Curcumin

Summary:
- GSH depletion, exhaustion of the antioxidant defense system
- higher concentrations (5-10 µM) of curcumin induce autophagy and ROS production
- Inhibition of TrxR, shifting the enzyme from an antioxidant to a prooxidant
- strong inhibitor of Glo1, causes depletion of cellular ATP and GSH
-Curcumin has been found to act as an activator of Nrf2 (bad), hence could be combined with Nrf2 knockdown(see below, example propolis) to improve efficacy
- upregulates BAX, downregulates Bcl-2

https://pubmed.ncbi.nlm.nih.gov/33656766/
Curcumin induces ferroptosis in non-small-cell lung cancer via activating autophagy 2021
Background: Emerging studies showed curcumin can inhibit glioblastoma and breast cancer cells via regulating ferroptosis. However, the role of ferroptosis in the inhibitory effect of curcumin on non-small-cell lung cancer (NSCLC) remains unclear.
Results: Curcumin inhibited tumor growth and cell proliferation, but promoted cell death. Characteristic changes of ferroptosis were observed in curcumin group, including iron overload, GSH depletion and lipid peroxidation.

https://www.researchgate.net/publication/377208738_Curcumin_and_Ferroptosis_a_Promising_Target_for_Disease_Prevention_and_Treatment
Curcumin and Ferroptosis: a Promising Target for Disease Prevention and Treatment 2024
Ferroptosis is caused by oxidative disturbances of the intracellular microenvironment, which are under the control of glutathione peroxidase 4 (GPX4) and can be prevented by lipophilic antioxidants and iron chelators. GPX4 is a selenoenzyme that can reduce lipid peroxides to lipid alcohol using glutathione (GSH) as a cofactor. Ferroptosis results from a substantial accumulation of lipid hydroperoxides when GPX4 activity is suppressed [6].
By modulating their activity, curcumin can influence redox homeostasis as a whole. In particular, it inhibits the activity of glutathione peroxidase, provoking cell death through ferroptosis [16,17]

https://www.cambridge.org/core/journals/british-journal-of-nutrition/article/improvement-of-sulphur-mustardinduced-chronic-pruritus-quality-of-life-and-antioxidant-status-by-curcumin-results-of-a-randomised-doubleblind-placebocontrolled-trial/67C0DC2E4FA22188D2973BCA3C0F14D1
Improvement of sulphur mustard-induced chronic pruritus, quality of life and antioxidant status by curcumin: results of a randomised, double-blind, placebo-controlled trial 2011
The present trial investigated the efficacy of curcumin in the alleviation of SM-induced chronic pruritic symptoms. A total of ninety-six male Iranian veterans (age 37–59 years) were randomised to receive either curcumin (1 g/d, n 46) or placebo (n 50) for 4 weeks. Serum concentrations of substance P and activities of antioxidant enzymes were measured at baseline and at the end of the trial.
Serum concentrations of substance P ($P < 0{\cdot}001$) as well as activities of superoxide dismutase ($P = 0{\cdot}02$), glutathione peroxidase ($P = 0{\cdot}006$) and catalase ($P < 0{\cdot}001$) were significantly reduced in the curcumin group, while no significant change was observed in the placebo group.

https://www.nature.com/articles/s41418-023-01178-1
Curcumin activates a ROS/KEAP1/NRF2/miR-34a/b/c cascade to suppress colorectal cancer metastasis 2023
Therefore, the accumulation of ROS presumably mediates curcumin-induced apoptosis. The diketone group of curcumin conjugates with glutathione-SH, leading to the depletion of the glutathione pool and exhaustion of the antioxidant defense system in cells [33]. Curcumin has both antioxidative and pro-oxidative properties depending on the dose and cell types. At very low concentrations (≤ 1 µM) curcumin functions as an antioxidant in non-cancerous cells [34]. However, in cancer cells higher concentrations (5-10 µM) of curcumin induce autophagy and ROS production [35, 36]. In addition, concentrations of curcumin beyond 5.8 ± 1.6 µM induce endoplasmic reticulum (ER) membrane destabilization [37, 34] and inhibit Ca2 + -ATPase (SERCA) [38], thereby leading to release of Ca2+ causing mitochondrial destabilization and thereby additional release of ROS [39].

https://aacrjournals.org/mct/article/3/9/1101/234394/Effect-of-curcumin-on-normal-and-tumor-cells-Role
Effect of curcumin on normal and tumor cells: Role of glutathione and bcl-2 2004
Glutathione (GSH), also known as γ-l-glutamyl-l-cysteine-glycine, is an ubiquitous tripeptide that functions as an important intracellular radical scavenger. It protects cells against reactive oxygen species (ROS) as well as against many toxins, mutagens, and drugs. GSH is also essential for the cellular metabolism of various enzymes, hormones, and amino acids. GSH also plays an important role in multidrug resistance either through its spontaneous reactions or through its function as a coenzyme in glutathione *S*-transferases (GST) reacting with the drug (1). Cellular redox potential is largely determined by GSH content, which accounts for >90% of the cellular nonprotein thiols (2). It is important for many biochemical functions including the regulation of gene transcription as well as modulation of apoptosis (3). GSTs belong to a family of enzymes that catalyze the conjugation of GSH to a wide variety of chemical toxins (4) and reactive electrophiles (5). Blockage of the GSH/GST detoxification system enhanced the chemosensitivity of several tumor cell lines (6). Generation of oxidative intermediates has been proposed to be a critical event in the process of programmed cell death induced by various agents. Consequently, depletion of GSH has been found to either precede the onset of apoptosis or render the cells more sensitive to cell death (7).
In the present study, we show that curcumin induced apoptosis in human breast carcinoma cell lines as well as in human hepatoma cells but failed to do so in normal rat hepatocyte primary cultures. Furthermore, our results indicate that GSH plays a vital role in the sensitivity of these cell lines to curcumin. Depletion of GSH further sensitized the cells to curcumin effects, and the cell death is caused by the generation of ROS.
Because depletion of GSH could either precede the onset of apoptotic cell death or render the cells more sensitive to apoptotic agents, we tested cell viability using propidium iodide staining after treating the cells with BSO (1 mmol/L) for 2 hours prior to curcumin treatment. Viability of cells depleted of GSH was significantly lower showing at least 30% increase in dead cells (Fig. 1C–E). Surprisingly, in MDAMB cells, no significant difference was seen in GSH-depleted or nondepleted cells (Fig. 1D). On the other hand, exogenous GSH protected all the cell lines from undergoing apoptosis (Fig. 1B).

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7356876/
The Cancer Chemopreventive and Therapeutic Potential of Tetrahydrocurcumin 2020
Notably, this study demonstrated that tetrahydrocurcumin decreased intracellular glutathione (GSH) in glioma cells: elevated GSH levels have been shown to increase the anti-oxidative capacity of cancer cells against oxidative stress, which plays a key role in therapeutic resistance [99]. The effect of tetrahydrocurcumin alone or in combination with radiation on decreasing GSH content may be related to its anti-proliferative effect in glioma cells [72]. Because tetrahydrocurcumin is unable to react with thiols and deplete cellular GSH—due to the lack of unsaturated β-diketone group present in curcumin—it is not clear how the reduction of GSH is mediated; further examination of this is needed.

https://www.sciencedirect.com/science/article/abs/pii/S0753332217311472

Curcumin inhibited growth of human melanoma A375 cells via inciting oxidative stress 2017
Here, we firstly found a fascinating result that Curcumin could reduce the proliferation and induced apoptosis of human melanoma A375 cells. Meanwhile, IC_{50} of Curcumin on A375 cells is 80 μM at 48 h. In addition, Curcumin caused oxidative stress through inducing further ROS burst, decreasing GSH, and wrecking mitochondria membrane potential (MMP), which were reversed by ROS inhibitor *N*-acetylcysteine (NAC).

https://pubmed.ncbi.nlm.nih.gov/15879598/
Thioredoxin reductase is irreversibly modified by curcumin: a novel molecular mechanism for its anticancer activity 2005
TrxRs (thioredoxin reductase (TrxR) isoenzymes) have been found to be overexpressed by a number of human tumors.
Inhibition of TrxR by curcumin added to cultured HeLa cells was also observed with an IC_{50} of around 15 μm. Modification of TrxR by curcumin provides a possible mechanistic explanation for its cancer preventive activity, shifting the enzyme from an antioxidant to a prooxidant.
The **thioredoxin system, composed of TrxR, Trx, and NADPH**, is the major disulfide reducing enzyme system in all cells responsible for maintaining the intracellular redox milieu with a high content of free protein thiols and rare disulfides (17-19).

https://www.sciencedirect.com/topics/nursing-and-health-professions/thioredoxin-reductase
Curcumin in cancer prevention and therapy 2020
Thioredoxin reductase (TrxR), as part of a major thiol regulating system, allows the redox metabolism to adjust to cellular requirements.
TrxR can scavenge ROS and directly inhibit proapoptotic proteins such as apoptosis signal-regulating kinase 1 (ASK1). Many tumor cells have elevated TrxR levels and TrxR has been shown to play a major role in drug resistance. Inhibition of TrxR and its related redox reactions thus contributes to a successful single, combinatory, or adjuvant cancer therapy. Curcumin inhibits TrxR1 activity in Trx-dependent disulfide to induce apoptosis in cancer cells (Fang et al., 2005; Urig and Becker, 2006).

https://pubmed.ncbi.nlm.nih.gov/33791015/
Cytotoxic, chemosensitizing and radiosensitizing effects of curcumin based on thioredoxin system inhibition in breast cancer cells: 2D vs. 3D cell culture system 2021
Targeting the thioredoxin/thioredoxin reductase (Trx/TrxR) system is a promising strategy to overcome cancer resistance to conventional therapy. The present study investigated the effect of curcumin on the Trx/TrxR system either alone or in combination with chemotherapy, or radiotherapy in human MCF-7 breast cancer cells seeded in 2 and 3D culture systems. Cell viability, thioredoxin reductase 1 (TrxR1) activity, and the genetic expression of Trx, TrxR1, Bcl2 and BAX genes were studied. The findings showed that the mode of culture significantly affected the response of cancer cells to different treatment modalities, as well as their gene expression patterns. Curcumin treatment resulted in a reduction of breast cancer cell proliferation and induction of apoptosis, an effect that may be mediated by manipulating Trx system components, mainly Trx expression, and to a lesser extent TrxR1 expression and concentration. Furthermore, curcumin increased the sensitivity of breast cancer cells to chemotherapy and radiotherapy by reducing Trx and TrxR1 expression levels. Thus, curcumin may have a potential role as a dose-modifying agent that can be used either to sensitize resistant cells to therapy or to reduce the dose of these therapeutic agents.

https://pmc.ncbi.nlm.nih.gov/articles/PMC2567432/
Curcumin Inhibits Glyoxalase 1—A Possible Link to Its Anti-Inflammatory and Anti-Tumor Activity 2008
The results described herein provide new insights into curcumin's biological activities as they indicate that inhibition of Glo1 by curcumin may result in non-tolerable levels of MGO and GSH, which, in turn, modulate various metabolic cellular pathways including depletion of cellular ATP and GSH content. This may account for curcumin's potency as an anti-inflammatory and anti-tumor agent. The findings support the use of curcumin as a potential therapeutic agent.
We found that curcumin acts as a strong inhibitor of Glo1, causes depletion of cellular ATP and GSH and thus has a potential impact at cellular metabolism predominantly in cells whose energy gain relies on the glycolytic pathway.

https://pmc.ncbi.nlm.nih.gov/articles/PMC11615379/
Nrf2 depletion enhanced curcumin therapy effect in gastric cancer by inducing the excessive accumulation of ROS 2024
Here, Cur showed a stronger inhibitory effect on GC cells AGS and HGC27. In addition, Cur's inhibition of GC cells growth was accompanied by increased ROS production, triggering of the Keap1-Nrf2 signaling pathway, and increased transcription of its downstream antioxidant genes HO-1, GCLM, and NQO1. However, when a ROS scavenger NAC was used, the inhibitory effect of Cur on GC cells was reversed. Nuclear factor erythroid 2-related factor 2 (Nrf2) is overexpressed or activated in cancers to shield cancer cells from oxidative damage by responding to oxidative stress (OS). Cur has been found to act as an activator of Nrf2.
Notably, compared with Nrf2 knockdown and Cur alone, the combination of the two dramatically increased Cur-induced ROS overaccumulation and inhibition of GC cells proliferation, migration, and invasive abilities. Consistent with in vitro experiments, Cur combined with Nrf2 knockdown significantly inhibited tumor growth in nude mice transplanted with AGS cells. Therefore, we concluded that Nrf2 depletion enhanced Cur therapy effect in GC by inducing the excessive accumulation of ROS, indicating that this is a promising treatment strategy.

https://biosignaling.biomedcentral.com/articles/10.1186/s12964-022-00906-3
An update of Nrf2 activators and inhibitors in cancer prevention/promotion 2022
Some examples of natural Nrf2 activators include curcumin, sulforaphane (SF), kahweol, resveratrol, garlic oganosulfur compounds, zerumbone, epigallocatechin-3-gallate, carnosol, cinnamonyl-based compounds, lycopene, and cafestol [75,76,77]. Magesh et al. [78] have categorized about 90 kinds of these synthetic or natural activators of Nrf2 in several groups: (1) isothiocyanates and sulfoxythiocarbamates; (2) oxidizable phenols and quinones; (3) Michael acceptors; (4) vicinal dimercaptans; (5) trivalent arsenicals; (6) dithiolethiones and diallyl sulfides; (7) heavy metals and metal complexes; (8) miscellaneous inducers; (9) selenium-based compounds; (10) polyenes; and (11) hydroxyl peroxides. By inducing the Nrf2-mediated defense response, these chemopreventive agents can activate the antioxidants, phase II detoxification factors, and transducers, and protect the cells from carcinogenic exposure [23].
CUR, at lower concentrations, had demethylating effects on the promoter region of Nrf2, which resulted in elevated expressions of Nrf2 and its target genes [153]. Besides epigenetic modifications in Nrf2, curcumin may indirectly phosphorylate Nrf2 at serine- and/or threonine-rich regions and facilitate the nuclear transition of Nrf2. In addition, it can directly interact with sensor cysteine thiol(s) of Keap1 and diminish its inhibitory effect on Nrf2 [154]. Interestingly, CUR is able to play radiation and chemotherapy sensitizer role in some of the human cancers such as prostate [155], colorectal [156, 157] and ovarian cancer [158]. Some of the clinical trial studies indicated that CUR is quite safe and probably has therapeutic applicability in cancer treatment. Curcumin consumption for 3 months could improve the pre-cancerous lesions of patients with resected uterine cervical intraepithelial neoplasia, intestinal metaplasia, oral leukoplakia, and bladder cancer [159]. Despite the activatory effect of CUR on Nrf2 signaling, it is able to exert inhibitory effects on some other signalings, such as Notch1 [160], NF-kappa B [158] and mitochondrial signaling pathways [161].
Nrf2 inhibitors
In contrast with the several agents that function as Nrf2 inducers, very few molecular components have been recognized as Nrf2 inhibitors. Since Nrf2 has multifaceted roles in cancer cells, Nrf2 inhibitors can be applied as anticancer agents [27, 77, 191]. Indirectly, Nrf2 inhibitors down-regulate drug detoxifying and

eliminating enzymes and sensitize cancer cells to chemotherapeutics [76, 176]. According to the Nrf2 deactivation mechanisms and their potential applications in cancer treatment, several small molecules have been characterized as Nrf2 pathway inhibitors (Fig. 3).

Brusatol (BRU)

Brusatol is a quassinoid which is extracted from Brucea Javanica (Simaroubaceae), an evergreen shrub grown in Northern Australia and Southeast Asia [27]

Luteolin (3′,4′,5,7-tetrahydroxyflavone (LUT))

Luteolin is a natural polyphenolic flavonoid which obtained from various kinds of plants for example broccoli, celery, parsley, perilla leaf, and peppers, and characterized as one of the Nrf2 inhibitors [193, 196].

Trigonelline (TRG)

Trigonelline, a heterocyclic compound, is widely existing in plants, coffee and fenugreek seed that in comparison with chemicals is less toxic to humans [201].

Ascorbic acid (vitamin C, L-ascorbic acid, AscA, AA)

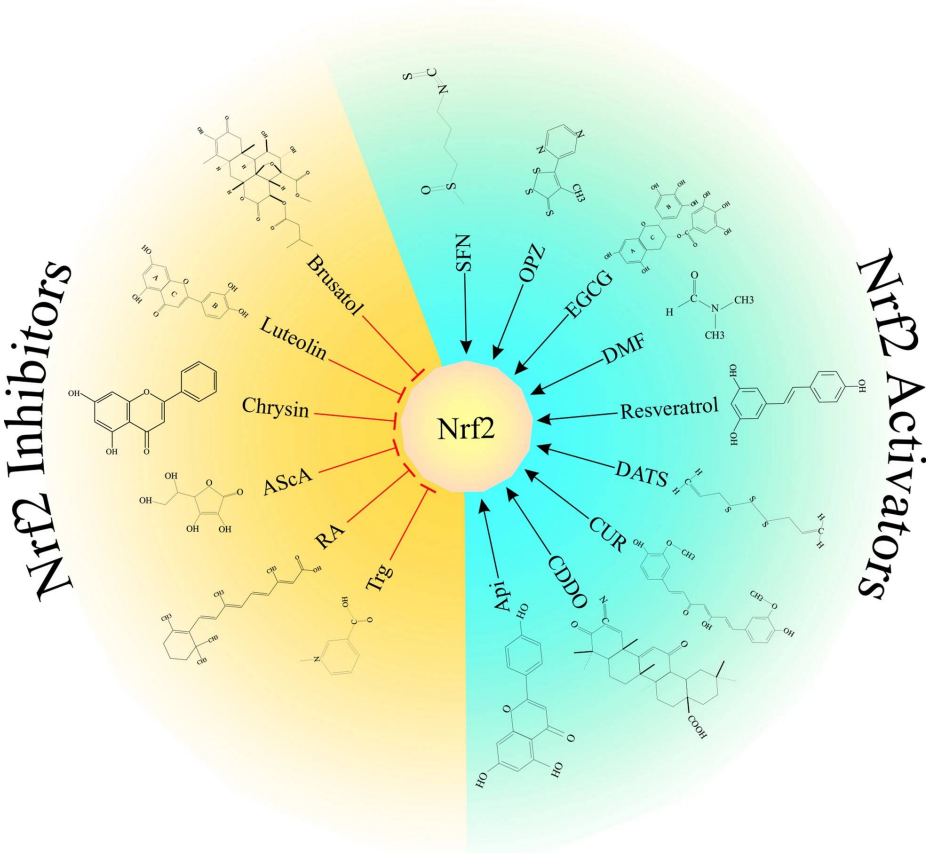
Ascorbic acid which generally known as an antioxidant agent [176], suppresses the Nrf2/DNA complex [78] and through inhibition of the nucleus translocation of Nrf2, reduces the cellular level of peroxides [176].

Retinoic acid (RA)

Retinoic Acid (RA), also known as All-trans-retinoic acid (ATRA), is a metabolite of vitamin A [207]. RA by ARE-inducing elements, for example, tBHQ, decreases the capability of Nrf2 to mediate the induction of ARE-regulated genes in both in vivo and ex vivo conditions [78].

Chrysin (5,7-dihydroxy-2-phenyl-4H-chromen-4-one (CHR))

Chrysin, a natural flavonoid, is found in many plant extracts including honey, propolis, mushroom, blue passion flower, vegetables, and fruits [212].



https://pmc.ncbi.nlm.nih.gov/articles/PMC9405286/

Effects and Mechanisms of Curcumin for the Prevention and Management of Cancers: An Updated Review 2022

The mechanisms of curcumin on cancers.

Study Type	Models	Dose & Duration	Effects	Mechanisms	Ref.
Breast cancer					
In vitro In vivo	MDA-MB-231 and MDA-MB-468 cells; female *BALB/c-nu/nu* mice with MDA-MB-231 adherent cells	10, 15, 20, 25, 30 and 35 μM, 24, 48 and 72 h	Inhibiting proliferation, invasion and migration, EMT and stemness	↓PTCH1, SMO, Gli1, Gli2, N-cadherin, vimentin, Oct4, Sox2	[27]
In vitro	MCF-7 and MDA-MB-231 cells	6.25, 25 and 100 μM, 24 h	Cytotoxicity and photosensitizing effect	↓PTP1B; ↑ROS	[28]
In vitro	MCF-7/TAMR cells	5, 10, 20, 30 and 40 μM, 48 h	Preventing cell migration and invasion, and EMT	↓N-cadherin, H19; ↑E-cadherin	[29]
In vitro	MCF-7 and MDA-MB-231 cells	5, 10, 20, 40, 60, 80, 100, 120 and140 μM, 24 and 48 h	Inhibiting cell viability; Promoting oxidative stress, ER stress, and ferroptosis	↑HO-1, Nrf2, ROS, HSPA5, ATF4, DDIT3, MDA, FTL, TFRC, FTH1, BACH1, RELA, USF1, NFE2L2; ↓GPX4, GSH	[30]
In vitro In vivo	MDA-MB-231 cell; BALB/c nude mice with MDA-MB-231 cells	5, 10, 20 and 50 μM, 24 h; 25 g/kg, 4 weeks	Inhibiting cell proliferation and cancer growth	↑GFPu, miR-142-3p; ↓PSMB5, PSMB1, P300, CT-1	[31]
In vitro, In vivo	MCF-7, MDA-MB-231 and MDA-MB-468 cells; female BALB/c nude mice with MDA-MB-231 cells	20 and 40 μM, 48 h; 100 mg/kg/2 days, 21 days	Inhibiting proliferation, migration and invasion; Promoting apoptosis; Blocking the cell cycle	↓cyclin A1, CDK1, Bcl-2, EZH2; ↑Caspase-9, DLC1	[32]

Study Type	Models	Dose & Duration	Effects	Mechanisms	Ref.
In vitro	MCF-7 and MDA-MB-231 cells	10, 15, 20, 25, 30, 35 and 40 μM, 24 and 48 h	Inhibiting cell viability, invasion and migration, mammosphere formation and differentiation abilities, stem cell properties	↓$CD44^+CD24^-$ subpopulation, vimentin, fibronectin, β-catenin, Oct4, Nanog, Sox2; ↑E-cadherin	[33]
In vitro	HCC-38, UACC-3199, and T47D cells	5 and 10 μM, 3 days	Suppressing proliferation and methylation	↓DNMT1, miR-29b, SNCG; ↑BRCA1, TET1, DNMT3	[34]
In vitro	MCF-7 and MDA-MB-231 cells	5, 10 and 25 μM, 48 h	Inhibiting cell vitality; Inducing apoptosis	↓TLR4, TRIF, IRF3, IFN-α/β	[35]
In vitro	MCF-7, MDA-MB-453 and MDA-MB-231 cells	5, 10, 15, 20, 25 and 30 μM, 24, 48 and 72 h	Inhibiting proliferation, invasion and metastasis; Inducing apoptotic cell death and cell cycle arrest	↓Src, pSTAT-1, p-Akt, p-p44/42, Ras, c-raf, vimentin, β-catenin, p53, Rb, p-Rb, Bax, Bcl-2, Bcl-xL, Mcl-1; ↑PIAS-3, SOCS-1, SOCS-3, ROS, NF-κB, PAO, SSAT, p21, Bak	[36]
In vitro	T47D, MCF7, MDA-MB-415, SK-BR-3, MDA-MB-231, MDA-MB-468 and BT-20 cells	10 and 30 μM, 24 and 48 h	Inhibiting proliferation; Inducing G2/M arrest and apoptosis	↓CDC25, CDC2, p-Akt, p-mTOR, p-S6, Bcl-2; ↑p21, Bax, Cleaved-caspase-3	[37]
In vitro	MDA-MB-231 and CAL-51 cells	5 μM, 48 h	Inhibiting proliferation; Inducing apoptosis	↓Bcl-2, RAD51; ↑ROS, Bax, γH2AX	[24]
Lung cancer					
In vitro In vivo	H1650, H1299, H460 and A549 cells; BALB/c nude mice with A549 cells	10, 20 and 40 μM, 24 h; 50 mg/kg, 22 days	Accelerating apoptosis; Inhibiting migration, invasion and xenograft tumor growth	↓circ-PRKCA, ITGB1; ↑miR-384	[38]
In vitro In vivo	H460, H1299, H1975, A549, SCC-827, PC-9 and CMT-64 cells; female C57bl/6j mice with CMT-64 cells	4, 8, 12, 16, 20, 24 and 28 μg/mL, 24 h; 5 mg/kg, 24 h	Inhibiting of tumor growth and volume; Ameliorating the immunosuppressive micro-environment	↓MDSCs cells, Treg cells, IL-10; ↑NK cells	[39]
In vitro	H1299 and A549 cells	2.5, 5 and 7.5 μM, 48 h	Decreasing migration, invasion and EMT Process	↑TAp63α, E-cadherin, ZO-1; ↓Vimentin, N-cadherin, miR-19a, miR-19b	[40]
In vitro In vivo	A549 and H1299 cells; female C57BL/6 mice with Lewis lung carcinomas cells	5, 10, 20, 30 and 40 μM, 24 h; 100 mg/kg/day, 15days	Inhibiting tumor growth; Inducing ferroptosis and autophagy	↓SOD, GSH, SLC7A11, GPX4, p62; ↑MDA, iron, ACSL4, Beclin1, LC3-II, autolysosome, mitochondrial damage	[41]
In vitro In vivo	A549/GR and H520/GR cells; BALB/c nude mice with A549/GR cells	50, 100 and 150 μM, 48 h; 100 mg/kg, 3 weeks	Suppressing proliferation; Promoting apoptosis	↑lncRNA-MEG3, PTEN	[42]
In vitro	A549, NCI-H1299	5, 25, 125 and 250 nM, 24, 48 and 72 h	Suppressing sphere size and number, and stemness	↓ALDH, CD133, Epcam, Oct4, TAZ; ↑Hippo pathway, p-TAZ	[43]
In vitro	H446 cells	5, 10, 15 and 20 μM, 24 and 48 h	Inducing cell apoptosis; Regulating cell cycle	↓Bcl-2, CCNF, LOX1, MRGPRF, and VEGFB; ↑Bax, cytochrome-C, miR-548ah-5p	[44]
In vitro	A549 cells	1, 2, 5, 10 and 20 μM, 24 and 48 h	Inhibiting migration and invasion	↓E-cadherin, sE-cad, vimentin, slug; ↑N-cadherin, snail, MMP-9	[45]
In vitro	A549 cells	25, 50 and 100 μM, 48 h	Inhibiting proliferation; Inducing apoptosis	↓14-3-3 proteins, p-Bad, p-AKT/AKT, Caspase-9, PARP; ↑Cleaved-caspase-9, Cleaved-PARP	[46]
In vitro	A549 cells	5, 10, 20 and 40 μM, 24, 48, 72 and 96 h	Inhibiting proliferation; Inducing apoptosis and autophagy	↓p-Akt, p-mTOR, p62, LC3-I; ↑Beclin1, LC3-II	[47]
In vitro	A549 cells	10, 20 and 40 μM, 12, 24 and 48 h	Inhibiting migration and invasion	↓miR-25-5p; ↑miR-330-5p	[48]
In vitro	A549 and H1299 cells	0.5, 1, 5, 10 and 20 μM, 24, 48 and 72 h	Inhibiting colony formation; Promoting apoptosis and autophagy	↓p-mTOR, p-S6, p-PI3K, p-Akt ↑LC3-II/ LC3-I, Beclin-1	
Colorectal cancer					
In vitro In vivo	TCO1 and TCO2 cells; SCID mice with organoid cells	0.6, 2, 6 and 20 μg/mL, 72 h; 20 mg/day, 21 days	Inducing necrotic lesions and apoptosis; Inhibiting stemness and proliferation	↓cyclin D1, c-MYC, p-ERK, CD44, CD133, LGR5	[49]
In vitro In vivo	CC531 cells; tumor-bearing rats with CC531 cells	15, 20, 25 and 30 μM, 24, 48 and 72 h; 200 mg/kg/day, 28 days	Reducing proliferation and migration; Diminishing global tumor progression	↑AST, ALP, albumin; ↓cholinesterase, cholesterol, and total protein	[50]
In vitro	SW620 cells	1, 5 and 25 μM, 48 h	Inhibiting tumor sphere formation; Inducing apoptosis and autophagy	↓GP1BB, COL9A3, COMP, AGRN, ITGB4, LAMA5, COL2A1, ITGB6, LGR5, TFAP2A, ECM; ↑Autolysosomes, autophagosomes	[51]
In vitro In vivo	SW480 and HT-29 cells; BALB/c nude mice with SW480 cells	10, 20, 30, 40, 50 and 60 μM, 24 h; 100 mg/kg/day, 3 weeks	Inhibiting proliferation and tumor volume and weight; Inducing apoptosis	↓NNMT, p-STAT3, G2/M phase cell cycle arrest; ↑ROS	[52]
In vitro	HCT-116/L-OHP cells	10, 20, 30 and 40 μM, 48 h	Inhibiting proliferation, migration and invasion; Arresting cell cycle distribution	↓ERCC1, Bcl-2, GST-π, MRP, P-gp; ↑miR-409-3p	[53]
In vitro	5-FU resistant HCT-116 cells	5, 10, 20 and 40 μM, 48 h	Inhibiting proliferation; Inducing apoptosis; Blocking G0/G1 phase	↓E-cadherin, β-catenin, TCF4, Axin; ↑TET1, NKD2, vimentin	[54]
In vitro	SW480 cells	0.1, 0.2 and 0.4 μM, 24 h	Inhibiting EMT and the expression of DNMTs	↑E-cadherin; ↓N-cadherin, twist, snail, vimentin, CDX2, DNMT1, DNMT3a, Wnt3a, β-catenin	[55]
In vitro In vivo	HCT8 and HCT8/DDP cells; Nude mice with HCT8/DDP cells	10 μM, 48 h; 1 g/kg/week, 42 days	Reducing tumor volume and weight; Promoting apoptosis	↓Bcl-2, KCNQ1OT1; ↑cytochrome C, Bax, Cleaved-caspase-3, Cleaved-PARP1, miR-497	[56]
In vitro	HCT116, HCT8, SW480 and SW620 cells	10 μM, 24 h	Reducing clone formation	↑NBR2, p-AMPK, p-ACC; ↓p-S6K/p-S6, Mtor, S-phase	[57]
In vitro	SW480 and 5FU-SW480 cells	5, 10, 15, 20, 25, 30, and 50 μM, 48 and 72 h	Inducing apoptosis; Decreasing colony formation and migration	↓insulin, IGF-1 receptors	[58]
In vitro, In vivo	HCT116/OXA and HCT116 cells; BALB/c nude mice with HCT116/OXA cells	1, 2, 4, 8, 16, 32 and 64 μM, 48 h; 60 mg/kg, 3 weeks	Inhibiting tumor volumes and weights; Decreasing the migratory ability	↓p-p65, Bcl-2, p-Smad2, p-Smad3, N-cadherin, TGF-β; ↑Cleaved-caspase3, E-cadherin	[59]
In vitro	HT-29 and DLD-1 cells	15, 20 and 25 μM, 48 h	Inducing apoptosis and G2/M cell cycle arrest	↓p-Akt, p-Bad, Bcl-2, GPX1, GPX4; ↑ROS, HSP27, Bad, cPARP, Beclin 1, p62	[60]
In vitro In	SW480 cells; female nude mice with	40 μM, 24 h; 200 mg/kg, 5 days	Suppressing proliferation	↓β-catenin, TCF4, miR-21, miR-130a;	[61]

Study Type	Models	Dose & Duration	Effects	Mechanisms	Ref.
vivo	SW480 cells			↑Nkd2	
In vitro	HCT-116 and HCT-8 cells	2.5, 5, 10, 20 and 40 μM, 24 h	Inhibiting proliferation, migration and stem-cell like characteristics	↑CD44	[62]
Head and Neck Cancer					
In vitro In vivo	HNSCC cell lines SNU1076, SNU1041, FaDu and SCC15; C57BL/6 mice with SCC15 cells	1, 2, 5, 10, 20, 40 and 80 μM, 1, 3, 6, 12 and 24 h; 50 mg/kg, 6 weeks	Inhibiting cell viability, invasion, EMT, and tumor formation and growth; Enhancing ability of effector T cells to kill cancer cells and immune response to tumors	↓p-STAT3, TIM-3$^+$CD4$^+$ T cells, PD-1$^+$CD8$^+$ T cells, TIM-3$^+$CD8$^+$ T cells, CD4$^+$CD25$^+$FoxP3$^+$ Treg cells, PD-1, TIM-3; ↑E-cadherin, CD8$^+$ T cells, IFN-γ	[63]
In vitro	SCC-9, FaDu and HaCaT cells	50, 25, 10, 5, 2.5, 1.25 and 0.75 μM, 24 and 48 h	Reducing cell viability; Inducing cell cycle arrest; Modifying cytoskeleton organization	↓procaspase-3, EGFR, PLD1, RPS6KA1, p-mTOR, p-AKT, PI3K; ↑Caspase-3, PRKCG, EGF	[64]
Gastric cancer					
In vitro	AGS cells	10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 μM, 24, 48 and 72 h; 50 mg/kg, 6weeks	Inducing apoptosis; Suppressing proliferation	↓Bcl-2, survivin; ↑Bax, the proportion of Sub-G1 cells	[65]
In vitro	MGC-803 cells	5, 10, 15, 20, 40 and 60 μM, 24, 48 and 72 h	Inhibiting proliferation and migration; Promoting mitochondrial and DNA damage, and apoptosis	↓Δ ψm, cyclin E1, DNMT1, p-Rb, methylated CpG sites; ↑ROS, ATM, ATR, GADD45A, p21, p-p53, p-γH2AX	[66]
In vitro	SGC-7901 cells	10, 20, 40 and 80 μM, 48 h	Suppressing proliferation, invasion, and cytoskeletal remodeling ability; Inducing apoptosis	↓Gli1, Foxm1, β-catenin, pseudopods, skeleton fibers, vimentin; ↑S stage, E-cadherin	[67]
In vitro In vivo	SGC-7901 cells; BALB/c male nude mice with SGC-7901 cells	50 μM, 24, 48 and 96 h	Decreasing migration, invasion and growth of transplanted tumors; Promoting cell apoptosis	↓Bcl-2, cyclin D1, CDK4; ↑miR-34a	[68]
In vitro	SGC-7901 and BGC-823 cells	10, 20 and 40 μM, 24 h	Inhibiting proliferation; Promoting apoptosis and autophagy	↓Bcl-2, Bcl-xL, LC3I, PI3K, p-Akt, p-mTOR; ↑Bax, Beclin1, ATG3, Cleaved-caspase-3, Cleaved-PARP, ATG5, LC3II, p53, p21	[69]
In vitro In vivo	SGC-7901 cells; Balc/c nude mice with SGC7901 cells	25 μM, 3, 5 and 7 days; 100 mg/kg, 2 weeks	Inhibiting proliferation, gastrin and gastric acid secretion; Promoting apoptosis	↑Caspase-3	[70]
Bladder cancer					
In vitro	T24 and RT4 cells	10, 15, 20 and 25 μM, 48 and 72 h	Inhibiting cell growth, migration and invasion; Inducing cell cycle arrest	↓Trop2, cyclin E1; ↑G2/M cell populations, p27	[71]
In vitro	J82, TCCSUP and T24 cells	1, 5, 10 and 20 μM, 24, 48 and 72 h	Decreasing invasion and tumorigenicity; Increasing apoptosis	↓miR-7641; ↑p16	[72]
Prostate Cancer					
In vitro	PC-3 and DU145 cells	10, 20, 30, 40 and 50 μM, 12, 24 and 48 h	Reducing cell viability, migration and invasion; Promoting apoptosis	↓PCLAF, Bcl-2, Caspase-3; ↑miR-30a-5p, Bax, Cleaved-caspase-3	[73]
In vitro	Prostate-CAFs, PC-3 and NAFs cells	10, 20 and 30 μM, 8, 12 and 24 h	Inducing apoptosis and ER stress; Regulating cell cycle	↓Bcl-2, ΔΨm; ↑Cleaved-caspase-3, Bax, Bims, Cleaved-PARP, Puma, p-p53, ROS, p-ERK, p-eIF2α, CHOP, ATF4	[74]
In vitro In vivo	LNCaP and 22Rv1 cells; male TRAMP mice	5, 25 and 50 μM, 24, 48 and 72 h; 200 mg/kg/day, 30days	Inhibiting growth; Inducing apoptosis	↓CYP11A1, HSD3B2, StAR, testosterone, dihydrotestosterone; ↑AKR1C2, SRD5A1, CYP17A1	[75]
In vitro	22RV1, PC-3 and DU145 cells	1, 5, 10 and 20 μM, 4 days	Suppressing proliferation	↓cyclin D1, PCNA, β-catenin, c-MYC; ↑p21, miR-34a	[76]
Thyroid cancer					
In vitro	K1, FTC-133, BCPAP and 8505C cells	10, 12.5, 20, 25, 30, 40 and 50 μM, 24 and 72 h	Inhibiting cell growth; Inducing autophagy	↑LC3-II, Beclin-1, p-p38, p-JNK, p-ERK1/2; ↓p62, p-PDK1, p-Akt, p-p70S6, p-p85S6, p-S6, p-4E-BP1	[77]
In vitro	TPC-1 and BCPAP-R cells	2.5, 5, 10, 20 and 40 μM, 24 h	Inhibiting cell viability, invasion, migration and EMT	↓MMP-9, MMP-2, N-cadherin, vimentin, fibronectin, p-JAK, p-JAK2, p-JAK3, p-STAT1, p-STAT2; ↑E-cadherin, miR-301a-3p	[78]
Liver cancer					
In vitro In vivo	HepG2, Huh-7 and MHCC-97H cells; BALB/c-nu nude mice with HepG2 cells	1.2, 2.4, 4.8 and 9.6 μg/mL, 24 and 48 h; 120 and 240 mg/kg/day, 15 days	Reducing tumor volume and weight, and angiogenesis	↓MDSCs, GM-CSF, G-CSF, TLR4, MyD88, p-IKKα, p-IKKβ, NF-κB, TNF-α, IL-6, IL-1β, PGE2, COX-2, VEGF, CD31, α-smooth	[79]
In vitro	HepG2 and HuT78 cells	5 and 10 μM, 24 h	Inducing cell death	↓lactate, ldh-a, mct-1, mdr-1, stat-3, HIF-1α, HCAR-1; ↑NO	[80]
In vitro	HepG2 cells	20, 50, 80 and 100 μM, 24, 48 and 72 h	Inhibiting proliferation, migration and invasion; Promoting apoptosis	↓HSP70, eHSP70, TLR4	[25]
In vitro In vivo	Bel-7,402 and HepG2 cells; male BALB/c mice with H22 cells	15 and 30 μM, 24, 48 and 72 h; 100 mg/kg/day, 14 days	Inducing apoptosis, G2/M cell cycle arrest; Modulating gut microbiota	↓p-PI3K, p-Akt, p-mTOR, tumors weights and sizes; ↑Cleaved-caspase-3, *Lactobacillus*, Epsilonbacteraeota, Helicobacterac-eae, Campylobacterales, *Helicobacter*, *Escherichia-shigella*, *Bifidobacterium*, *Campylobacteria*	[81]
In vitro In vivo	HepG2 and SK-HEP1 cells; male BALB/c mice H22 and HepG2 cells	20, 40, 60, 80, 100, 120 and 140 nM, 24 h; 100 mg/kg curcumin or Zn (II)-curcumin, 2 weeks	Inhibiting tumor growth; Regulating gut microbiota; Improving intestinal permeability	↓*Firmicutes*, *unclassified Lachnospiraceae*, *Clostridium cluster XIVa*, *Pseudoflavonifractor*, *Oscillibacter*; ↑*Bacteroidetes*, *Barnesiella*, *Unclassified_Porphyromonadaceae*, *Paraprevotella*, *Prevotella*, zonula occludens-1, occludin	[82]
Ovarian cancer					
In vitro	SKOV3 cells	10, 20, 30, 40 and 50 μM, 6, 12 and 24 h	Inhibiting migration and invasion	↓STAT3, fascin	[83]
In vitro	SKOV3 cells	20 μM, 96 h	Inhibiting cell migration and EMT	↓DNMT3a, β-catenin, cyclin D1, c-Myc, fibronectin, vimentin; ↑SFRP5, E-cadherin	[84]
In vitro	SK-OV-3 and A2780 cells	5, 10, 20, 40 and 80 μM, 24, 48 and 72 h	Inducing apoptosis and autophagy	↓p62, p-AKT, p-mTOR, p-p70S6K; ↑Caspase-9, PARP, Atg3, Beclin-1, LC3B-I/II	[85]
In vitro In	SKOV3 and A2780 cells; BALB/c	10, 20 and 40 μM, 24, 48 and	Inhibiting proliferation;	↓PCNA, miR-320a;	[86]

Study Type	Models	Dose & Duration	Effects	Mechanisms	Ref.
vivo	athymic mice with A2780 cells	72 h; 15 mg/kg/2days, 5 weeks	Promoting apoptosis	↑Bax, Cleaved-caspase-3, circ-PLEKHM3, SMG1	
Oral Cancer					
In vitro	HSC-4 and Ca9-22 cells	15 μM, 48 h	Decreasing invasion, migration and EMT	↓vimentin, p-c-Met, p- ERK, pro-MMP9; ↑E-cadherin	[87]
Pancreatic Cancer					
In vitro	Panc-1 and MiaPaCa-2 cells	6, 10 and 12 μM, 24 h	Reducing cell survival; Inducing apoptosis and DNA damage	↓G0/G1-fraction; ↑γH2AX-MFI, G2/M-fraction, S-phase cells	[88]
In vitro	PANC-1 cells	2.5, 5, 10 and 20 μM, 72 h	Inducing apoptosis	↑Cleaved-caspase-3, miR-340, Cleaved-PARP; ↓PARP, XIAP	[89]
In vitro	Patu8988 and Panc-1 cells	5, 10, 15 and 20 μM, 48 and 72 h	Inhibiting migration and invasion; Inducing apoptosis	↓NEDD4, p-Akt, p-mTOR; ↑PTEN, p73, β-TRCP	[90]
Cervical Cancer					
In vitro	Siha cells	5, 15, 30 and 50 μM, 6, 12, 24 and 48 h	Inhibiting proliferation; Inducing G2/M cell cycle arrest, apoptosis, autophagy	↓cyclins B1, cdc25; ↑ROS, p62, LC3I/II, Cleaved-caspase-3, Cleaved-PARP, p53, p21	[91]
In vitro	Siha cells	20 μM, 72 h	Decreasing EMT and migration	↓N-cadherin, vimentin, slug, Zeb1, PIR, pirin; ↑E-cadherin	[92]
Tongue Cancer					
In vitro	CAL 27 cells	10, 25, 50 and 100 μM, 16 and 24 h	Inhibiting proliferation and migration; Promoting apoptosis and S-phase cell cycle arrest	↓Bcl-2; ↑Bax, Cleaved-caspase-3, S-phase cells	[93]
Brain Cancer					
In vitro	SNB19 and A1207 cells	10, 15, 20 and 25 μM, 48 and 72 h	Suppressing proliferation, migration and invasion; Inducing apoptosis and cell cycle arrest	↓NEDD4, Notch1, p-Akt; ↑G2/M phase	

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Flavonoid-induced glutathione depletion: Potential implications for cancer treatment 2014

In the case of flavonoids, however, their chemopreventive properties may rather rely on eliminating precancerous cells due to their prooxidant properties in vivo. This is likely the case of HCs, apigenin, genistein, and chrysin, where their cytotoxicity may result from a combination of interference with the mitochondrial respiratory chain and MRP-mediated GSH depletion [4–6,10]. It is worth noting that the bee product propolis, which is known to exert antimicrobial, antiviral, and cancer preventive properties, contains chrysin, a poor antioxidant, as one of its major components [54].

When measuring intracellular GSH levels, chrysin also induced GSH depletion in the presence of curcumin (Fig. 5B). PC-3 cells were particularly sensitive to curcumin-induced toxicity, which was also potentiated by chrysin (Fig. 5C). The combination of curcumin and chrysin resulted in GSH depletion in PC-3 cells as well (data not shown). Apigenin potentiated the toxicity of curcumin, but unexpectedly less than chrysin.

**** Added Note chrysin is an Nrf2 inhibitor which would improve the pro-oxidant effect of curcumin ***