anastasiou CA, Yannakoulia M, Kosmidis MH, Dardiotis E, Hadjigeorgiou GM, Sakka P, et al. Mediterranean diet and cognitive health: Initial results from the Hellenic Longitudinal Investigation of Ageing and Diet. PLoS One. 2017;12:e0182048. [DOI] [PubMed] [PMC]

37. Keramati M, Kheirouri S, Etemadifar M. Dietary approach to stop hypertension (DASH), but not Mediterranean and MIND, dietary pattern protects against Parkinson's disease. Food Sci Nutr. 2023; 12:943–51. [DOI] [PubMed] [PMC]

38. Strikwerda AJ, Dommershuijsen LJ, Ikram MK, Voortman T. Diet Quality and Risk of Parkinson's Disease: The Rotterdam Study. Nutrients. 2021;13:3970. [DOI] [PubMed] [PMC]

39. Knight E, Geetha T, Burnett D, Babu JR. The Role of Diet and Dietary Patterns in Parkinson's Disease. Nutrients. 2022;14:4472. [DOI] [PubMed] [PMC]

40. Maraki MI, Yannakoulia M, Xiromerisiou G, Stefanis L, Charisis S, Giagkou N, et al. Mediterranean diet is associated with a lower probability of prodromal Parkinson's disease and risk for Parkinson's disease/dementia with Lewy bodies: A longitudinal study. Eur J Neurol. 2023;30:934–42. [DOI] [PubMed]

41. Safe S, Kothari J, Hailemariam A, Upadhyay S, Davidson LA, Chapkin RS. Health Benefits of Coffee Consumption for Cancer and Other Diseases and Mechanisms of Action. Int J Mol Sci. 2023;24:2706. [DOI] [PubMed] [PMC]

42. Kontou N, Psaltopoulou T, Soupos N, Polychronopoulos E, Linos A, Xinopoulos D, et al. The role of number of meals, coffee intake, salt and type of cookware on colorectal cancer development in the context of the Mediterranean diet. Public Health Nutr. 2013;16:928–35. [DOI] [PubMed] [PMC]

43.   Henn M, Babio N, Romaguera D, Vázquez-Ruiz Z, Konieczna J, Vioque J, et al. Increase from low to moderate, but not high, caffeinated coffee consumption is associated with favorable changes in body fat. Clin Nutr. 2023;42:477–85. [DOI] [PubMed]

44.   Chapkin RS, Davidson LA, Park H, Jin U, Fan Y, Cheng Y, et al. Role of the Aryl Hydrocarbon Receptor (AhR) in Mediating the Effects of Coffee in the Colon. Mol Nutr Food Res. 2021;65:e2100539. [DOI] [PubMed] [PMC]

45.   Wu M, Luo Q, Nie R, Yang X, Tang Z, Chen H. Potential implications of polyphenols on aging considering oxidative stress, inflammation, autophagy, and gut microbiota. Crit Rev Food Sci Nutr. 2021;61:2175–93. [DOI] [PubMed]

46.   Bondonno NP, Dalgaard F, Kyrø C, Murray K, Bondonno CP, Lewis JR, et al. Flavonoid intake is associated with lower mortality in the Danish Diet Cancer and Health Cohort. Nat Commun. 2019;10: 3651. [DOI] [PubMed] [PMC]

47.   Hertog MGL, Kromhout D, Aravanis C, Blackburn H, Buzina R, Fidanza F, et al. Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. Arch Intern Med. 1995;155:381–6. [DOI]

48.   Ivey KL, Hodgson JM, Croft KD, Lewis JR, Prince RL. Flavonoid intake and all-cause mortality. Am J Clin Nutr. 2015;101:1012–20. [DOI] [PubMed]

49.   Mérida DM, Vitelli-Storelli F, Moreno-Franco B, Rodríguez-Ayala M, López-García E, Banegas JR, et al. Polyphenol intake and mortality: A nationwide cohort study in the adult population of Spain. Clin Nutr. 2023;42:1076–85. [DOI] [PubMed]

50.   Liu X, Liu Y, Huang Y, Yu H, Yuan S, Tang B, et al. Dietary total flavonoids intake and risk of mortality from all causes and cardiovascular disease in the general population: A systematic review and meta-analysis of cohort studies. Mol Nutr Food Res. 2017;61:1601003. [DOI] [PubMed]

51.   Ivey KL, Jensen MK, Hodgson JM, Eliassen AH, Cassidy A, Rimm EB. Association of flavonoid-rich foods and flavonoids with risk of all-cause mortality. Br J Nutr. 2017;117:1470–7. [DOI] [PubMed] [PMC]

52.   Rienks J, Barbaresko J, Oluwagbemigun K, Schmid M, Nöthlings U. Polyphenol exposure and risk of type 2 diabetes: dose-response meta-analyses and systematic review of prospective cohort studies. Am J Clin Nutr. 2018;108:49–61. [DOI] [PubMed]

53.   Chen Z, Qian F, Hu Y, Voortman T, Li Y, Rimm EB, et al. Dietary phytoestrogens and total and cause-specific mortality: results from 2 prospective cohort studies. Am J Clin Nutr. 2023;117:130–40. [DOI] [PubMed] [PMC]

54.   Murillo G, Mehta RG. Cruciferous vegetables and cancer prevention. Nutr Cancer. 2001;41:17–28. [DOI] [PubMed]

55.   Key TJ. Fruit and vegetables and cancer risk. Br J Cancer. 2011;104:6–11. [DOI] [PubMed] [PMC]

56.   Rodríguez-García C, Sánchez-Quesada C, Gaforio JJ. Dietary Flavonoids as Cancer Chemopreventive Agents: An Updated Review of Human Studies. Antioxidants (Basel). 2019;8:137. [DOI] [PubMed] [PMC]

57.   Abbaszadeh H, Keikhaei B, Mottaghi S. A review of molecular mechanisms involved in anticancer and antiangiogenic effects of natural polyphenolic compounds. Phytother Res. 2019;33:2002–14. [DOI] [PubMed]

58.   Khater M, Greco F, Osborn HMI. Antiangiogenic Activity of Flavonoids: A Systematic Review and Meta-Analysis. Molecules. 2020;25:4712. [DOI] [PubMed] [PMC]

59.   Abotaleb M, Samuel SM, Varghese E, Varghese S, Kubatka P, Liskova A, et al. Flavonoids in Cancer and Apoptosis. Cancers (Basel). 2018;11:28. [DOI] [PubMed] [PMC]

60.   Bisol Â, de Campos PS, Lamers ML. Flavonoids as anticancer therapies: A systematic review of clinical trials. Phytother Res. 2020;34:568–82. [DOI] [PubMed]

61.   Niedzwiecki A, Roomi MW, Kalinovsky T, Rath M. Anticancer Efficacy of Polyphenols and Their Combinations. Nutrients. 2016;8:552. [DOI] [PubMed] [PMC]

62. Kopustinskiene DM, Jakstas V, Savickas A, Bernatoniene J. Flavonoids as Anticancer Agents. Nutrients. 2020;12:457. [DOI] [PubMed] [PMC]

63. Kikuchi H, Yuan B, Hu X, Okazaki M. Chemopreventive and anticancer activity of flavonoids and its possibility for clinical use by combining with conventional chemotherapeutic agents. Am J Cancer Res. 2019;9:1517–35. [PubMed] [PMC]

64. Grosso G, Godos J, Lamuela-Raventos R, Ray S, Micek A, Pajak A, et al. A comprehensive meta-analysis on dietary flavonoid and lignan intake and cancer risk: Level of evidence and limitations. Mol Nutr Food Res. 2017;61:1600930. [DOI] [PubMed]

65. Fike LT, Munro H, Yu D, Dai Q, Shrubsole MJ. Dietary polyphenols and the risk of colorectal cancer in the prospective Southern Community Cohort Study. Am J Clin Nutr. 2022;115:1155–65. [DOI] [PubMed] [PMC]

66. Monllor-Tormos A, García-Vigara A, Morgan O, García-Pérez M, Mendoza N, Tarín JJ, et al. Mediterranean diet for cancer prevention and survivorship. Maturitas. 2023;178:107841. [DOI] [PubMed]

67. Taliou A, Zintzaras E, Lykouras L, Francis K. An open-label pilot study of a formulation containing the anti-inflammatory flavonoid luteolin and its effects on behavior in children with autism spectrum disorders. Clin Ther. 2013;35:592–602. [DOI] [PubMed]

68. Myers G, Prince RL, Kerr DA, Devine A, Woodman RJ, Lewis JR, et al. Tea and flavonoid intake predict osteoporotic fracture risk in elderly Australian women: a prospective study. Am J Clin Nutr. 2015; 102:958–65. [DOI] [PubMed]

69. Burden S, Jones DJ, Sremanakova J, Sowerbutts AM, Lal S, Pilling M, et al. Dietary interventions for adult cancer survivors. Cochrane Database Syst Rev. 2019;2019:CD011287. [DOI] [PubMed] [PMC]

70. El-Rayes BF, Philip PA, Sarkar FH, Shields AF, Ferris AM, Hess K, et al. A phase II study of isoflavones, erlotinib, and gemcitabine in advanced pancreatic cancer. Invest New Drugs. 2011;29:694–9. [DOI] [PubMed]

71. Shafei LKIA, Ibrahim MIM, Billa N. Is Curcumin at the Threshold of Therapeutic Effectiveness on Patients with Colon Cancer? Front Pharmacol. 2021;12:707231. [DOI] [PubMed] [PMC]

72. Panknin TM, Howe CL, Hauer M, Bucchireddigari B, Rossi AM, Funk JL. Curcumin Supplementation and Human Disease: A Scoping Review of Clinical Trials. Int J Mol Sci. 2023;24:4476. [DOI] [PubMed] [PMC]

73. de Waure C, Bertola C, Baccarini G, Chiavarini M, Mancuso C. Exploring the Contribution of Curcumin to Cancer Therapy: A Systematic Review of Randomized Controlled Trials. Pharmaceutics. 2023;15: 1275. [DOI] [PubMed] [PMC]

74. Beishline K, Azizkhan-Clifford J. Sp1 and the 'hallmarks of cancer'. FEBS J. 2015;282:224–58. [DOI] [PubMed]

75. Safe S, Abbruzzese J, Abdelrahim M, Hedrick E. Specificity Protein Transcription Factors and Cancer: Opportunities for Drug Development. Cancer Prev Res (Phila). 2018;11:371–82. [DOI] [PubMed]

76. Orzechowska-Licari EJ, LaComb JF, Mojumdar A, Bialkowska AB. SP and KLF Transcription Factors in Cancer Metabolism. Int J Mol Sci. 2022;23:9956. [DOI] [PubMed] [PMC]

77. Vizcaíno C, Mansilla S, Portugal J. Sp1 transcription factor: A long-standing target in cancer chemotherapy. Pharmacol Ther. 2015;152:111–24. [DOI] [PubMed]

78. Safe S. Specificity Proteins (Sp) and Cancer. Int J Mol Sci. 2023;24:5164. [DOI] [PubMed] [PMC]

79. Oh J, Han J, Hwang ES. Downregulation of transcription factor, Sp1, during cellular senescence. Biochem Biophys Res Commun. 2007;353:86–91. [DOI] [PubMed]

80. Adrian GS, Seto E, Fischbach KS, Rivera EV, Adrian EK, Herbert DC, et al. YY1 and Sp1 transcription factors bind the human transferrin gene in an age-related manner. J Gerontol A Biol Sci Med Sci. 1996;51:B66–75. [DOI] [PubMed]

81. Zhu Y, Cui J, Liu J, Hua W, Wei W, Sun G. Sp2 promotes invasion and metastasis of hepatocellular carcinoma by targeting TRIB3 protein. Cancer Med. 2020;9:3592–603. [DOI] [PubMed] [PMC]

82. Lou Z, O'Reilly S, Liang H, Maher VM, Sleight SD, McCormick JJ. Down-regulation of overexpressed sp1 protein in human fibrosarcoma cell lines inhibits tumor formation. Cancer Res. 2005;65: 1007–17. [DOI]

83. McCormick JJ, Maher VM. Malignant transformation of human skin fibroblasts by two alternative pathways. Adv Exp Med Biol. 2011;720:191–207. [DOI] [PubMed]

84. Jin H, Xu J, Guo X, Huang H, Li J, Peng M, et al. XIAP RING domain mediates miR-4295 expression and subsequently inhibiting p63α protein translation and promoting transformation of bladder epithelial cells. Oncotarget. 2016;7:56540–57. [DOI] [PubMed] [PMC]

85. Zhong X, Zheng L, Shen J, Zhang D, Xiong M, Zhang Y, et al. Suppression of MicroRNA 200 Family Expression by Oncogenic KRAS Activation Promotes Cell Survival and Epithelial-Mesenchymal Transition in KRAS-Driven Cancer. Mol Cell Biol. 2016;36:2742–54. [DOI] [PubMed] [PMC]

86. Kwon Y, Baek H, Ye D, Shin S, Kim D, Chun Y. CYP1B1 Enhances Cell Proliferation and Metastasis through Induction of EMT and Activation of Wnt/β-Catenin Signaling via Sp1 Upregulation. PLoS One. 2016;11:e0151598. [DOI] [PubMed] [PMC]

87. He J, Liu W, Ge X, Wang G, Desai V, Wang S, et al. Arsenic-induced metabolic shift triggered by the loss of miR-199a-5p through Sp1-dependent DNA methylation. Toxicol Appl Pharmacol. 2019;378: 114606. [DOI] [PubMed] [PMC]

88. Naini S, Etheridge KT, Adam SJ, Qualman SJ, Bentley RC, Counter CM, et al. Defining the cooperative genetic changes that temporally drive alveolar rhabdomyosarcoma. Cancer Res. 2008;68:9583–8. [DOI] [PubMed] [PMC]

89. Chadalapaka G, Jutooru I, Sreevalsan S, Pathi S, Kim K, Chen C, et al. Inhibition of rhabdomyosarcoma cell and tumor growth by targeting specificity protein (Sp) transcription factors. Int J Cancer. 2013; 132:795–806. [DOI] [PubMed] [PMC]

90. Hedrick E, Cheng Y, Jin U, Kim K, Safe S. Specificity protein (Sp) transcription factors Sp1, Sp3 and Sp4 are non-oncogene addiction genes in cancer cells. Oncotarget. 2016;7:22245–56. [DOI] [PubMed] [PMC]

91. Wei D, Wang L, He Y, Xiong HQ, Abbruzzese JL, Xie K. Celecoxib inhibits vascular endothelial growth factor expression in and reduces angiogenesis and metastasis of human pancreatic cancer via suppression of Sp1 transcription factor activity. Cancer Res. 2004;64:2030–8. [DOI] [PubMed]

92. Hedrick E, Crose L, Linardic CM, Safe S. Histone Deacetylase Inhibitors Inhibit Rhabdomyosarcoma by Reactive Oxygen Species-Dependent Targeting of Specificity Protein Transcription Factors. Mol Cancer Ther. 2015;14:2143–53. [DOI] [PubMed] [PMC]

93. Nair V, Sreevalsan S, Basha R, Abdelrahim M, Abudayyeh A, Hoffman AR, et al. Mechanism of metformin-dependent inhibition of mammalian target of rapamycin (mTOR) and Ras activity in pancreatic cancer: role of specificity protein (Sp) transcription factors. J Biol Chem. 2014;289: 27692–701. [DOI] [PubMed] [PMC]

94. Nair V, Pathi S, Jutooru I, Sreevalsan S, Basha R, Abdelrahim M, et al. Metformin inhibits pancreatic cancer cell and tumor growth and downregulates Sp transcription factors. Carcinogenesis. 2013;34: 2870–9. [DOI] [PubMed] [PMC]

95. Hsu T, Wang M, Chen S, Huang S, Yeh Y, Su W, et al. Betulinic acid decreases specificity protein 1 (Sp1) level via increasing the sumoylation of sp1 to inhibit lung cancer growth. Mol Pharmacol. 2012;82:1115–28. [DOI] [PubMed]

96. Lo W, Hsu T, Yang W, Kao T, Wu M, Huang Y, et al. Betulinic Acid-Mediated Tuning of PERK/CHOP Signaling by Sp1 Inhibition as a Novel Therapeutic Strategy for Glioblastoma. Cancers (Basel). 2020; 12:981. [DOI] [PubMed] [PMC]

97. Chintharlapalli S, Papineni S, Ramaiah SK, Safe S. Betulinic acid inhibits prostate cancer growth through inhibition of specificity protein transcription factors. Cancer Res. 2007;67:2816–23. [DOI] [PubMed]

98. Liu X, Jutooru I, Lei P, Kim K, Lee S, Brents LK, et al. Betulinic acid targets YY1 and ErbB2 through cannabinoid receptor-dependent disruption of microRNA-27a:ZBTB10 in breast cancer. Mol Cancer Ther. 2012;11:1421–31. [DOI] [PubMed] [PMC]

99. Chintharlapalli S, Papineni S, Lei P, Pathi S, Safe S. Betulinic acid inhibits colon cancer cell and tumor growth and induces proteasome-dependent and -independent downregulation of specificity proteins (Sp) transcription factors. BMC Cancer. 2011;11:371. [DOI] [PubMed] [PMC]

100. Li W, Liu M, Xu Y, Feng Y, Che J, Wang G, et al. Combination of quercetin and hyperoside has anticancer effects on renal cancer cells through inhibition of oncogenic microRNA-27a. Oncol Rep. 2014;31:117–24. [DOI] [PubMed]

101. Huang K, Chen Y, Zhang R, Wu Y, Ma Y, Fang X, et al. Honokiol induces apoptosis and autophagy via the ROS/ERK1/2 signaling pathway in human osteosarcoma cells in vitro and in vivo. Cell Death Dis. 2018;9:157. [DOI] [PubMed] [PMC]

102. Kim DW, Ko SM, Jeon YJ, Noh YW, Choi NJ, Cho SD, et al. Anti-proliferative effect of honokiol in oral squamous cancer through the regulation of specificity protein 1. Int J Oncol. 2013;43:1103–10. [DOI]

103. Jutooru I, Guthrie AS, Chadalapaka G, Pathi S, Kim K, Burghardt R, et al. Mechanism of action of phenethylisothiocyanate and other reactive oxygen species-inducing anticancer agents. Mol Cell Biol. 2014;34:2382–95. [DOI] [PubMed] [PMC]

104. Li C, Zhou Y, Tu P, Zeng K, Jiang Y. Natural carbazole alkaloid murrayafoline A displays potent anti-neuroinflammatory effect by directly targeting transcription factor Sp1 in LPS-induced microglial cells. Bioorg Chem. 2022;129:106178. [DOI] [PubMed]

105. Chae J, Cho JH, Lee K, Choi N, Seo KS, Kim S, et al. Role of transcription factor Sp1 in the quercetin-mediated inhibitory effect on human malignant pleural mesothelioma. Int J Mol Med. 2012;30: 835–41. [DOI] [PubMed]

106. Slika H, Mansour H, Wehbe N, Nasser SA, Iratni R, Nasrallah G, et al. Therapeutic potential of flavonoids in cancer: ROS-mediated mechanisms. Biomed Pharmacother. 2022;146:112442. [DOI] [PubMed]

107. Wang Y, Hu J. Natural and synthetic compounds for glioma treatment based on ROS-mediated strategy. Eur J Pharmacol. 2023;953:175537. [DOI] [PubMed]

108. Khan F, Pandey P, Verma M, Ramniwas S, Lee D, Moon S, et al. Emerging trends of phytochemicals as ferroptosis modulators in cancer therapy. Biomed Pharmacother. 2024;173:116363. [DOI] [PubMed]

109. Zhang J, Duan D, Song Z, Liu T, Hou Y, Fang J. Small molecules regulating reactive oxygen species homeostasis for cancer therapy. Med Res Rev. 2021;41:342–94. [DOI] [PubMed]

110. Liu Y, Fan C, Pu L, Wei C, Jin H, Teng Y, et al. Phloretin induces cell cycle arrest and apoptosis of human glioblastoma cells through the generation of reactive oxygen species. J Neurooncol. 2016; 128:217–23. [DOI] [PubMed]

111. Choi J, Kim J, Lee H, Pak J, Shim BS, Kim S. Reactive Oxygen Species and p53 Mediated Activation of p38 and Caspases is Critically Involved in Kaempferol Induced Apoptosis in Colorectal Cancer Cells. J Agric Food Chem. 2018;66:9960–7. [DOI] [PubMed]

112. Seydi E, Salimi A, Rasekh HR, Mohsenifar Z, Pourahmad J. Selective Cytotoxicity of Luteolin and Kaempferol on Cancerous Hepatocytes Obtained from Rat Model of Hepatocellular Carcinoma: Involvement of ROS-Mediated Mitochondrial Targeting. Nutr Cancer. 2018;70:594–604. [DOI] [PubMed]

113. Raza W, Meena A, Luqman S. 3,5,7-trihydroxyflavone restricts proliferation of androgen-independent human prostate adenocarcinoma cells by inducing ROS-mediated apoptosis and reduces tumour growth. J Biochem Mol Toxicol. 2023;37:e23474. [DOI] [PubMed]

114. Li X, Zhu Q, Ma M, Guo H. Quercetin inhibits the progression of endometrial HEC-1-A cells by regulating ferroptosis-a preliminary study. Eur J Med Res. 2022;27:292. [DOI] [PubMed] [PMC]

115. Bhosale PB, Abusaliya A, Kim HH, Ha SE, Park MY, Jeong SH, et al. Apigetrin Promotes TNFα-Induced Apoptosis, Necroptosis, G2/M Phase Cell Cycle Arrest, and ROS Generation through Inhibition of NF-κB Pathway in Hep3B Liver Cancer. Cells. 2022;11:2734. [DOI] [PubMed] [PMC]

116. Pandey P, Khan F, Maurya P. Targeting Jab1 using hesperidin (dietary phytocompound) for inducing apoptosis in HeLa cervical cancer cells. J Food Biochem. 2021;45:e13800. [DOI] [PubMed]

117. Zhang Z, Pan Y, Zhao Y, Ren M, Li Y, Lu G, et al. Delphinidin modulates JAK/STAT3 and MAPKinase signaling to induce apoptosis in HCT116 cells. Environ Toxicol. 2021;36:1557–66. [DOI] [PubMed]

118. Shendge AK, Chaudhuri D, Mandal N. The natural flavones, acacetin and apigenin, induce Cdk-Cyclin mediated G2/M phase arrest and trigger ROS-mediated apoptosis in glioblastoma cells. Mol Biol Rep. 2021;48:539–49. [DOI] [PubMed]

119. Shendge AK, Chaudhuri D, Basu T, Mandal N. A natural flavonoid, apigenin isolated from *Clerodendrum viscosum* leaves, induces G2/M phase cell cycle arrest and apoptosis in MCF-7 cells through the regulation of p53 and caspase-cascade pathway. Clin Transl Oncol. 2021;23:718–30. [DOI] [PubMed]

120. Kaushik S, Shyam H, Agarwal S, Sharma R, Nag TC, Dwivedi AK, et al. Genistein potentiates Centchroman induced antineoplasticity in breast cancer via PI3K/Akt deactivation and ROS dependent induction of apoptosis. Life Sci. 2019;239:117073. [DOI] [PubMed]

121. Choi YH. Isorhamnetin induces ROS-dependent cycle arrest at G2/M phase and apoptosis in human hepatocarcinoma Hep3B cells. Gen Physiol Biophys. 2019;38:473–84. [DOI] [PubMed]

122. Lee YJ, Kim WI, Kim SY, Cho SW, Nam HS, Lee SH, et al. Flavonoid morin inhibits proliferation and induces apoptosis of melanoma cells by regulating reactive oxygen species, Sp1 and Mcl-1. Arch Pharm Res. 2019;42:531–42. [DOI] [PubMed]

123. Schomberg J, Wang Z, Farhat A, Guo KL, Xie J, Zhou Z, et al. Luteolin inhibits melanoma growth in vitro and in vivo via regulating ECM and oncogenic pathways but not ROS. Biochem Pharmacol. 2020;177:114025. [DOI] [PubMed]

124. O'Hagan HM, Wang W, Sen S, Shields CD, Lee SS, Zhang YW, et al. Oxidative damage targets complexes containing DNA methyltransferases, SIRT1, and polycomb members to promoter CpG Islands. Cancer Cell. 2011;20:606–19. [DOI] [PubMed] [PMC]

125. Kasiappan R, Jutooru I, Karki K, Hedrick E, Safe S. Benzyl Isothiocyanate (BITC) Induces Reactive Oxygen Species-dependent Repression of STAT3 Protein by Down-regulation of Specificity Proteins in Pancreatic Cancer. J Biol Chem. 2016;291:27122–33. [DOI] [PubMed] [PMC]

126. Kasiappan R, Jutooru I, Mohankumar K, Karki K, Lacey A, Safe S. Reactive Oxygen Species (ROS)-Inducing Triterpenoid Inhibits Rhabdomyosarcoma Cell and Tumor Growth through Targeting Sp Transcription Factors. Mol Cancer Res. 2019;17:794–805. [DOI] [PubMed] [PMC]

127. Piccolo M, Ferraro MG, Iazzetti F, Santamaria R, Irace C. Insight into Iron, Oxidative Stress and Ferroptosis: Therapy Targets for Approaching Anticancer Strategies. Cancers (Basel). 2024;16:1220. [DOI] [PubMed] [PMC]

128. Wang J, Li J, Liu J, Chan K, Lee H, Lin KN, et al. Interplay of Ferroptosis and Cuproptosis in Cancer: Dissecting Metal-Driven Mechanisms for Therapeutic Potentials. Cancers (Basel). 2024;16:512. [DOI] [PubMed] [PMC]

129. Bian W, Li H, Chen Y, Yu Y, Lei G, Yang X, et al. Ferroptosis mechanisms and its novel potential therapeutic targets for DLBCL. Biomed Pharmacother. 2024;173:116386. [DOI] [PubMed]

130. Lei G, Gan B. Exploring Ferroptosis-Inducing Therapies for Cancer Treatment: Challenges and Opportunities. Cancer Res. 2024;84:961–4. [DOI] [PubMed]

131. Chen X, Li J, Kang R, Klionsky DJ, Tang D. Ferroptosis: machinery and regulation. Autophagy. 2021;17:2054–81. [DOI] [PubMed] [PMC]

132. Xu G, Wang H, Li X, Huang R, Luo L. Recent progress on targeting ferroptosis for cancer therapy. Biochem Pharmacol. 2021;190:114584. [DOI] [PubMed]

133. Stepanić V, Kučerová-Chlupáčová M. Review and Chemoinformatic Analysis of Ferroptosis Modulators with a Focus on Natural Plant Products. Molecules. 2023;28:475. [DOI] [PubMed] [PMC]

134. Xie Y, Song X, Sun X, Huang J, Zhong M, Lotze MT, et al. Identification of baicalein as a ferroptosis inhibitor by natural product library screening. Biochem Biophys Res Commun. 2016;473:775–80. [DOI] [PubMed]

135. Zhao X, Wang X, Pang Y. Phytochemicals Targeting Ferroptosis: Therapeutic Opportunities and Prospects for Treating Breast Cancer. Pharmaceuticals (Basel). 2022;15:1360. [DOI] [PubMed] [PMC]

136. Maxwell MA, Muscat GEO. The NR4A subgroup: immediate early response genes with pleiotropic physiological roles. Nucl Recept Signal. 2006;4:e002. [DOI] [PubMed] [PMC]

137. Pearen MA, Muscat GEO. Minireview: Nuclear hormone receptor 4A signaling: implications for metabolic disease. Mol Endocrinol. 2010;24:1891–903. [DOI] [PubMed] [PMC]

138. Lith SC, van Os BW, Seijkens TTP, de Vries CJM. 'Nur'turing tumor T cell tolerance and exhaustion: novel function for Nuclear Receptor Nur77 in immunity. Eur J Immunol. 2020;50:1643–52. [DOI] [PubMed] [PMC]

139. Safe S, Karki K. The Paradoxical Roles of Orphan Nuclear Receptor 4A (NR4A) in Cancer. Mol Cancer Res. 2021;19:180–91. [DOI] [PubMed] [PMC]

140. Safe S. Natural products and synthetic analogs as selective orphan nuclear receptor 4A (NR4A) modulators. Histol Histopathol. 2024;39:543–56. [DOI] [PubMed]

141. Shrestha R, Mohankumar K, Martin G, Hailemariam A, Lee S, Jin U, et al. Flavonoids kaempferol and quercetin are nuclear receptor 4A1 (NR4A1, Nur77) ligands and inhibit rhabdomyosarcoma cell and tumor growth. J Exp Clin Cancer Res. 2021;40:392. [DOI] [PubMed] [PMC]

142. Zhang L, Mohankumar K, Martin G, Mariyam F, Park Y, Han SJ, et al. Flavonoids Quercetin and Kaempferol Are NR4A1 Antagonists and Suppress Endometriosis in Female Mice. Endocrinology. 2023;164:bqad133. [DOI] [PubMed]

143. Zhang L, Martin G, Mohankumar K, Hampton JT, Liu WR, Safe S. Resveratrol Binds Nuclear Receptor 4A1 (NR4A1) and Acts as An NR4A1 Antagonist in Lung Cancer Cells. Mol Pharmacol. 2022;102: 80–91. [DOI] [PubMed] [PMC]

144. Lee H, Kim S, Kim B, Safe S, Lee S. Broussochalcone A Is a Novel Inhibitor of the Orphan Nuclear Receptor NR4A1 and Induces Apoptosis in Pancreatic Cancer Cells. Molecules. 2021;26:2316. [DOI] [PubMed] [PMC]

145. Lee H, Kim DH, Lee I, Park J, Martin G, Safe S, et al. Plant Alkaloid Tetrandrine Is a Nuclear Receptor 4A1 Antagonist and Inhibits Panc-1 Cell Growth In Vitro and In Vivo. Int J Mol Sci. 2022;23:5280. [DOI] [PubMed] [PMC]

146. Lee M, Upadhyay S, Mariyam F, Martin G, Hailemariam A, Lee K, et al. Flavone and Hydroxyflavones Are Ligands That Bind the Orphan Nuclear Receptor 4A1 (NR4A1). Int J Mol Sci. 2023;24:8152. [DOI] [PubMed] [PMC]