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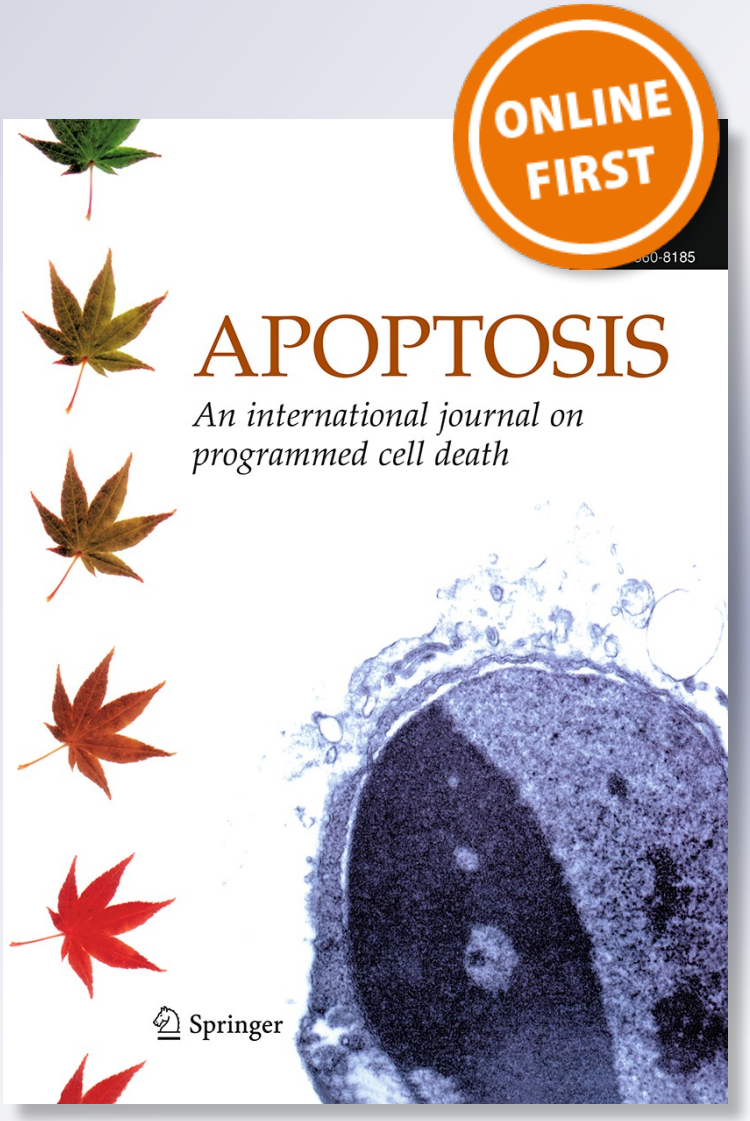

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REVIEW

Cell death mechanisms of plant-derived anticancer drugs: beyond apoptosis

Hala Gali-Muhtasib[1,2] · Raed Hmadi[3] · Mike Kareh[1] · Rita Tohme[1,2] · Nadine Darwiche[2,3]

Abstract Despite remarkable progress in the discovery and development of novel cancer therapeutics, cancer remains the second leading cause of death in the world. For many years, compounds derived from plants have been at the forefront as an important source of anticancer therapies and have played a vital role in the prevention and treatment of cancer because of their availability, and relatively low toxicity when compared with chemotherapy. More than 3000 plant species have been reported to treat cancer and about thirty plant-derived compounds have been isolated so far and have been tested in cancer clinical trials. The mechanisms of action of plant-derived anticancer drugs are numerous and most of them induce apoptotic cell death that may be intrinsic or extrinsic, and caspase and/or p53-dependent or independent mechanisms. Alternative modes of cell death by plant-derived anticancer drugs are emerging and include mainly autophagy, necrosis-like programmed cell death, mitotic catastrophe, and senescence leading to cell death. Considering that the non-apoptotic cell death mechanisms of plant-derived anticancer drugs are less reviewed than the apoptotic ones, this paper attempts to focus on such alternative cell death pathways for some representative anticancer plant natural compounds in clinical development. In particular, emphasis will be on some promising polyphenolics such as resveratrol, curcumin, and genistein; alkaloids namely berberine, noscapine, and colchicine; terpenoids such as parthenolide, triptolide, and betulinic acid; and the organosulfur compound sulforaphane. The understanding of non-apoptotic cell death mechanisms induced by these drugs would provide insights into the possibility of exploiting novel molecular pathways and targets of plant-derived compounds for future cancer therapeutics.

Keywords Anticancer drugs · Medicinal plants · Cell death · Apoptosis · Necrosis · Autophagy · Senescence

Introduction

Cancer is a major public health problem and the second leading cause of mortality around the world with an incident rate of more than 2.6 million cases per year [1, 2]. Research indicates that most cancers are caused by a dysfunction of many genes coding for proteins such as growth factors, growth factor receptors, antiapoptotic proteins, transcription factors, and tumor suppressors, all of which constitute a target for cancer treatment [3, 4]. The increase in the incidence of cancer along with the undesirable side effects observed with chemotherapy urges the discovery of new agents from natural sources. In fact, the concept of chemoprevention by naturally derived compounds has gained increasing attention especially that prevailing cancer treatments have shown limited therapeutic success [5].

The plant kingdom has provided an endless source of medicinal plants that were first used as herbal medicines in their crude forms as syrups, infusions, and ointments [6]. Traditional or folk medicine is the use of medicinal plants as the main medical resource which according to World Health Organization (WHO) includes knowledge, skills,

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and practices for the prevention and treatment of diseases [7]. Herbal medicine can be classified into four basic systems: Traditional Chinese Herbalism, Ayurvedic Herbalism, Western Herbalism, which originally came from Greece and Rome to Europe and then spread to North and South America, and Arab Traditional Medicine, which forms the basis for alternative and herbal medicine in use [8–11]. Today approximately 25 % of all prescriptions contain one or more active ingredients from plants [5].

Cancer chemoprevention by phytochemicals has shown promising results against various malignancies [12]. Secondary metabolites derived from plants potentially represent an inexhaustible source of chemicals for the discovery of new drugs. The actual compound isolated from the plant may not directly serve as the drug but leads to the development of potential novel agents [13]. Recent studies have focused on the effects of plant-derived compounds on cell cycle regulatory and apoptotic pathways [14, 15], yet little is known about their effects on non-apoptotic pathways e.g. autophagy, mitotic catastrophe, senescence leading to cell death, and programmed necrosis or "necroptosis" [16]. Polyphenolics, flavonoids, alkaloids, terpenoids, and sulfhydryl compounds represent a large and diverse group of plant-derived agents used against cancer [4]. Compounds like taxol, vinblastine, vincristine, the campothecin derivatives, topotecan, irinotecan, and etoposide are used clinically all over the world to treat cancer [17]. Other promising anticancer agents include flavopiridol, roscovitine, combretastatin A-4, betulinic acid, and silvestrol [14]. From this list one can well deduce the predominance of polyphenols, flavonoids, and their synthetic analogues. Therefore, emphasis in this review article will be on promising phenolics, alkaloids, and terpenoids as well on the organosulfur compound sulforaphane. From each group of plant-derived anticancer agents, we have chosen to highlight non-apoptotic cell death mechanisms of one of the three highly researched compounds namely resveratrol (Fig. 1a), curcumin (Fig. 1b), and genistein (Fig. 1c) from polyphenolics; berberine (Fig. 1d), noscapine (Fig. 1e), and colchicine (Fig. 1f) from alkaloids; and parthenolide (Fig. 1g), triptolide (Fig. 1h), and betulinic acid (Fig. 1i) from terpenoids. Our better understanding of the cell death mechanisms of action of these drugs would enhance the possibility of exploiting novel targets for the development of lead plant-derived anticancer compounds that are more effective and less toxic in the clinic.

Plant polyphenols

Plant polyphenols are rich in the human diet and are known to possess anticancer properties by interfering with the different stages of cancer development [18]. These molecules are synthesized in plants as secondary metabolites to protect against diseases, parasites, ultraviolet radiation, and predators and do play crucial roles in plant development, reproduction, and pigmentation [19–21]. Dietary polyphenolic compounds are ubiquitous in fruits, vegetables, and beverages and are considered safe and well tolerated by humans with only few reported cases of toxicity and adverse effects [22]. The polyphenol family is subdivided into different groups as a function of the number of phenol rings that they contain and of the structural elements that bind these rings to one another [23]. Polyphenols are divided into flavonoids, stilbenes, coumarins, tannins, and phenolic acids with flavonoids constituting the largest and most abundant group [21]. Here we will review three polyphenolic compounds (Table 1) that are best studied for their anticancer properties, namely the stilbene resveratrol, the phenolic acid curcumin, and the flavonoid genistein.

The strong antioxidant effect of polyphenols is the key property responsible for their cancer chemopreventive and therapeutic effects [24]. Studies have linked the antioxidant properties of polyphenols to the number of galloyl groups and their positions on the glucose core [25]. The reactive hydroxyl groups of polyphenols allow them to inactivate free radicals by losing an electron or a hydrogen atom [26], produce hydrogen peroxide by losing protons [27], chelate metal ions [26], and precipitate proteins [28]. Other mechanisms of cancer inhibition by polyphenols include antiproliferation, induction of cell cycle arrest, induction of apoptosis, anti-inflammation, and inhibition of metastasis and angiogenesis [29]. Polyphenols have been found to inhibit key signaling pathways that contribute to proliferation, such as nuclear factor kappa-B (NF-κB), hypoxia inducible factor 1, phosphoinositide 3 kinase/Akt, Wnt signaling proteins, among others [30]. Current evidence suggests that polyphenolic compounds may exert their cancer therapeutic effects by the regulation of autophagy [31] and of microRNAs [32].

Resveratrol

Resveratrol is a polyphenolic phytoalexin that exists naturally in plant cells and certain fungi as two structural isomers: *cis*-(Z) and *trans*-(E). The *trans* isomer is the predominant and active form [33, 34]. Resveratrol is abundant in grape skins, peanuts, berries, in the roots of Japanese Knotweed (*Polygonum cupsidatum*), and various herbs which produce resveratrol mainly to overcome environmental stress and/or fight infections [34]. The International Union of Pure and Applied Chemistry (IUPAC) name of *trans* resveratrol is 5-((E)-2-(4-hydroxyphenyl)vinyl)-1,3-benzenediol (Fig. 1a). Also known as

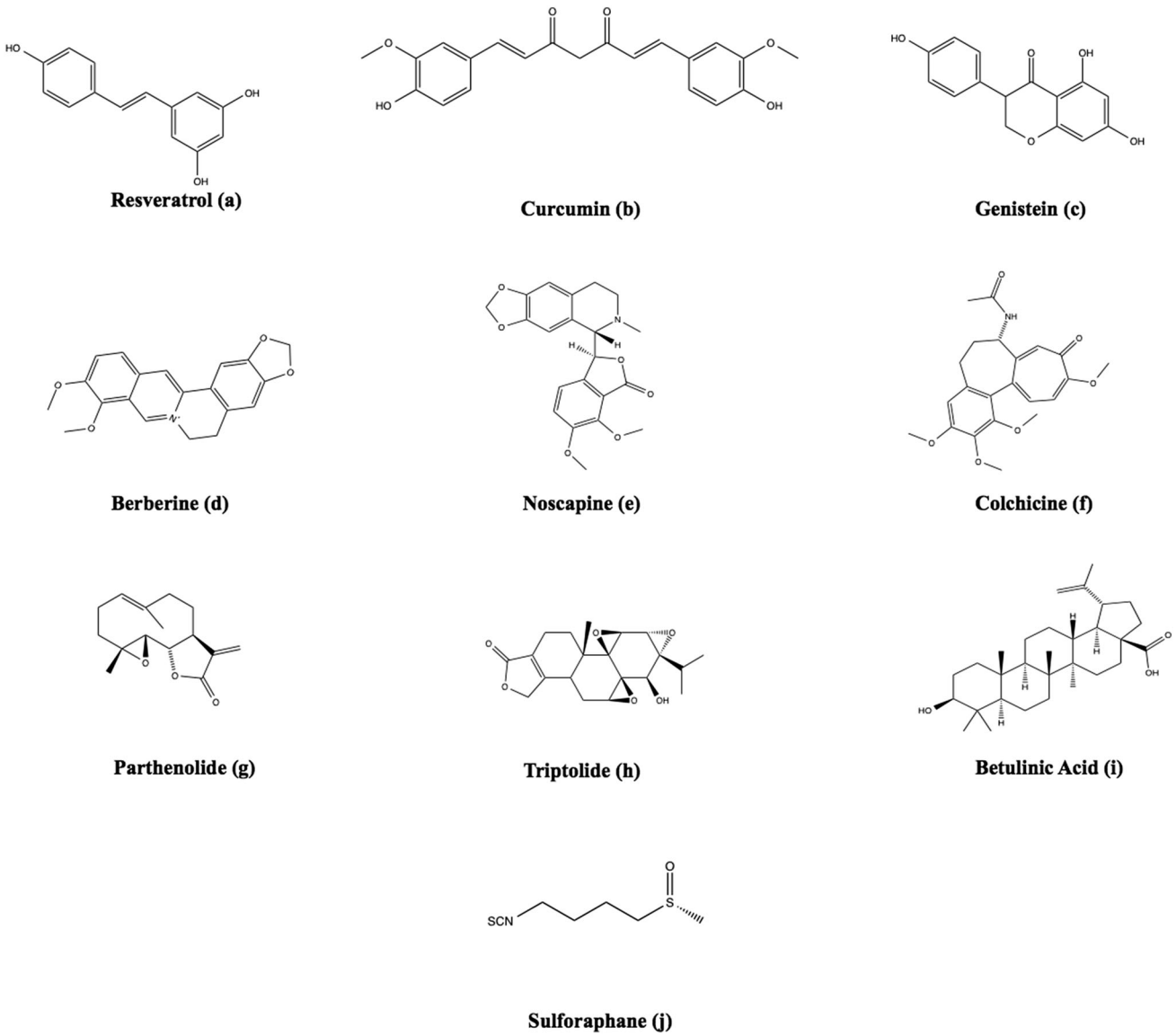

Fig. 1 Chemical structures of selected polyphenols (**a–c**), alkaloids (**d–f**), terpenoids (**g–i**), and organosulfurs (**j**)

3,5,4-trihydroxystilbene, resveratrol is a stilbenoid that consists of a *trans* ethene double bond with a phenyl group on each of the carbon atoms of the double bond. Resveratrol is produced by the action of the stilbene synthase enzyme [35]. Resveratrol's multiple cell death mechanisms, particularly in lymphoma and leukemia, have been extensively reviewed [36]. Numerous biological activities have been attributed to resveratrol including antiviral, antiinflammatory, neuroprotective, cardioprotective, and chemopreventive effects [37]. Resveratrol's ability to modulate reactive oxygen species (ROS), control cellular calcium homeostasis, and to interact with key molecular targets accounts for its wide range of activities [37, 38]. Resveratrol has been shown to cause cell cycle arrest in different phases depending on the nature of the cell lines, the concentrations applied, and the time of exposure [39–42]. The mechanisms of cell cycle arrest by resveratrol include the activation of Chk1/Chk2 kinases of the Ataxia Telangiectasia-mutated (ATM)/ATR pathway, phosphorylation of Cdc25C, increased expression of p21, p27, and p53 proteins as well as increased expression of the cell cycle regulators cyclin-dependent kinase 2 (CDK2), cyclin A, cyclin E, and cyclin B1 [33, 40, 41].

Autophagy by resveratrol

Controversies exist on the function of autophagy in cancers. On one hand, autophagy has been shown to have a prosurvival effect on tumor cells when nutrients are

Table 1 Plant-derived anticancer polyphenols

Purified plant compound	Plant origin	Cancer system	Cell death signaling mechanisms	References
Polyphenols				
Resveratrol and analogues	*Fallopia japonica* (roots of the Japanese Knotweed)	Autophagy		
		Ovarian (A2780, CaOV3, ES-2, TOV112D, A1947)		[39]
		Ovarian (A2780, OVCA432, OVCA429, CaOV3, SKOV3)	Inhibit pAkt and mTOR	[43]
		Lung (A549, H460), salivary gland adenocarcinoma (HSG, HSY)	Induce estrogen receptor coactivator PELP1 accumulation in autophagosomes	[41]
		Breast (MCF-7)	Inhibit Akt phosphorylation and mTOR	[42]
		Colorectal (DLD1, HT-29, COLO 201)		[44]
		Leukemia (K562), primary myeloid leukemia cells	Activate JNK and AMPK; induce JNK-dependent p62 accumulation; inhibit mTOR	[46]
		Glioma (U373)	Localize LC3 and p62 to autophagosomes	[47]
		Breast cancer stem like cells from (MCF-7, SUM159)	Suppress Wnt/β-catenin	[51]
		Leukemia (HL-60)		[52]
		Necrosis		
		Breast (MCF-7)		[42]
		Glioma (C6)	Induce DNA damage	[34]
		Senescence		
		Colorectal (HCT-116)	Activate ATM/ATR pathway and p38/MAPK; ROS generation	[53]
		Non-small cell lung (A549, H460)	Phosphorylate p53; induce p21 and Nox5; reduce EF1A	[54]
		Glioma (U87, U118)	Inhibit H2B ubiquitin ligase RNF20	[55]
		Gastric (AGS, BGC-823, SGC-7901), BGC-823 mouse xenograft	Deregulate p16, p21, cyclin D1	[56]
Curcumin and analogues	*Curcuma longa* (Rhizome of Turmeric)	Autophagy		
		Glioma (U87, U118), leukemia (KBM-5), glioma mouse xenografts	Inhibit Akt/mTOR/P70S6; activate ERK1/2	[67]
		Colorectal (HCT-116), breast (MCF-7), osteosarcoma (U2OS)		[68]
		Oral (YD10B)	Generate ROS	[69]
		Pleural mesothelioma (ACC-MESO-1)		[70]
		Leukemia (K562)		[71]
		Colorectal (HCT-116)	Generate ROS	[72]
		Oral (KYSE450), esophageal (OE19)		[74]
		Epidermoid (A431)	Degrade p53	[76]
		Breast (MCF-7), liver (HepG2), colorectal (HCT-116), cervical (HeLa)		[77]
		Colorectal (HCT-116)		[78]
		Ovarian (HO8910)		[79]
		Lung (A549)		[80]
		Primary glioblastoma cells		[90]

Table 1 continued

Purified plant compound	Plant origin	Cancer system	Cell death signaling mechanisms	References
		Necrosis		
		Prostate (DU-145)	Generate ROS	[82]
		Bladder cancer xenografts	Downregulate NF-κB; decrease cyclin D1; increase p21 expression	[83]
		Mitotic catastrophe		
		Esophageal (OE21, OE33, KYSE450)	Induce monopolar spindles and duplicated unseparated chromosomes; cause nuclear fragmentation; accumulate cells in G_2/M	[74]
		Colorectal (HCT-116)	Trigger Chk1 and Chk2; generate ROS; induce MMR-dependent double-strand breaks	[84]
		Medulloblastoma (DAOY, D283Med, D341 Med), transgenic medulloblastoma models	Reduce HDAC1, increase tubulin acetylation	[73]
		Pancreatic (AsPC-1, MiaPaCa-2, Panc-1, BxPC-3, Pan02)	Increase COX-2 and VEGF; increase CUGBP2; induce caspase activation and mitochondrial membrane polarization	[86]
		Cervical (HeLa), colorectal (HCT-116), fibrosarcoma (HT1080), osteosarcoma (U20S)	Suppress DNA damage response; inhibit HATs and ATR-Chk1 signaling cascade	[87]
		Senescence		
		Breast (CAF-87)	Upregulate p16, p21, and p53; inhibit JAK2/STAT3; downregulate Lamin B1	[88]
		Breast (MCF-7)	Generate ROS; reduce mitochondrial membrane potential, activate p53/p21 and Rb/p16 pathways	[89]
Genistein	*Genista tinctoria* (drying twigs of Dyer's broom)	Autophagy		
		Ovarian (A2780, CaOV3, ES2)	Hinders glucose uptake by inhibiting Akt; causes competitive inhibition of Glut1	[91]
		Necrosis		
		Cervical (HeLa)		[93]
		Mitotic catastrophe		
		Breast (W525, W780)	Induces G_2/M arrest; causes chromosomal structural abnormalities including aneuploidy and chromosome tangling and stickiness; inhibits topoisomerase II	[92]
		Senescence		
		Breast (MCF-7), ovarian (SKOV-3), prostate (DU-145, LNCaP)	Inhibits telomerase activity	[98]

AMPK AMP-activated kinase, *ATM* ataxia-telangiectasia-mutated, *ATR* ataxia telangiectasia and Rad3-related protein, *Casp*-3 caspase 3, *Chk1* checkpoint kinase 1, *Chk2* checkpoint homolog, *COX-2* cyclooxygenase-2, *EF1A* elongation factor 1-alpha, *ERK1/2* extracellular signal-regulated kinases, *Glut1* glucose transporter 1, *HATs* histone acetyltransferases, *HDAC1* histone deacetylase 1, *JAK2* janus kinase 2, *JNK* c-Jun N-terminal kinase, *MAPK* mitogen-activated protein kinase, *MMR* mismatch repair, *mTOR* mammalian target of rapamycin, *NF-κB* nuclear factor kappa-light-chain-enhancer of activated B cells, *Nox5* NADPH oxidase, EF-hand calcium binding domain 5, *PELP1* proline-, glutamic acid- and leucine-rich protein 1, *pRb* retinoblastoma protein, *ROS* reactive oxygen species, *STAT3* signal transducer and activator of transcription 3, *VEGF* vascular endothelial growth factor

limited, and on the other hand, it can lead to non-apoptotic cell death. Resveratrol's antitumor effects have been demonstrated in various in vitro and in vivo models. In many cancers including leukemia, skin, ovarian, breast, lung, colorectal, and gastric cancers as well as adenoma and glioma, resveratrol's antiproliferative effects have been attributed to its capacity to induce cell death by autophagy. Treatment of ovarian cell lines (A2780, CaOV3, ES-2, TOV112D, A1947) with resveratrol induced growth inhibition and cell death by apoptotic (cytochrome c release,

caspase 9 cleavage) and non-apoptotic mechanisms [39]. Morphological analyses determined by light interference microscope as well as electron microscopic characterization in addition to Hoechst staining revealed that resveratrol induced the formation of cytoplasmic autophagosomes and consequently cell death by autophagy [39]. Few years later, the same group demonstrated that the autophagy response triggered by resveratrol in ovarian cancers involved blocking glucose uptake and lactate production by inhibition of the glycolytic regulators pAkt and mTOR [43]. The formation of autophagosomes in response to treatment with resveratrol was further evidenced by confirming the relocalization of the microtubule-associated protein 1 light chain 3 (LC3) proteins from the cytoplasm to the autophagic vacuoles [43]. Autophagy by resveratrol could be reversible [41] or irreversible [39] depending on the cell system and conditions.

Resveratrol was also shown to induce autophagy in lung cancer and salivary gland adenocarcinoma cell lines as evidenced by the presence of autophagosomes and upregulation of the LC3 levels in a time-dependent manner as well as its accumulation in the phagocytic vacuoles [41]. Additionally, resveratrol induced the accumulation of the estrogen receptor coactivator, proline-, glutamic acid-, and leucine-rich protein-1 (PELP1), in the autophagosomes along with the LC3 protein by the intermediary molecule, hepatocyte growth factor-regulated tyrosine kinase substrate, which is a known PELP1-binding protein [41].

Cell death by autophagy, as evidenced by increased expression of LC3-II proteins and their relocalization to the phagocytic structures following cytosolic LC3-I protein conversion, was also demonstrated in breast cancer cells [42]. Resveratrol triggered autophagy in both caspase-3-competent (MCF-7casp-3) and caspase-3-incompetent (MCF-7vc) cells, suggesting that the mechanism involved in resveratrol- induced autophagy was caspase -independent. Cell death in MCF-7casp-3 on the other hand involved both caspase-dependent and caspase- independent pathways. Suppression of Beclin 1 and other autophagic proteins such as ATG-7 decreased LC3-II protein levels, with ATG7 small interfering RNA (siRNA) completely abolishing vacuole formation and LC-3 II induction levels in MCF-7casp-3 cells. The inhibition of Akt phosphorylation and the mTOR signaling pathway was also associated with autophagy induction by resveratrol in the breast cancer cell lines [42].

Unlike the effect on ovarian cancers, exposure of colorectal DLD1 cancer cells to resveratrol induced a reversible autophagy which was dependent on the expression and cytosolic redistribution of Beclin1 and LC-3 II. In fact, the use of dominant-negative lipid kinase-deficient class III phosphoinositide 3-phosphate kinase, or RNA interference knockdown of Beclin1 resulted in autophagy suppression [44]. LC3-II levels were also increased in HT-29 and COLO 201 colon cancer cells with subsequent accumulation of the proteins in autophagosomes [44]. Ultimately, autophagy and specifically the increase in production of LC3-II proteins in HT-29 cells, was further proven to lead to cell death by apoptosis in a caspase and ROS-dependent manner [45]. ROS generation was upstream in the signaling pathway leading to LC3-II expression and autophagy as well as caspase activation and apoptosis.

Mechanistically, autophagy in imatinib-sensitive or resistant K562 chronic myeloid leukemia (CML) cells depended on c-Jun-N-terminal kinase (JNK) activation, p62 knock-down as well as AMPK activation and subsequent mTOR pathway inhibition [46]. Additionally, co-immunoprecipitation studies revealed that LC-3 and p62 proteins could potentially bind together especially that they both colocalized in the autophagosomes. Similarly, resveratrol-induced autophagy was associated with an increase in the expression of p62 in $CD34^{+}$ progenitors from CML patients [46]. In contrast, in U373 glioma cells, resveratrol-induced autophagy was inhibited after pretreatment with p38 or extracellular signal-regulated kinases (ERK)1/2 inhibitors but not with Akt/mTOR signaling pathway inhibitors [47].

In some instances, resveratrol decreased autophagy even if LC-3 II and lysosome associated membrane protein (lamp-2) levels were increased following treatment. This decrease in autophagy was due to a deficiency in autolysosome formation and the triggering of premature senescence in human epidermoid carcinoma A431 cells as well as to the downregulation of $Akt1^{S473}$ phosphorylation and mTOR signaling components including Rictor and the downstream effector activity of Rho-GTPase [48]. A recent phosphoproteomic analysis in serum starved MCF-7 cells showed that resveratrol inhibits the potential effectors of autophagy, namely Akt/mTORC1/SK1 signaling [49]. In HGC-27 gastric cancer cells, autophagy induction by resveratrol did not trigger cell death [50].

The promising anticancer potential of resveratrol has been further confirmed in breast cancer stem-like cells (BCSC) isolated from MCF-7 and SUM159 [51]. In vitro treatment with 20 or 40 μM of resveratrol significantly decreased the number and size of mammospheres and inhibited the proliferation of BCSC thus reducing the overall population [51]. Mechanistically, resveratrol's anticancer potential in BCSC involved the formation of autophagocytic vacuoles and the increase in LC3-II, Beclin 1, and Atg 7 protein levels. Resveratrol's activity was subsequently found to depend on the suppression of the Wnt/β-catenin signaling pathway [51].

The naturally occurring structural analogue to resveratrol, pterostilbene, has also been reported to induce autophagy, cell cycle arrest, and apoptosis in different cancer types including bladder, breast, and leukemic cancer

cells [52]. In HL-60 leukemic cells, pterostilbene's anti-cancer mechanism of action resulted in G_0/G_1 cell cycle arrest as well as the induction of autophagy by conversion of LC3-I proteins and formation of autophagic vacuoles. Subsequently, the cells underwent cell death as evidenced by phosphatidylserine externalization, DNA fragmentation, and caspase-3 and caspase-9 activation as well as by the disruption of mitochondrial membrane potential [52].

Necrosis and apoptosis by resveratrol

Resveratrol's chemotherapeutic mechanism has been attributed, to a large extent, to its ability to induce programmed necrosis and apoptosis by regulating key targets in these pathways. Treatment with resveratrol increased the ratio of terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL)-positive U373 glioma cells [47]. Caspase-dependent apoptosis was also induced in DLD-1 colorectal cancer cells in response to exposure to resveratrol [44]. Similarly, resveratrol induced apoptosis in HT-29 colorectal cancer cells in a time-dependent manner. In these cells, ROS signaling triggered autophagy which was required for caspase-8 and caspase-3 activation, PARP cleavage, and induction of apoptosis [45]. Apoptosis and cell death in leukemic cells was also found to be caspase-dependent [46].

In breast cancer, resveratrol triggered apoptosis in MCF-7casp-3 cells by the activation of caspase 3 and subsequent PARP cleavage [42]. Signs of necrosis, namely the early plasma membrane rupture and cell dilatation were also present in the MCF-7casp-3 and MCF-7vc cell lines with a more pronounced necrotic profile observed in the former cells [42]. Another study showed that when MCF-7 cells were treated with concentrations higher than 50 μM of resveratrol, S phase effect of resveratrol was reduced and was almost reversed at 150 μM concentrations [33]. High concentrations of resveratrol (150 μM) were associated with an increase in the apoptotic profile of the cells as evidenced by the presence of hypodiploid cells, chromatin condensation, and nuclear fragmentation [33]. MDA-MB-231 breast cancer cells on the other hand were more sensitive to treatment with resveratrol at 50 μM concentrations and were found to lead to cell death through non-apoptotic processes that involved regulation of cyclin D1 and cyclin E, CDK4, but not the tumor suppressors p21, p27, and p53 proteins [33].

The activity of resveratrol in some cancer cell lines was found to be mainly due to its metabolic activation by the cells [34]. Metabolically active H4IIE rat hepatoma cells and C6 rat glioma cells with poor metabolic activity differentially reacted to treatment with resveratrol [34]. At 24 h following incubation with 100 μM resveratrol, necrosis was detected in C6 cells but not in H4IIE cells. On the other hand, at 48 h post-treatment, nuclear fragmentation, which is indicative of apoptosis, was only detected in H4IIE cells. Apoptosis in H4IIE cells following DNA damage involved the activation of caspases 2 and 8/10 after which caspase 3 but not caspase 9 was activated [34]. This difference in drug response to apoptotic versus necrotic cell death is believed to be due to the difference in the cellular metabolism of resveratrol, among other factors [34].

Senescence by resveratrol

In addition to inducing cell death by autophagy, apoptosis, and programmed necrosis, resveratrol was shown to induce irreversible cell growth arrest or senescence mainly through the activation of the ATM/ATR pathway. In HCT-116 colorectal cancer cells, 30 μM of resveratrol caused complete growth arrest 30–40 days after treatment with no signs of apoptosis or DNA damage [53]. Senescence was evidenced by the enlarged cellular morphology, the expression of senescence-associated-β-galactosidase (SA-β-gal), the abrogated bromodeoxyuridine (BrdU)-labeling index as well as the decreased sensitivity to growth factors [53]. Mechanistically, both p53 phosphorylation and p21 induction were required for senescence as demonstrated by the inability of resveratrol to induce irreversible growth arrest when either protein was knocked out. Inhibitors of the ATM/ATR pathway abrogated both senescence and the phosphorylation of p53 and induction of p21, suggesting that senescence occurs through the activation of this pathway. The activation of ATM/ATR as well as senescence was also inhibited when cells were pre-incubated with the strong antioxidant N-acetylcysteine (NAC), confirming that ROS production by resveratrol was critical for the triggering of senescence. ROS further induced the activation of p38 MAPK which has been reported to positively regulate senescence [53].

Similarly, low concentrations of resveratrol (10–50 μM) induced senescence in A549 and H460 non-small cell lung cancer (NSCLC) cell lines through ROS-dependent p53 phosphorylation and p21 induction [54]. In these cells, resveratrol decreased the expression levels of the EF1A protein indicative of premature triggering of senescence. The elevated expression of the NAPDH oxidase-5 (Nox5) protein in response to treatment with resveratrol was suggested to account, in part, for the increased ROS production [54]. Senescence by resveratrol was also reported in glioma cells whereby mono-ubiquitination of histone H2B at K120 was inhibited through the depletion of the H2B ubiquitin ligase RNF20 and subsequent senescence induction [55].

Furthermore, treatment of gastric cancer cell lines namely, AGS, BGC-823, and SGC-7901with resveratrol at concentrations of 25 and 50 μM inhibited their proliferation in a dose-dependent manner in addition to reducing their cell viability and clonogenic potential [56]. The mechanism of action involved deregulation of major cell cycle regulators including cyclin D1, CDK4 and 6, p21, and p16 and subsequent G_1 phase arrest. β-Gal staining also revealed that resveratrol induces senescence in vitro.

In vivo cell death effects of resveratrol

Evaluating resveratrol in tumor animal models has shown promising therapeutic effects, neutral as well as negative effects depending on the mode of drug administration, dosage used and animal tumor model [57]. Exposure to resveratrol has been found to inhibit tumor initiation and promotion and suppress angiogenesis and metastasis [56]. In both UV-irradiated mice and in 7,12-dimethylbenz(a)anthracene (DMBA)-treated rats, resveratrol was able to suppress skin and mammary tumorigenesis, respectively [37, 48]. Not only did resveratrol and its nanoparticle bovine serum albumin formulations inhibit tumor growth in nude mice transplanted with ovarian carcinoma, but apoptosis was also induced in tumor tissues of treated animals along with an increase in the expression of cytochrome c, caspase 9, and caspase 3 proteins [58]. In gastric cancer BGC-823 xenograft mouse model, treatment with 40 mg/kg/day of resveratrol was also effective in inhibiting tumor growth and cell proliferation by deregulation of major cell cycle regulators and induction of senescence. Sirtuin (Sirt)1 silencing significantly reduced the inhibitory effect of resveratrol on cell viability and colony formation as well as on cell cycle arrest and senescence induction both in vitro and in vivo [56]. Recently, the anticancer effect of resveratrol was further confirmed in vivo in a mouse xenograft model of SUM159 breast cancer cells, where treatment with 100 mg/kg/day of resveratrol for 2 weeks significantly inhibited tumor growth and reduced the BCSC population in tumor tissues [51]. In addition, when assessing for the ability of residual cancer cells to initiate tumors, resveratrol-treated mice only developed 1/6 compared to 6/6 initiated tumors for the untreated control group [51].

Clinical trials on resveratrol

The cancer chemopreventive effects of resveratrol, which have been well documented in animals, have not been confirmed in humans yet. One drawback of resveratrol for development into an anticancer molecule is its poor bioavailability, and rapid metabolism which limits its presence in the bloodstream [59]. Moreover, the actual metabolite(s) responsible for the anticancer effects of resveratrol are still unknown. For this reason, intense research on its encapsulation alone or in combination with standard chemotherapeutic drugs has been conducted to enhance its bioavailability [60]. Another challenge for resveratrol's development for human use is that the maximum safe dose is not known yet. Although resveratrol was found to be well tolerated in colorectal cancer patients who ingested eight daily doses of resveratrol at 0.5 or 1.0 g before surgical resection [61], there have been concerns about the safety of consuming high doses of a nutraceutical rich in phenolics which could cause adverse effects and interact with other drugs (reveiwed in [59]). This is why the safety and efficacy of this plant-based drug should be documented before clinical use. Despite these challenges, a number of clinical trials are ongoing to test the effects of resveratrol on human health (www.clinicaltrials.gov). The main characteristics, objectives, outcomes, strengths, and limitations of these randomized clinical trials have been critically reviewed [59]. These trials will shed light on resveratrol's efficacy and safety of use in humans and will ultimately allow either its development into an anticancer molecule or its use as a scaffold for the development of other synthetic compounds.

Curcumin

Curcumin (diferuloylmethane)-(1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-hepadiene-3,5-dione) (Fig. 1b) is a natural polyphenolic compound extracted from the rhizomes of *Curcuma longa*. Curcumin first characterized in 1910, is a bis-α,β-unsaturated β-diketone that exists in balance with its enol form in a pH-dependent manner. It is the main active compound in the South Asian spice turmeric, and is also found in small amounts in ginger, giving both turmeric and ginger their yellow color. Curcumin possesses the potential to release electrons facilitating the scavenging activity of polyphenolic antioxidants. This property is mainly responsible for the anticancer, anti-inflammatory, and anti-oxidant activities of curcumin. In addition, curcumin has been shown to exert cytotoxic effects on cancer stem cells (CSCs) without being toxic to normal stem cells [62–64]. At the molecular level, curcumin has been shown to target miRNAs involved in epithelial to mesenchymal transition, Wnt-beta catenin, protein kinases, NF-κB, STAT3, anti-apoptotic proteins and to modulate cell cycle through epigenetic regulation [63, 65, 66]. Recent studies have also shown that the suppression of Sp-1 and its downstream targets, namely calmodulin and SEPP1 may account for curcumin's anti-tumor effects [65].

Autophagy by curcumin

Among all cell death mechanisms, which are triggered by anticancer agents, autophagy is the most dominant biological process which leads to notable effects on tumors after curcumin treatment. In malignant gliomas, curcumin was found to modulate the Akt/mTOR/P70S6 and ERK1/2 pathways, both of which regulate autophagic cell death [67]. In glioblastomas, curcumin induced autophagy by inhibiting the Akt pathway and activating the ERK1/2 signaling pathway. When the Akt pathway was activated, autophagy by curcumin was inhibited in U87-MG and U373-MG glioma cell lines, while blockage of the ERK1/2 pathway inhibited curcumin-induced autophagy and cytotoxicity in these cell lines [67]. Curcumin was also shown to induce autophagy in HCT-116 human colorectal cancer cells [68], MCF7 breast cancer cells, U2OS osteosarcoma cells and KBM-5 CML human leukemia cells.

Another autophagic cell death mechanism by curcumin in oral squamous cell carcinoma (SCC) involves the up-regulation of ROS production. In the presence of the antioxidant NAC, autophagy induced by curcumin was blocked, suggesting that ROS accumulation plays a key role in autophagy activation [69]. This curcumin-induced ROS production activated and accelerated active autophagosome formation along with high conversion of LC3I protein to its LC3II form, a marker of autophagy [69]. Furthermore, it was found that curcumin treatment contributed to a similar increase in LC3B II/LC3 I ratio and autophagosome formation in human malignant pleural mesothelioma ACC-MESO-1 cell line [70]. In addition to LC3II up-regulation by curcumin, Beclin 1 levels increased in CML K562 cells [71]. Following application of bafilomycin A1, an autophagy inhibitor, and the pan-caspase inhibitor, Z-VAD-fmk, on K562 cells, it was evident that cell death by curcumin was suppressed, which reveals that cell death in K562 is through both apoptosis and autophagy [71]. Other studies further confirmed the involvement of ROS accumulation in cell death mechanisms by curcumin. In HCT-116 human colorectal cancer cells, curcumin treatment resulted in ROS accumulation [72], an increase in LC3I conversion to LC3II and in sequestome 1, a marker of autophagosome degradation that binds to LC3 [73]. Testing the causality between ROS production and autophagy induction, it was noted that curcumin-induced autophagy in HCT-116 cells was not enhanced by ROS-activated ERK1/2 and p38 MAPK, suggesting that ROS production by curcumin might influence autophagy by other means [73]. Additionally, autophagy induction following treatment with curcumin was verified by various morphological aspects described in treated KYSE450 human esophageal SCC and human Caucasian OE19 esophageal carcinoma cells, such as the increase in vacuolization of the cytoplasm associated with complete loss of the cytoplasmic membrane, the nuclear membrane being intact, and nuclear pyknosis [74]. Recently, curcumin was found to inhibit the acetyltransferase activity of recombinant E1A-binding protein p300 (EP300), which suggested EP300 as an endogenous repressor of autophagy [75].

Curcumin treatment of A431 SCC expressing the p53R273H mutant caused increased degradation of p53, autophagy and apoptosis [76]. mTOR inhibition by rapamycin synergized with curcumin to result in p53 degradation, as well as increased LC3II levels and autophagosome formation. Autophagy-dependent degradation of mutant p53 shed light on the potential effectiveness of curcumin treatment on chemoresistant cells harboring p53 mutations [76].

Combination of curcumin with clinically approved anticancer drugs commonly used in chemotherapy showed antagonism between curcumin and the topoisomerase II inhibitor, etoposide [77]. Antagonism was thought to be caused by the action of curcumin and etoposide on the same target topoisomerase II, therefore one compensating the effect of the other. Along with the involvement of DNA damage response, electron microscopy examinations revealed that cell death mechanisms are implicated in resistance to genotoxic effects of both curcumin and etoposide. While curcumin treatment alone caused autophagy in MCF-7 human breast cancer cells, HepG2 human hepatocellular carcinoma, HCT-116 colorectal cancer, and HeLa cervical cancer cells, the combination of curcumin and etoposide caused apoptosis and programmed necrosis [77].

Various studies have reported the cytotoxic effects of curcumin derivatives and analogues. A stable curcumin derivative, bis-Dehydroxycurcumin (bDHC), caused irreversible cytotoxicity in HCT-116 cancer but not in normal cells [78]. An increase in p53 in the cytoplasm was found in response to treatment of HCT-116 cancer cells with bDHC, suggesting that this derivative acts on p53-dependent cytoplasmic mechanisms [78]. In addition, in response to bDHC, cells exhibited endoplasmic reticulum (ER) stress, revealed by dilated morphology of ER and deregulation in mRNA levels of transcriptional activators of the anti-apoptotic gene *Bcl-2* [78]. Therefore, it is by the activation of the ER stress response that bDHC induces apoptosis in p53-independent manner, in which activation of the transcriptional activator CHOP downregulates Bcl-2. Autophagy is another cell death mechanism triggered by bDHC-induced ER stress which reflects the crosstalk between apoptosis and autophagy. When apoptosis is inhibited, autophagy can still induce death in bDHC-treated cells whereas inhibition of autophagy led to a strong decrease in apoptotic response, suggesting the upstream position of autophagy with respect to apoptosis [78]. It was also shown that bDHC and curcumin can act as proteasome

inhibitors promoting the accumulation of poly-ubiquitinated proteins. Further studies are ongoing to overcome the high IC_{50} of bDHC in HCT-116 and LOVO cell lines [78]. Another curcumin analogue, B19, is subject to new investigation [79]. B19 is a monocarbonyl analogue of curcumin which was also shown to induce ER stress responses and to activate autophagy in HO8910 ovarian cancer cells. B19 increased the autophagosome formation and the conversion of light chain protein from LC3I to LC3II, which enhanced epithelial ovarian cancer cell death by autophagy [79]. Moreover, inhibition of autophagy promoted cancer apoptosis using 3-methyladenine, suggesting that autophagy induces ER stress which in turn increases B19-induced apoptosis [79].

The curcumin derivative 2E, 6E-2-(1H-indol-3-yl) methylene)-6-(4-hydroxy-3-methoxy benzylidene)-cyclohexanone (IHCH) has been found to inhibit the growth of A549 lung cancer cells by inducing autophagy. The occurrence of autophagy was evidenced by a higher number of autophagic vacuoles observed with respect to control cells and by the elevated conversion of LC3I to LC3II protein levels in IHCH-treated cells [80].

Necrosis and apoptosis by curcumin

Controversies still exist regarding the exact mechanisms of cell death induced by curcumin, although studies have mainly focused on autophagy and apoptosis. Programmed necrosis was found to be a common cell death mechanism involved in curcumin's anticancer effects, which was characterized by the induction of ROS and caspase-independent cell death [81].

DU-145 human prostate cancer cells were found to respond to curcumin treatment by ROS production [82]. However, a complex signaling network is switched on during different stages of curcumin treatment, where ROS promotes cell survival in early times of treatment along with an increase in Akt or p38/JNK phosphorylation, while at later times of treatment, necrotic cell death and caspase degradation occur [82]. In contrast, in human skin normal fibroblast BJ cells, curcumin treatment induced less necrotic effects compared to prostate cancer cells based on low levels of ROS production, low activities of survival signals and less caspase degradation, all of which are required for programmed necrosis [82].

Curcumin was found to act on other pathways leading to programmed necrosis and apoptosis. It down-regulated the activity of NF-κB, thus suppressing transformation of tumor cells [83]. Curcumin-induced down-regulation of NF-κB was evident by a decrease in cyclin D1 protein levels and an increase in p21 expression levels, which explain curcumin's effects on cell proliferation and apoptosis induction through the p53 pathway [83]. Traditionally, Bacillus Calmette-Guerin (BCG) intravesicular administration is being used as a treatment for bladder cancer, in early stages or after removal of the tumor, because it stimulates immune responses that can destroy -cancer cells within the bladder. The advantage of using curcumin as an alternative therapy lies in that it has the same efficacy without the side-effects accompanying BCG treatment. Moreover, BCG is being rejected in one-third of the patients, and curcumin characterized by low absorptivity in the vesicles, both make curcumin an attractive venue for potential intravesicular treatment [83].

Mitotic catastrophe by curcumin

Curcumin treatment of tumor cells triggers not only apoptosis, but also mitotic catastrophe in which defective cell cycle checkpoints and various disturbances during mitosis result in mitotic failure [74]. Exposure of human esophageal SCC cells (OE21, OE33) and esophageal adenocarcinoma KYSE450 cells to curcumin revealed mitotic catastrophe induction as evidenced by visible distinct chromosomes, monopolar spindles and duplicated yet not separated chromosomes [74]. Curcumin-treated cells displayed many aspects of mitotic catastrophe such as nuclear fragmentation, the absence of the nuclear envelope, the presence of an extra nucleus, and other abnormal morphologies specific to cell lines. In addition, all three esophageal cancer cell lines have shown an increase in percentage of cells arrested in G_2/M phases after curcumin treatment and an increase in mitoses-specific antibodies staining, an early prophase marker commonly used to assess mitotic perturbance [74].

Furthermore, curcumin has the potential to induce mitotic catastrophe by a mismatch repair (MMR)-dependent process [84]. The MMR-dependent process is known as the system, which corrects post-replicative errors and was shown to be involved in HCT-116 cancer cells sensitivity to curcumin by triggering the serine/threonine protein kinase DNA damage checkpoints, Chk1 and Chk2. Curcumin was defined as a genotoxic agent that produces ROS leading to DNA double strand breaks (DSB) in the genome, which ultimately results in the activation of G_2/M cell cycle checkpoint through Chk1/Chk2 pathway [84]. Although ROS generation is not affected by the MMR system, however DNA double strands fail to break if MMR is deficient in cells. Thus, DSB are reduced in MMR deficient cells treated with curcumin and fail to activate the G_2/M checkpoint, which explains the resulting cell death through mitotic catastrophe [84].

Recent studies have shown that human medulloblastoma cell lines, DAOY, D283 Med and D341 Med, are responsive to curcumin treatment by a reduction in histone

deacetylase 1 (HDAC1) expression and activity, and an increase in tubulin acetylation [73]. This increase in tubulin acetylation resulted in modifications in microtubular organization affecting spindle formation, since increased tubulin acetylation is mainly associated with increased microtubule stability [85]. Hence, curcumin was shown to affect tubulin stability and organization along with its direct binding to tubulin in the mitotic spindle.

The response of pancreatic cancer cells to curcumin treatment further confirms the induction of mitotic catastrophe by this anticancer drug. Studies have shown that curcumin acts on translation inhibition in pancreatic cancer cells by increasing mRNA expression, but not protein levels, of the cyclooxygenase COX-2 and the vascular endothelial growth factor (VEGF) [86]. Curcumin was found to increase the expression of the RNA binding protein-CUGBP2, which in turn inhibits translation by an increased binding to VEGF and COX-2 transcripts. Consequently, mitotic catastrophe induction by curcumin is due to caspase activation and mitochondrial membrane polarization in pancreatic cancer cells [86].

In summary, following curcumin treatment mitotic catastrophe was induced either by triggering abnormalities in chromosome segregation, MMR-dependent DNA DSB, translation inhibition, or tubulin stability and organization. One additional approach of inducing mitotic catastrophe by curcumin in cervical cancer HeLa, colorectal cancer HCT-116, HT1080 fibrosarcoma, and U20S osteosarcoma cells is to suppress the DNA damage response pathway [87]. Curcumin was shown to inhibit histone acetyltransferases (HATs) responsible for histone acetylation at DSB sites and for inhibition of ATR kinase, thereby disturbing ATR-Chk1 signaling cascade [87].

Senescence by curcumin

An additional studied tumor suppressor process triggered by curcumin is senescence which implicates morphological, functional, and behavioral modifications and an irreversible growth arrest. Furthermore, cells undergoing senescence exhibit a senescence-associated secretory phenotype, which can be marked by SA-β-gal activity, Ki-67, Lamin B1 levels, and BrdU incorporation [88]. A recent study showed that curcumin inactivates breast cancer-associated fibroblasts (CAFs) by inducing senescence, in turn reducing their pro-carcinogenic competence [88]. Upon curcumin treatment on CAF-87 cells, the levels of the tumor suppressor proteins p16, p21, and p53 were upregulated and JAK2/STAT3 pathway, which is responsible for the pro-carcinogenic potential of CAFs, was inhibited. Cells treated with curcumin also showed decreased Ki67 index and lower BrdU incorporation when compared to untreated cells confirming the inhibition of proliferation. In addition, SA-β-gal activity was higher in curcumin-treated cells whereas Lamin B1 level was downregulated which implicates curcumin-induction of senescence in breast fibroblasts [88]. The induction of senescence in stromal breast fibroblasts by curcumin suggests that it may be a potential anticancer drug targeting stromal cells along with tumor cells.

A natural derivative of curcumin known as bisdemethoxycurcumin (BDMC) was also investigated for its anticancer effects [89]. BDMC treatment of MCF-7 breast cancer cells inhibited cell proliferation in parallel with a rapid increase of intracellular ROS levels and reduced mitochondrial membrane potential. MCF-7 cells treated with BDMC showed upregulation of p53/p21 and p16/Rb pathways, thereby inducing activation of p53, p21, p16, and Rb proteins involved in pro-apoptotic signaling. When the antioxidant NAC was added to BDMC-treated cells, the aforementioned modifications were counteracted [89].

In vivo cell death effects of curcumin

Curcumin was found to inhibit tumor growth in animal models by several mechanisms. In the syngeneic murine orthotopic bladder tumor model, the smaller tumor size in curcumin-treated mice was associated with a higher percentage of necrotic cells [83]. Tubulin acetylation and mitotic catastrophe were responsible for reduced tumor size and increased survival by curcumin in the transgenic medulloblastoma murine model [73]. Further in vivo studies showed that autophagy induction by curcumin was the mechanism of growth inhibition of subcutaneously injected human malignant glioma cells as evidenced by the high detection of LC3-II protein in curcumin-treated mice compared to controls [67].

In glioblastoma patient samples, curcumin toxicity was also found to decrease the excessive proliferation of glioblastoma-initiating cells responsible for initiation and relapse of this cancer [90]. In lipopolysaccaride -induced brain inflammation model and GL26 brain tumor model, intranasal administration of exosome-containing curcumin significantly protected from brain inflammation and delayed brain tumor growth, respectively. Glioblastoma-initiating cells showed higher differentiation in counterpart of slower proliferation and elevated LC3II protein levels, indicative of autophagy [90].

Genistein

Genistein is a natural isoflavonoid (4′,5,7-trihydroxyisoflavone) (Fig. 1c) found in soybean seeds. It is also found in legumes as a precursor for the biosynthesis of

antimicrobial phytoalexins and phytoanticipins. Genistein is one of the most widely studied drugs [91–93] that exerts its antitumor mechanisms through the regulation of ROS production [94]. It was first isolated from the drying twigs of Dyer's broom *Genista tinctoria,* hence its name.

Genistein is known to provide health benefits regarding cognitive functions, cardiovascular diseases, bone loss, and cancer treatment [95]. It is effective against several tumor types such as breast cancer, prostate, ovarian, colon, gastric, lung, and pancreatic adenocarcinomas and lymphomas [91]. Genistein was reported to be an enhancer of anticancer treatments as it is involved in altering the regulation of gene expression of processes such as proliferation, cell cycle progression, transcription, apoptosis, oncogenesis, cancer cell invasion, and metastasis [93, 95]. Genistein has a structure similar to the principal mammalian estrogen and at low concentrations below 10 μM it induced cell proliferation in breast cancer cells [92]. Despite such estrogen-like effects, studies have shown that increased consumption of soy beans, which have high levels of genistein, is inversely related to the occurrence of breast cancer [96].

Autophagy by genistein

Genistein has been shown to trigger autophagy in several types of cancer cell lines. Treatment with genistein inhibited the proliferation of both cisplatin-sensitive A2780 and cisplatin-insensitive (CaOV3, ES2) ovarian cancer cells, with a more pronounced effect in the former [91]. The mechanism behind this inhibition was found to be due to genistein's ability to hinder glucose uptake via inhibition of Akt kinase, an oncogenic kinase that, when activated, plays an important role in glucose metabolism by allowing the uptake of glucose and its metabolism. Under these conditions of low glucose, genistein was shown to induce autophagy as evidenced by the increase of LC3-II levels within autophagosomes [91, 97]. This effect of glucose inhibition by genistein is thought to be achieved through several mechanisms such as competitive inhibition of the glucose transporter Glut-1 or targeting the upstream activators of Akt like growth factor receptors, PI3 kinase, PDK-1 or even directly targeting the Akt kinase [91]. Considering that invasive cancers usually have altered glucose metabolic pathways and mainly rely on glycolysis, which results in an exaggerated glucose uptake by the cells, there is no doubt that the limitation of glucose intake by genistein would have detrimental effects on tumor cell proliferation.

Aside from inhibiting Akt kinase itself, genistein was found to abrogate the NF-κB pathway through Akt signaling mechanisms. NF-κB is known to control cell growth, differentiation, apoptosis, and stress-response [95]. The inhibition of NF-κB by genistein holds great promise for combination therapy, since chemotherapeutic agents such as cisplatin, gemcitabine, and docetaxel have been reported to activate NF-κB in cancer cells, thus causing resistance to therapy and re-emergence of cancer. In vitro and in vivo studies have shown that treatment with genistein followed by treatment with lower doses of these chemotherapeutic agents dramatically inhibited growth in a greater number of cells [95].

Necrosis and apoptosis by genistein

To study the apoptotic effects of genistein, HeLa cells were separated into two categories: ethanol stressed and non-stressed cells [93]. After treatment with genistein, it was found that only stressed cells developed apoptotic features including nuclear condensation, disruption of mitochondria, cell shrinkage, caspase activation and phosphatidyl serine externalization, ultimately leading to cell death. Higher concentrations of genistein were required to induce these effects in unstressed HeLa cells, suggesting that stressed cells were more sensitive to genistein [93]. This greater sensitivity to genistein was found to be related to the alteration of transcription and translation by this drug. The antiapoptotic genes, *XIAP* and *MDM2*, which were overexpressed upon removal of stress conditions, were inhibited in the presence of genistein. In addition, treatment with genistein led to an increase in p53 protein levels which eventually activated its target genes *NOXA* and *PUMA* leading to apoptosis [93]. Surprisingly, treatment with the caspase inhibitor, Z-VAD fmk, did not inhibit cell death, rather the cells displayed late apoptotic and necrotic features. The results of this study suggested that the recovery of cancer cells from apoptosis after stress removal may be relevant for the state of proliferation of cancer cells after cessation of chemotherapy.

Mitotic catastrophe by genistein

Among other processes induced by genistein is the induction of mitotic catastrophe in some tumor cell lines [92]. Treatment of normal breast cells and breast cancer cells with genistein caused a more significant G_2/M arrest in cancerous cells as compared to their normal counterpart. In addition, when the mitotic fraction was measured, genistein-treated cells were found to be lower in number than control cells at any time, suggesting that cancer cells are inhibited from completing mitosis. Although genistein-treated cells were able to enter prophase and metaphase, they exhibited a high level of chromosomal structural abnormalities during anaphase, namely chromosome

bridges and/or absent chromosomes in addition to a major reduction in the number of telophase cells [92]. To understand the basis of this cell cycle arrest, a spread of the chromosomes showed that genistein caused aneuploidy, chromosome tangling and stickiness during metaphase, which could explain the abortive exit from mitosis. Further analysis showed that this was due to the inhibition of topoisomerase II, an enzyme necessary for normal chromosomal separation during mitosis. To confirm these findings, the same cells were treated with the specific topoisomerase II inhibitor, ICRF-193, which was found to cause polyploidy by allowing continuous duplication of DNA content in contrast to the blocking of endoreduplication and the 4n or 8n stop caused by genistein. Interestingly, the effect of genistein was found to be dominant over ICRF-193 when both were present [92].

Senescence by genistein

Apoptosis was found to constitute only 20–25 % of the observed cell death effects of genistein, therefore, there was a need to study other cell death mechanisms induced by this drug and which may account for its cytotoxic activities [91]. Treatment of breast (MCF-7), ovarian (SKOV-3), and prostate (DU-145, LNCaP) cancer cells with low concentrations of genistein (0.5–1 μM) were shown to activate telomerase activity at the transcriptional level [98]. However, higher concentrations of genistein (50 μM) reversed this effect and caused a significant inhibition of telomerase activity. This inhibition of telomerase was one of the major triggers of cell aging and senescence in the studied tumor cells. The extreme variation in genistein's effects depending on the concentrations applied proposes a wide frame of activities by which it can act on the cell [98]. In addition, genistein was shown to induce senescence by the activation of the tumor suppressor protein p53 [99].

In conclusion, genistein treatment provides the prospect for the primary prevention of common cancers through its pleiotropic effects. Further studies will reveal whether genistein is a promising chemopreventive and/or therapeutic agent against other human cancers. In fact, most anticancer drugs and therapeutic agents kill cancer cells by inducing apoptosis, a mechanism that has been recently shown not to be as effective since chemoresistence frequently arises by mutations in apoptotic pathways. About 70–80 % of ovarian cancer patients are treated effectively by chemotherapy, but the rest shows a relapse and eventual metastasis [91]. Therefore, non-apoptotic mechanisms of cell death induced by genistein seem to be a promising approach for improving cancer treatment and therapy.

In vivo cell death effects of genistein

The chemopreventive and chemotherapeutic effects of genistein in animal preclinical models have been recently summarized [100]. Genistein has been shown to reduce tumor incidence and multiplicity of several cancers in vivo, including leukemia, when targeted by linkage to a monoclonal antibody, and chemically-induced mammary tumors when administered neonatally [96]. The mechanisms of suppression of tumor formation by genistein include the inhibition of epidermal growth factor (EGF)-mediated tyrosine phosphorylation in the human epidermal carcinoma animal model [101], as well as enhanced death of B cell precursor leukemia cells by selectively inhibiting the CD-19-associated tyrosine kinase [101]. Also, genistein was found to act as a poison for Topoisomerase II in the colon of rats when applied at high levels [102]. The supplementation of genistein containing soy isoflavones induced interesting anticancer effects like preventing pulmonary metastasis of melanoma cells in mice, decreasing the rate of rise in prostate serum antigen levels with no toxicity in prostate cancer patients, and reducing oxidative damage in human volunteers [103].

Alkaloids as a rich source of anticancer drugs

Alkaloids are a diverse group of naturally occurring compounds known to be rich in biological activities [104]. They are broadly distributed in the plant kingdom, mainly in higher plants. Alkaloids contain a ring structure and a basic nitrogen atom; however some neutral and mildly acidic members exist [105, 106]. These nitrogen-containing compounds are not easily classified and there are no clear boundaries to distinguish them. Recent alkaloid classifications rely mostly on biosynthetic pathways and on the carbon skeleton [107].

Alkaloids have served as the main source of well-known anticancer drugs in the clinic such as the taxanes, vinca alkaloids, and camptothecins [14] and many other compounds are in clinical development [104]. The anticancer mechanisms of the most commonly used alkaloids involve arresting cells at G_2/M phase, inducing apoptosis by microtubule destabilization, preventing microtubule polymerization, and inhibiting topoisomerase I [14].

Recently, alkaloids derived from marine sources such as sponges, algae, aquatic fungi, cyanobacteria, and tunicates have been found to be a rich reservoir of anticancer compounds [108]. A PuBMed and Medline database search showed that there are more reports of non-apoptotic tumor cell death mechanisms by the alkaloids berberine, colchicine, and noscapine, all three of which are in clinical

development (Table 2). Based on this finding, we opted to review these three naturally occurring alkaloids.

Berberine

Berberine is an isoquinoline alkaloid (Fig. 1d) that has been extensively used in Ayurvedic and old Chinese medicine and is widely distributed in several medicinal plants such as goldenseal (*Hydrastis canadensis*), Indian Barberry (*Berberis aristata*), Chinese Goldthread (*Coptis chinensis*, *Coptis rhizome*, and *Coptis japonica*), and Amur cork tree (*Phellondendron amurense*), among others [109]. Berberine has a broad range of medicinal properties including antibacterial, antidiabetic, anti-inflammatory, hepatoprotective and neuroprotective effects, expansion of blood vessels, inhibition of platelet aggregation, and protection of myocardial ischemia and, therefore, has a wide spectrum of clinical applications [110]. Berberine is commonly used in Traditional Medicine for the treatment of diarrhea, diabetes, hyperlipidemia, metabolic syndrome, obesity, fatty liver disease, arrhythmia, and pain [111].

The wide spectrum of pharmacological properties of berberine indicates that it has a biologically interesting carbon skeleton which constitutes an attractive lead compound for several modifications [110]. Recently, berberine's anticancer properties have been highlighted in several reviews [104, 110, 112, 113]. Research has shown that berberine has potent apoptotic properties in tumor cells and interferes with several stages of tumor progression and metastasis. The molecular targets of berberine are diverse as this drug interacts directly with nucleic acids on specific DNA sequences [114] and with several crucial proteins such as DNA topoisomerases, telomerase, COX-2, p53, NF-κB, and metalloproteinases (MMPs) [112]. The antitumor effects of berberine are observed in several types of solid and hematological cancers and result in cell cycle arrest at G_1 or G_2/M phases, DNA damage, intrinsic and extrinsic apoptosis, production of ROS, and involvement of Bcl-2 family members [110]. Only recently alternative mechanisms of cell death have been uncovered for berberine ranging from autophagy, ER stress, programmed necrosis, mitotic catastrophe, and senescence as presented next.

Autophagy by berberine

Berberine was first demonstrated to induce autophagic cell death in vitro and in vivo when used in combination with radiotherapy in lung cancer [115]. Berberine enhanced radiosensitivity in A549 NSCLC cells through autophagy as evidenced by increased autophagosome formation, acridine orange-stained positive cells, LC3 modification, and mitochondrial disruption. This observation was extended in vitro and showed that berberine can induce autophagic and apoptotic cell death in hepatocellular carcinoma (HCC) HepG2, MHCC97-1, and SMMC7721 cells [116, 117]. In berberine-induced autophagy, the role of Beclin-1 activation and mTOR inhibition was mainly by suppressing the activity of Akt and up-regulating p38 MAPK signaling [116]. Cell death by berberine was diminished with the autophagy inhibitor 3-methyladenine (3-MA) or following interference with the essential autophagy gene *Atg5* [116, 117]. Berberine-induced cell death in HCC cell lines was also abrogated by the apoptosis inhibitor, z-DEVD-fmk, which further indicated the involvement of autophagy and apoptosis [117]. CD147, a glycosylated immunoglobulin super family transmembrane protein, is overexpressed in HCC cancer cells and is shown to promote angiogenesis, invasion, metastasis, and to confer resistance to chemotherapeutic drugs [117]. In HCC cells, berberine-induced autophagic cell death reduced the expression of CD147 [117]. Recently, berberine was shown to induce apoptotic and autophagic cell death in HepG2 cells as shown by Annexin V^+ and by the two autophagy regulators Beclin-1 and LC3BII [118]. Inhibitors of autophagy (3-MA and Bafilomycin A1) abrogated berberine-induced cytotoxicity whereas the caspase inhibitor z-VAD-fmk only partially reversed it. siRNA-mediated silencing of LC3B or Beclin-1 also reversed berberine-induced cell death [118], further emphasizing the role of autophagy activation in berberine-mediated cell death. Interestingly, the role of AMPK activation was also highlighted in berberine-induced autophagy and apoptosis where its inhibition by RNA interference (RNAi) or compound C abrogated berberine induced caspase-3 cleavage, apoptosis, and autophagy in HepG2 cells [118].

Necrosis and mitotic catastrophe by berberine

Berberine was shown to induce cell death in U937 promonocytic and B16 melanoma cell lines but had no effect on the cell cycle distribution [119]. Berberine induced apoptosis in U937 cells, however, it resulted in necrotic but not apoptotic cell death in B16 melanoma cells at concentrations as low as 1 μg/ml [119]. Doxorubicin is a potent anticancer drug that causes side effects namely hepatocellular degeneration, and liver fibrosis and necrosis. Interestingly, pretreatment with berberine reduced doxorubicin-induced hepatotoxicity and liver necrosis in mice, highlighting the hepatoprotective effect of this alkaloid when used in combination of hepatotoxic drugs [120].

Berberine was shown to inhibit the proliferation and induce cell death in several types of cancer cells either G_1

Table 2 Plant-derived anticancer alkaloids

Purified plant compound	Plant origin	Cancer system	Cell death signaling mechanisms	References
Alkaloids				
Berberine and analogues	*Hydrastis canadensis* (goldenseal), *Berberis aristata* (Indian Barberry), *Coptis chinensis*, *Coptis rhizome*, *Coptis japonica* (Chinese Goldthread), *Phellondendron amurense* (Amur cork tree)	Autophagy		
		Non-small cell lung (A549, H460), Lewis lung carcinoma models	Enhance radiation effect through autophagy; induce mitochondrial disruption	[115]
		Liver (HepG2, MHCC97-1, SMMC7721)	Inhibit mTOR and Akt; upregulate p38 MAPK	[116]
		Liver (SMMC7721, HepG2)	Reduce CD147 expression	[117]
		Liver (HepG2)	Activate AMPK	[118]
		Necrosis and mitotic catastrophe		
		Melanoma (B16)		[119]
		Prostate (RM-1)	Induce DNA damage and G_2/M arrest; activate ATM-Chk1	[121]
		Senescence		
		Leukemia (U937)	Inhibit telomerase activity; increase chromosomal abnormalities	[123]
		Breast (SK-BR-3)	Increase expression of p53, p21, p16, and plasminogen activator inhibitor-1	[124]
		Glioblastoma (U87, U251, U118), gliobastoma xenografts	Reduce EGFR and RAF-MEK-ERK signaling	[126]
Noscapine and analogues	*Lachryma papaveris* (opium)	Autophagy		
		Prostate (PC-3)	Generate ROS	[132]
		Mitotic Catastrophe		
		Breast (MCF10A-transformed by cigarette smoke condensate)	Induce polyploidy and telomeric association; downregulate cdc2/cyclin B1 and cdc2 kinase activity	[133]
		Cervical (HeLa)	Impair CLIP-170 and EB1 function; upregulate Aurora A, Plk4, and cyclin B1	[132]
		Prostate (PC-3)	Generate ROS; induce DNA damage	[134]
Colchicine and analogues	*Colchicum autumnale* (meadow saffron), *Gloriosa superba* (glory lily)	Autophagy		
		Breast (MCF-7, MDA-MB-231)	Bind colchicine site of tubulin	[141]
		Melanoma (B16/F10), colorectal (HCT-116)	Decrease p62 levels	[142]
		Pancreas (PANC-1), leukemia (Jurkat)	Generate ROS	[143]
		Necrosis		
		Lung, colorectal, ovarian, prostate, and breast mouse models		[144]
		Fibrosarcoma (HT1080)		[140]
		Melanoma (B16/F10) mouse model	Cause vascular disruption	[142]

AMPK AMP-activated kinase, *ATM* ataxia-telangiectasia-mutated, *cdc2* cell division cycle protein 2 homolog, *Chk1* checkpoint kinase 1, *CLIP-170* cytoplasmic linker protein 170, *EB1* end binding protein-1, *EGFR* epidermal growth factor receptor, *ERK* extracellular signal-regulated kinase, *LC3* light chain 3, *MAPK* mitogen-activated protein kinase, *MEK* mitogen-activated protein kinase kinase, *mTOR* mammalian target of rapamycin, *Plk4* polo-like kinase 4, *RAF* rapidly accelerated fibrosarcoma, *ROS* reactive oxygen species

arrest, G_2/M arrest or apoptosis depending on the cell type, drug dose, and treatment duration. Concentrations of berberine, as low as 5 μM, caused G_1 arrest and apoptosis due to activation of the p53-p21 cascade in the prostate cancer cells RM-1 [121]. On the other hand, ten-fold higher concentrations caused extensive DNA damage leading to

G_2/M arrest and mitotic catastrophe to avoid genomic instability [121, 122]. Pharmacological inhibition of ATM or Chk1 abrogated berberine-induced G_2/M arrest. The involvement of Chk1 in the G_2/M arrest by berberine was further confirmed by RNAi of this kinase. Interestingly, inhibition of ATM by caffeine or KU55933 abolished G_2/M arrest but caused apoptosis due to berberine treatment [121].

Senescence by berberine

Berberine was first shown to induce tumor cellular senescence by using COMPARE analysis, a bioinformatics approach for identifying antitumor agents according to their growth-inhibitory against a panel of human tumor cell lines [123]. Berberine was identified as a moderate telomerase inhibitor with 50 % inhibition at approximately 35 μM concentrations in the U937 monoblastoid leukemia cells. Using berberine as a seed alkaloid in COMPARE resulted in the identification of the berberine-like compounds, the rhodacyanine derivatives MKT077 and FJ5002, with 50 % telomerase inhibition at approximately 5 and 2 μM, respectively [123]. Long-term treatment of U937 cells with nanomolar (nM) concentrations of FJ5002 resulted in progressive telomere erosion, increased chromosome abnormalities, and senescence/crisis-like features. Berberine and its synthetic derivatives were shown to induce growth arrest, apoptosis, and cellular senescence in HER-1/neu overexpressing SK-BR-3 breast cancer cells [124]. Berberine and derivatives increased the expression of some senescence players namely the tumor suppressors p53, p21, p16, and the p53 target gene plasminogen activator inhibitor-1 which is an essential mediator of replicative senescence [125]. Interestingly, berberine and its derivatives reduced the expression and phosphorylation of the oncogene HER/neu indicating possible efficacy against aggressive breast cancer cells [124]. Recently, the antitumor effect of berberine in glioblastoma was demonstrated to be mediated through the induction of cellular senescence in vitro and in vivo [126]. Berberine resulted in reduced levels of epidermal growth factor receptor (EGFR) and RAF-MEK-ERK signaling which are usually upregulated in human glioblastoma [126]. Inhibition of EGFR by pharmacological means or by the use of RNAi resulted in senescence induction in these cells.

It is interesting to note that depending on the proliferating cell type, berberine may be used as an anti-aging or gerosuppressive agent. In fact, berberine was shown to suppress the level of constitutive mTOR, DNA damage, ROS as well as senescence in several types of proliferating tumor cells such as A549 lung, WI-38 lung fibroblast, TK6 lymphoblastoid, and stimulated human lymphocytes [127]. Berberine can also reduce premature and stress-induced senescence caused by the anticancer drug, mitoxantrone by targeting mTOR/S6 inhibition and ROS signaling underscoring the beneficial effects of this alkaloid in age-related diseases [128].

In vivo cell death effects of berberine

Berberine has demonstrated significant antitumor properties in several animal models [112]. Berberine hydrochloride treatment increased the life span in Dalton's lymphoma ascites bearing mice where intraperitoneal administration was shown to be more effective than the oral route [129]. In addition, berberine sulfate exhibited antipromoting properties in the two-stage epidermal carcinogenesis model [130]. Berberine enhanced radiosensitivity of lung cancer cells in vitro as well as in vivo [115]. This synergistic effect was demonstrated using the Lewis lung carcinoma model in mice, showing that berberine combined with irradiation caused tumor shrinkage, induced autophagy, increased expression of Beclin-1, and decreased Bcl-2 protein levels. Interestingly, berberine inhibited the growth of glioblastoma xenografts and induced senescence in tumors [126].

Noscapine

Opium is known through prehistoric ages for its sedative and medicinal properties. Noscapine, a phthalideisoquinoline alkaloid (Fig. 1e), is one of the most abundant alkaloids in opium constituting up to 10 % of its total content. This orally bioavailable drug has low toxicity, good tolerance, and low addiction and has been commonly used as a cough suppressant and in the treatment of strokes and cancer [131]. Noscapine antitumor properties extend to several in vitro cancer models ranging from lymphoma, breast cancer, ovarian carcinoma, glioblastoma, colon cancer, and NSCLC [131]. Noscapinoids, or noscapine analogues, are microtubule-modulating agents that have been developed with promising chemotherapeutic properties but evading the side effects of conventional tubulin-targeting drugs. Noscapinoids include several haloderivatives namely 9-bromonoscapine (Br-nos) and its reduced derivative (Red-Br-nos); they have higher tubulin binding activities at concentrations much lower than noscapine, and have limited effects on microtubule dynamics, and are therefore less toxic. They are more potent than noscapine; induce G_2/M arrest, and a variety of cell death mechanisms including autophagy and mitotic catastrophe.

Autophagy by noscapine

The noscapine analogue Red-Br-nos was shown to exert autophagy in prostate PC-3 cells as demonstrated by numerous acidic vascular organelles, conversion of LC3-I to LC3-II, and upregulation of Beclin-1 expression followed by caspase-independent apoptotic cell death [132]. Red-Br-nos treated PC-3 cells resulted in ROS generation and its attenuation by ROS scavengers was found to reduce autophagy and apoptosis. Given the contradictory functions of autophagy in cell death and survival, the authors have used a variety of genetic and biochemical approaches such as Beclin-1 siRNA and 3-MA, to respectively elucidate the role of autophagy and apoptosis in inducing cell death by this noscapine analogue in PC-3 cells [132]. Unexpectedly, enhanced cell death induction by apoptosis was observed upon inhibition of autophagy in treated cells, highlighting the role of autophagy suppression in the anticancer effects of this noscapine analogue.

Mitotic catastrophe by noscapine

Several reports have implicated noscapinoids as strong inducers of mitotic catastrophe in breast, prostate, and cervical cancer cells. In fact, Br-nos induced polyploidy and telomeric association in cigarette smoke condensate (csc)-transformed normal breast epithelial cells (MCF10A-csc3) while no effect was observed in normal MCF10A cells [133]. Br-nos treatment resulted in irreversible mitotic catastrophe in MCF10A-csc3 cells which exhibited aberrant multipolar spindle formation and subsequent impaired alignment of the replicated chromosomes to the equatorial plate and apoptotic cell death. Decreased levels of cdc2/cyclin B1 and cdc2 kinase activity were suggested to play a role in Br-nos-induced mitotic cell arrest leading to cell death [133]. Further studies in HeLa cervical cancer cells have shown that Br-nos induced mitotic catastrophe and that inhibition of microtubule dynamics was accompanied with impaired association of microtubule plus-end tracking proteins such as CLIP-170 and EB1 [132]. Br-nos treatment resulted in the formation of multipolar spindles due to centrosome amplification and upregulation of Aurora A and Plk4 protein levels. Metaphase arrested cells subsequently underwent either extensive apoptosis or mitotic slippage due to elevated cyclin B1 levels eventually leading to an inappropriate exit from the cell cycle, while other cells formed massive G_1-like interphase cells [132]. Red-Br-nos was shown to induce ROS-dependent DNA damage that resulted in widespread centrosome amplification in prostate cancer PC-3 cells [134]. Unlike other DNA damaging agents such as doxorubicin which cause DSB, prolonged G_2 arrest, and "template" centrosome amplification, Red-Br-nos causes de novo centrosome amplification and a transient S/G_2 arrest, followed by aberrant mitotic entry resulting in aberrant mitotic figures with spindle multipolarity [134]. ROS-mediated DNA damage was partly responsible for centrosome amplification and spindle multipolarity in the treated prostate cancer cells. Most mitotically arrested cells died following "metaphase catastrophe" by caspase-independent apoptosis which was marked by extensive membrane blebbing while minority of cells underwent aberrant anaphase or "anaphase catastrophe" and progressed through mitosis with multiple lobes followed by complete or incomplete cytokinesis [134].

In vivo cell death effects of noscapine

Noscapine was first recognized as a potent inducer of apoptosis when administered orally, injected intraperitoneally or intragastrally against solid murine lymphoid tumors and against xenografts of breast and bladder tumor cells implanted in nude mice [135]. Noscapine was shown to arrest cells in mitosis by binding stoichiometrically to tubulin, altering its conformation, and affecting microtubule assembly [135]. Noscapine potent antitumor properties extended to several in vivo models ranging from lymphoma, breast cancer, ovarian carcinoma, glioblastoma, colon cancer, and NSCLC with minimal toxicity to normal tissues [131]. The oral bioavailability of noscapine as well as its ability to cross the blood brain barrier, and to interfere with microtubule dynamics with minimal cytotoxicity to normal tissues including those harboring rapidly proliferating cells, highlights its chemotherapeutic potential [136].

Colchicine

Colchicine, a nitrogen-containing bioactive alkaloid (Fig. 1f), is a natural compound that is one of the oldest antimitotic drugs still currently available that can be isolated from plants belonging to the lily family such as meadow saffron (*Colchicum autumnale*) or glory lily (*Gloriosa superba*) [137]. Colchicine has been used in the treatment of several diseases namely gout, familial Mediterranean fever, cirrhosis, Behcet's disease, and other crystal arthritis [138]. Its potent antitumor properties are due to the fact that it binds irreversibly to tubulin which hinders microtubule formation, inhibits cell cycle progression, and induces apoptosis. Unfortunately, colchicine's action is not very specific as it targets rapidly dividing tumor cells as well as normal cells halting their progression into clinical applications. As a result,

derivatives of colchicine as well as colchicinoid prodrugs were developed with less toxicity and more specific targeting to tumor cells and with potential drug delivery strategies [139]. Some of these latter compounds are vascular disrupting agents (VDA) which cause rapid shutdown and disruption of the tumor vasculature leading to tumor starvation and excessive cell death [140].

Autophagy and necrosis by colchicine

The first report linking colchicine to autophagy was observed with the substituted pyrrole colchicine mimetic, JG-03-14, that binds to the colchicine site of tubulin [141]. This microtubule poison promotes cell death at nM concentrations mostly through autophagy in *p53* wild type breast cancer cells (MCF-7) and in mutant *p53* cells (MDA-MB-231). JC-03-14 induced autophagic cell death was extended to additional tumor cells, the B16/F10 melanoma and the HCT-116 colon cancer cells [142]. Recently, a novel derivative of allocolchicine (S)-3,8,9,10-tetramethoxyallocolchicine, known as Green 1, was shown to trigger selective autophagic cell death and programmed necrosis in pancreatic cancer (PANC-1) and acute T cell leukemia cells (E6-1 or Jurkat), while having minor effect on normal human fibroblasts [143]. Unlike colchicine, Green 1 does not seem to target tubulin polymerization to a great extent; however, it selectively results in increased ROS production from isolated mitochondria only in cancer cells and is, therefore, more tolerated in mice.

In vivo cell death effects of colchicine

ZD6126, an allocolchicine analogue, caused extensive tumor cell necrosis in mouse models of human lung, colorectal, ovarian, prostate, and breast tumors [144]. Unfortunately, this compound resulted in extensive associated cardiotoxicity in humans which halted its clinical development in Phase II [145]. Consequently, novel ZD6126 analogues were developed with more selective toxicity to tumor versus normal cells and were shown to be well tolerated in mice [143].

One efficient chemotherapeutic strategy is to disrupt the vasculature inside the tumor and to deprive it of nutrients and oxygen leading to massive tissue necrosis [146]. Several VDAs, including colchicine, target the colchicine-binding site of tubulin and cause rapid destruction of tumor vasculature leading to tumor necrosis; however, their clinical application has been compromised by intrinsic systemic toxicity, in particular to the cardiovascular system [147]. A colchicinoid prodrug (ICT2588) was developed that is selectively metabolized by specific membrane-type 1 MMPs (MT1-MMPs) that are overexpressed in tumor cells into an active VDA [140]. This prodrug was shown to be selectively activated in HT1080 fibrosarcoma cells that overexpress MT1-MMP but not in MCF cells that did not express this MMP. The prodrug ICT2588 was selectively activated in the tumor but not in normal tissues in vivo and resulted in decreased toxicity, reduction of tumor vasculature, and necrosis of the tumor. Using nanomedicine drug delivery systems to bypass the toxicity problem of VDAs, a polymeric colchicinoid prodrug with reduced toxicity and improved cancer vascular disruption was synthesized by conjugating colchicine to PEG_{5000} [148]. Favorable hydrolysis and vascular disruption potential was observed with this prodrug using human umbilical vein endothelial cells and in B16F10 melanoma bearing mice. Specifically only tumor bearing mice treated with this colchicinoid prodrug but not with free cholchicine resulted in vascular disruption and consequent tumor necrosis [148]. Recently, the colchicine-mimetic JG-03-14 reduced the size of tumor nodules in a murine model of B16/F10 melanoma cells that metastasizes to the lungs [142].

Terpenoids as a rich source of anticancer drugs

Terpenoids are the largest class of natural products and constitute a rich source of compounds in drug discovery particularly in the cancer field [149]. Terpenoids are derived from five carbon isoprene units and are divided into five categories based on structures including monoterpenoids, sesquiterpenoids, diterpenoids, triterpenoids, and tetraterpenoids. The anticancer properties of terpenoids have been recently the focus of intense research. In fact, several sesquiterpene lactones were tested in cancer clinical trials due to their selectivity toward tumor and CSCs [150]. Terpenoids inhibit several stages of tumor formation including cell proliferation, cell death, angiogenesis, and metastasis. They induce tumor cell death by targeting several specific molecular mechanisms, namely blocking the sarco/ER calcium ATPase pump, inhibiting NF-κB, JAK-STAT, AP-1, MMPs, DNA topoisomerase I and II, and the proteasome, activating p53, binding to DNA minor grooves, and modulating epigenetic mechanisms [149, 150]. Most terpenoids induce tumor cell death by targeting apoptotic pathways [151]. Alternative cell death mechanisms have been emerging only recently for this class of natural compounds specifically for parthenolide, triptolide, and betulinic acid (Table 3).

Parthenolide

Sesquiterpene lactones are 15-C terpenoids that are commonly used in traditional medicines against inflammation and cancer [152]. Several sesquiterpene lactones such as

Table 3 Plant-derived anticancer terpenoids and organosulfurs

Purified plant compound	Plant origin	Cancer system	Cell death signaling mechanisms	References
Terpenoids				
Parthenolide and analogues	*Tanacetum parthenium* (shoots of feverfew)	Autophagy		
		Breast (MDA-MB-231)	Activate NADP oxidase and JNK; generate ROS; deplete thiol and glutathione levels; downregulate NF-κB	[157]
		Liver (HepG2)		[159]
		Cervical (HeLa), leukemia (HL60)	Deplete 4E-BP1; generate ROS	[160]
		Necrosis		
		Leukemia (HL60, Jurkat)		[161]
		Breast (MDA-MB-231)	Generate ROS; induce dissipation of mitochondria membrane potential; activate RIP-1	[157]
Triptolide and analogues	*Tripterygium wilfirdii* (roots of Huangteng and gelsemium elegan)	Autophagy		
		Pancreas (S2-013, S2-VP10, Hs766T)	Require *atg-5* or *Beclin-1*; inactivate Akt/mTOR/p70S6K pathway; upregulate ERK 1/2 pathway; downregulate GRP78	[171, 172]
		Lung (A549)		[166]
		Neuroblastoma (SH-SY5Y)	Induce intracellular calcium levels; inhibit NF-κB and heat shock protein levels	[173]
		Senescence		
		Primary prostate cells		[175]
Betulinic Acid and analogues	*Betula alba* (bark of white birch tree)	Autophagy		
		Bladder (KU7, 253JB-V)	Decrease Akt phosphorylation	[182]
		Glioblastoma (U87MG)		[183]
Organosulfurs				
Sulforaphane and analogues	*Brassica oleracea* (cruciferous vegetables)	Autophagy		
		Prostate (PC3)	Disrupt Bcl-2/Beclin-1 interaction	[197]
		Breast (MDA-MB-231, MCF7, MDA-MB-468, SKBR-3)	Decrease Akt activity	[204]
		Mitotic catastrophe and necrosis		
		Breast (MCF7)	Induce aneuploidy	[199]
		Breast (F3II) and (MCF-7)	Activate cdc2	[198]
		Colorectal (Caco-2)		[208]

atg-5 autophagy protein 5, *Bcl-2* B-cell lymphoma 2, *cdc2* cell division cycle protein 2 homolog, *eIF4E* eukaryotic translation initiation factor 4E, *4E-BP1* translation initiation factor eIF4E binding protein, *ERK1/2* extracellular signal-regulated kinase1/2, *GRP78* 78 kDa glucose-regulated protein, *JNK* c-Jun N-terminal kinase, *LC3* light chain 3, *mTOR* mammalian target of rapamycin, *NADP* nicotinamide adenine dinucleotide phosphate, *NF-κB* nuclear factor kappa-light-chain-enhancer of activated B cells, *p70S6K* ribosomal protein S6 kinase beta-1, *RIP-1* receptor-interacting serine/threonine-protein kinase 1, *ROS* reactive oxygen species

parthenolide, artemisinin, and thapsigargin have been tested in cancer clinical trials [150, 153]. The sesquiterpene lactone parthenolide "4a,5b-epoxy-germacra-1-(10),11-(13)-dien-12,6a-olide" (Fig. 1g) originally purified from the shoots of feverfew (*Tanacetum parthenium*) has received considerable attention in cancer drug discovery [154]. Interestingly, parthenolide is the first small molecule found to be selective against CSCs by targeting specific signaling pathways and by modulating epigenetic mechanisms [154, 155]. This led to the derivatization of an orally bioavailable analogue, dimethylamino-parthenolide (DMAPT) with improved pharmacokinetic properties [156]. Parthenolide induced intrinsic and extrinsic apoptosis in a variety of tumor cells by inhibiting NF-κB, activating p53 signaling, regulating MAPK and JNK, interfering with STAT3, regulating Bcl-2 family members, and generating ROS [153].

Autophagy by parthenolide

The first report showing that the cytotoxic activities of parthenolide are due to autophagy was in the triple-negative MDA-MB231 breast cancer cells [157]. Parthenolide and DMAPT activated NADP oxidase which resulted in the accumulation of ROS, the depletion of the thiol and glutathione levels and activation of JNK while downregulating NF-κB. Parthenolide and DMAPT both induced autophagic cell death as shown by vacuole staining with monodansylcadaverine (MDC) as distinct dot-like structures, increased expression of Beclin-1, and the formation of LC3-II [157]. ROS generation later caused the dissipation of the mitochondrial membrane and caspase-independent necrotic cell death. Parthenolide was also shown to induce ROS-dependent cell death in MCF-7 cells through activated AMPK, apoptosis, and autophagy which led to M phase arrest [158]. However, parthenolide induced autophagy as a survival rather than a cell death mechanism in these breast cancer cells. In fact, suppression of either AMPK using a selective compound C inhibitor or a retroviral knockdown shAMPK or inhibiting autophagy by 3-MA, potentiated parthenolide-induced apoptosis [158]. In other tumor cells such as HepG2 HCC, parthenolide treatment resulted in mitochondrial-dependent apoptosis, caspase 9 and 3 cleavage, and increased p53 and Bax while decreasing Bcl-2 [159]. In these latter cells, parthenolide induced autophagic cell death as evident by MDC and acridine orange staining of autophagy positive cells and decreased expression of the proliferation marker Ki67 [159]. It remains to be determined whether autophagy in parthenolide-treated HepG2 cells acts as a survival or cell death pathway. Recently, the translation initiation factor eIF4E binding protein 1 (4E-BP1) was shown to be involved in parthenolide-mediated autophagic cell death in HL-60 and HeLa cells which was independent of its role in protein translation [160]. The use of shRNA 4E-BP1 provided evidence for autophagy being a downstream event of parthenolide-mediated 4E-BP1 reduction and autophagy induction.

Necrosis and apoptosis by parthenolide

Parthenolide was observed to induce mixed cell necrosis and apoptosis simultaneously in treated HL60 acute promyelocytic cells (APL) and Jurkat T lymphoma cells [161]. Apoptosis was confirmed by caspase-3 activation and propidium iodide-stained DNA while necrosis was determined by the rapid loss of plasma membrane integrity and subsequent DNA content. The interaction of parthenolide with the plasma membrane led to membrane rupture and resulted in rapid necrotic cell death, a property of several sesquiterpene lactones that directly target plasma membranes affecting their lipid oxidation and rupture [162]. Later parthenolide and DMAPT were shown to induce autophagy as reported earlier and mixed apoptosis and necrosis in treated MDA-MB231 breast cancer cells due to the dissipation of mitochondrial membrane potential and subsequent ROS generation [157]. Recently, receptor-interacting protein 1 (RIP1) kinase emerged as a key upstream regulator at the "crossroads" of inflammatory signaling and the activation of cell death by apoptosis or caspase-independent programmed necrosis [163]. Interestingly, parthenolide-mediated necrotic cell death in MDA-MB231 cells was suppressed by the use of the antioxidant NAC and the RIP-1 kinase inhibitor, nec-1, implying the role of ROS and RIP-1 activation in the induction of necrotic cell death [157].

In vivo cell death effects of parthenolide

Various in vivo tumor models and drug administration routes have been tested for parthenolide and DMAPT [154]. Oral treatment with DMAPT in mice bearing xenografts of MDA-MB231 cells resulted in a reduction of tumor volume, NF-κB, MMP-2 and MMP-9, VEGF, and lung metastasis while it upregulated p-JNK and enhanced animal survival [157]. Parthenolide injections in AML xenograft model increased apoptosis in tumor cells, decreased Bcl-2 and cyclin D1 expression, and inhibited NF-κB [164]. In general, it was documented that when the drug is injected in the form of parthenolide or when administered orally in the form of DMAPT, both compounds, as single agents, do not eradicate substantially tumor volumes. In particular, they do not seem to affect all the subpopulations in a tumor but seem to preferentially target the CSCs, which often constitute a small fraction of the tumor volume and eradicate them mostly by apoptosis [154, 156]. This was obvious when both drugs consistently inhibited tumor metastasis and engraftment for which CSCs are crucial, therefore, emphasizing the need for combination therapies with other drugs that target the tumor. In fact, parthenolide or DMAPT have been successfully used in combination treatment in murine breast, lung, prostate, and pancreatic cancer models [153].

Although most of the reported cell death pathways induced by parthenolide and DMAPT in various in vivo models are apoptotic, however it remains to be determined whether these drugs cause other modes of cell death in animal tumors by targeting CSC.

Triptolide

Triptolide is a diterpenoid with a unique triepoxide structure (Fig. 1h) extracted from the roots of *Tripterygium wilfordii*, also known as Huangteng and gelsemium elegan.

Triptolide has a long history in Chinese medicine because it has numerous pharmacological activities including potent neuroprotective, anti-rheumatism, and anti-inflammatory properties [165]. Consequently, triptolide has been used in the treatment of several diseases including nephritic syndrome, arthritis, lupus, central nervous system diseases, and cancer [165].

Remarkably triptolide inhibited the proliferatin of all 60 US National Cancer Institute cancer cell lines at nM IC_{50} values. Furthermore, its anticancer activities were confirmed in a variety of xenograft animal models resulting in the development of several triptolide and more water-soluble and less toxic derivatives such as PG490-88 or F60008 that entered clinical trials [166]. This diterpenoid is a strong modulator of the cell transcriptional machinery and was shown to interact with RNA polymerase and modulate the transcription of crucial transcription factors such as NF-κB, p53, NF-AT, and HSF-1 [167]. More recently triptolide was demonstrated to inhibit global gene transcription in cancer cells due to the proteasomal-mediated degradation of the large subunit of RNA polymerase II [168]. This drug is known to be an inducer of apoptosis which affects a broad spectrum of tumors that contain both wild-type and mutant forms of *p53* [169]. This diterpenoid was shown to inhibit the expression of the negative p53 regulator, MDM2, and to downregulate the inhibitor of apoptosis XIAP in a p53-independent manner as well as the heat shock proteins (HSP70) which are upregulated in tumor formation [165, 169, 170]. However, autophagy has emerged recently as a new mechanism of cell death for this drug.

Autophagy by triptolide

Triptolide was shown to inhibit the growth of pancreatic cancers in vitro and in vivo, although pancreatic adenocarcinomas are among the most lethal and chemotherapy resistant cancers [169, 171, 172]. Depending on the pancreatic adenocarcinoma cell type, triptolide may induce caspase-dependent apoptosis in MiaPaCA-2, Capan-1, and BxPC-3 or caspase-independent autophagic cell death in S2-013, S2-VP10, and Hs766T [171]. Triptolide induced autophagy specifically because the increase in LC3-II was time- and dose-dependent and the increase in acridine orange in tumor cells upon triptolide treatment was reversed by the addition of the autophagy inhibitor 3-MA and the knock down of the autophagy specific genes *atg-5* or *Beclin-1*. Triptolide-induced autophagy required *atg5* and *Beclin-1* and was associated with the inactivation of the Protein Kinase B (Akt)/mammalian target of Rapamycin/p70S6K pathway and the upregulation of the ERK 1/2 pathway [171]. Finally, inhibition of autophagy in S2-013 and S2-VP10 treated cells enhanced apoptotic cell death while abrogating autophagy or apoptosis rescued the cells from the cytotoxic effect of the drug. Recently, triptolide was shown to activate the unfolded protein response leading to chronic ER stress in pancreatic cancer cells [172]. A major player in triptolide-induced cell death is the survival glucose-regulated protein (GRP78) which is elevated in pancreatic cancer cells and downregulated in pancreatic cancer cells upon triptolide treatment leading to either apoptosis in MIA PaCa-2 cells or autophagy in S2-VP10 cells [172].

A derivative of triptolide, tripchlorolide, was shown to suppress the growth and induce autophagic cell death in A549 lung cancer cells with no observed signs of apoptosis [166]. Cell death in A549 cells treated cells was alleviated by pretreatment with the autophagy inhibitor 3-MA implying that autophagy rather than apoptosis is the principal cell death pathway. In other tumor cells such as in neuroblastoma, triptolide-mediated cell death was cell type specific where it induced apoptosis in IMR-32 cells and autophagic cell death in SH-SY5Y [173]. Triptolide treatment of both types of neuroblastoma cells induced intracellular calcium levels while inhibiting NF-κB activity and heat shock protein expression levels. It was recently demonstrated that autophagosome formation requires elevated calcium levels [174]; however the role of elevated intracellular calcium levels in autophagy and apoptosis upon triptolide treatment remains to be determined.

Senescence by triptolide

Triptolide and its water soluble derivative PG490-88 exhibited potent antiproliferative, apoptotic, or senescence activities in primary prostate adenocarcinoma cells [175]. Low concentrations of the drugs (1 ng/ml) abrogated growth completely in clonal assays and induced senescence as demonstrated by SA-β-gal activity, enlarged and flattened shaped cells, and development of vacuoles. However, senescence induction was not associated with the upregulation of the senescence players p53, p21, p27, or p16 [175]. Ten-fold higher concentrations of triptolide (10 ng/ml) resulted in a robust induction of apoptosis and nuclear p53 accumulation and reduced levels of Bcl-2.

In vivo cell death effects of triptolide

Triptolide has shown potent antitumor activities in several types of solid tumor xenografts in mice (B16 melanoma, MDA-435 breast cancer, TSU bladder cancer, and MGC80-3 gastric carcinoma) with wild-type and mutant forms of *p53* [169]. This drug inhibited the formation of

metastasis in B16 melanoma xenograft mouse model [169]. Triptolide also decreased the growth of tumors in cholangiocarcinoma hamster model [176]. In an orthotopic neuroblastoma tumor model, triptolide was effective in inhibiting tumor growth [177]. Neuroblastoma tumors have elevated levels of HSP70 especially in those that are poorly differentiated and metastatic. Interestingly, triptolide treatment reduced HSP70 transcripts and proteins in neuroblastoma tumors through an unknown mechanism [177]. The modes of cell death induced by triptolide in vivo remain to be determined.

Betulinic acid

Betulinic acid is a triterpenoid (Fig. 1i), originally isolated from the bark of the white birch tree, has potent antitumor properties in several tumors in vitro and in vivo and is relative nontoxic to normal cells and healthy tissues [178, 179]. Because of its poor solubility and pharmacokinetic properties, several betulinic acid analogues have been developed and have shown potent antitumor properties in cancer cells and animal models [180]. Betulinic acid and its analogues are known to induce intrinsic apoptosis by affecting the mitochondrial membrane permeability complex, enhancing the release of cytochrome c, regulating Bcl-2 family members, inhibiting NF-κB activity, and to possess anti-angiogenic and anti-metastatic activities [179, 180]. Furthermore, betulinic acid treatment in HeLa cells was shown to induce apoptosis through both the ER pathway and the ROS-mediated mitochondrial pathway [181].

Autophagy by betulinic acid

Recently, betulinic acid was shown to inhibit bladder cancer cell growth and to downregulate specificity protein (Sp) transcription factors in particular Sp1 and Sp3 and EGFR that are commonly upregulated in bladder tumor cells [182]. Betulinic acid also decreased the phosphorylation of Akt and induced autophagic cell death. This was also observed in glioblastoma cells using B10, a semisynthetic derivative of betulinic acid [183]. In these latter cells, B10 induced a mixed mode of cell death including apoptosis and autophagy. This was followed by the abrogation of the autophagic flux leading to lysosomal cell death as evidenced by lysotracker red staining and by cathepsin release from lysosomes into the cytoplasm [183].

However, in KM3 multiple myeloma cells, betulinic acid treatment blocked autophagy and enhanced apoptosis as evidenced by reduced Beclin-1 but induced caspase 3-dependent apoptosis [184]. Recently, betulinic acid was demonstrated to induce apoptosis in HeLa cells directly through the mitochondria which could be abrogated by cyclosporin A (CsA), an inhibitor of the permeability transition pore [185]. The use of general caspase inhibitors and necrostatin blocked apoptosis and necroptosis, respectively, but not cell death in HeLa cells, implicating other alternate non-caspase dependent mechanisms of cell death by betulinic acid in these cells [185]. Autophagy was shown to be massively induced in various betulinic acid-treated tumor cells, but was prevented by CsA, suggesting that autophagy occurred downstream of the betulinic acid-induced mitochondrial damage. Downregulating or knocking out key regulators of autophagy, provided evidence that autophagy serves as a rescue pathway and not as a cell death mechanism by betulinic acid in HeLa, MCF7 and MEFs derived from *Atg5* or *Atg7* knockout mice [185].

In vivo cell death effects of betulinic acid

Betulinic acid exhibited potent antitumor activities in several animal models of human cancer [179]. Betulinic acid was first shown to inhibit tumor formation in mouse skin two-stage carcinogenesis [186]. This triterpenoid suppressed tumor growth in a melanoma xenograft model [187] and cooperated with chemotherapeutic drugs to reduce lung metastasis in a metastatic melanoma model [188]. Betulinic acid increased the survival of animals with ovarian cancer xenografts and no systemic toxicities or weight loss was noted in betulinic-acid treated mice [189]. Furthermore, betulinic acid suppressed tumor growth, induced apoptosis, and inhibited the expression of Bcl-2 and Ki-67 protein in U14 cervical carcinoma tumor-bearing mice [190].

Specificity proteins, namely Sp1, Sp3, and Sp4, are overexpressed in tumors and enhance the proliferation and angiogenic potential of cancer cells [191]. Betulinic acid inhibited tumor growth in mice bearing RKO colon cancer cells xenografts and reduced Sp1, Sp3, and Sp4 expression in colon tumors [192] as well as in a prostate model of human cancer [193]. Betulinic acid treatment also decreased tumor size and weight in nude mice with MDA-MB-231 xenografts [194]. This drug also reduced mRNA and protein levels of Sp1, Sp3 and Sp4, as well as mRNA of Sp-regulated gene products, such as VEGFR and survivin, and of the oncogenic miRNA-27a that targets genes that regulate Sp factors and the G_2-M checkpoint in these triple negative breast cancer cells [194].

Several derivatives of betulinic acid were developed to enhance its anticancer potency and pharmacokinetic properties. C-3 modified betulinic acid derivatives have shown enhanced in vivo antitumor efficacy and pharmacokinetic properties as compared to betulinic acid against human colon cancers [195].

Organosulfurs and sulforaphane

Organosulfurs are plant-derived drugs that were found to possess significant anticancer effects. These compounds are characterized by their sulfur group and they naturally occur in a large variety of fungi and plants, mainly, garlic, nuts, coffee and some tropical fruits. Isothiocyanates are a major subcategory of organosulfurs characterized by their isothiocyanate group (–N=C=S) of which the central carbon renders the compound highly electrophilic and readily reacting with sulfur-, nitrogen-, and oxygen-based nucleophiles [196]. These compounds are generated by the myrosinase-mediated hydrolysis of their corresponding glucosinolates [197].

Sulforaphane (1-isothiocyanato-4-(methylsulfinyl)-butane) (Fig. 1j), one type of isothiocyanates, is found in cruciferous vegetables. It is present in high amounts in broccoli, cabbage, cauliflower and hoary weed. It is produced from the precursor glucoraphanin (a glucosinolate) by the myrosinase enzyme when the plant is damaged upon cooking or chewing. Sulforaphane occurs naturally as an L-isomer and its D,L analogue has been successfully synthesized. It is involved in several biological processes, and has the ability to inhibit cancer cell proliferation by different mechanisms. Sulforaphane exerts its antitumor effects by causing cell cycle arrest in G_2/M phase which was found to be associated with altered microtubule dynamics [198–200]. Other mechanisms of cell cycle arrest by sulforaphane included altered activity of cdc2 kinase, increased protein expression of cyclin B1, p21 and histone H1 phosphorylation [198, 201].

Autophagy by sulforaphane

Among the biological processes associated with sulforaphane treatment is autophagy. A study carried out on PC-3 human prostate cancer cells showed that sulforaphane led to the formation of autophagosome-like structures containing remnants of mitochondria after 16 h of treatment [197]. The formation of these membranous structures increased progressively with exposure time to this drug. In addition, treatment with sulforaphane led to the formation of acidic vesicular organelles, a unique feature of cells undergoing autophagy [197].

Several studies aimed to decipher the mechanisms by which sulforaphane induces autophagy. When investigating the effect of sulforaphane on the localization and processing of LC3 proteins in PC-3 cells, it was found that it affects these proteins in three different ways [197]. First, sulforaphane induced a significant increase in LC3I protein levels. Second, this increase in LC3 was shown to be associated with its processing into the membranous form LC3II. Third, in contrast to control cells which showed a diffused distribution of LC3 across the cell; cells treated with sulforaphane showed a punctuate pattern of LC3II proteins which reflected their recruitment into autophagosomes. Thus, sulforaphane-induced autophagy appears to be associated with LC3I induction and processing into LC3II, the latter being recruited into autophagosomes.

Another mechanistic approach for deciphering sulforaphane-induced autophagy was to study its effect on the Bcl-2 proteins. It was previously shown that the binding of Bcl-2 to Beclin-1 interrupts autophagy [202]. Consequently, autophagy is triggered when this association is disrupted. It was found that sulforaphane-induced autophagy is promoted by disrupting the Bcl-2/Beclin-1 interaction since it is able to reduce Bcl-2 levels in PC-3 cells which possibly promoted the Beclin-1 dissociation [197].

Another approach to unravel the mechanisms by which sulforaphane induces autophagy was to study the mTOR autophagy regulator. In its active form, mTOR inhibits autophagy [203]. In turn, mTOR itself is negatively regulated by the TSC1/TSC2 complex. It is known that the phosphorylated active form of Akt kinase inactivates the TSC1/TSC2 complex, thus activating mTOR and inhibiting autophagy. In this context, breast cancer cells treated with different concentrations of sulforaphane showed a decreased level of activated Akt kinase, Akt^{Ser473}, as the sulforaphane concentrations increased. This decrease in the activated Akt kinase by sulforaphane was highly correlated with initiation of the drug-induced autophagic process [204].

Sulforaphane was also found to inhibit apoptotic cell death by preventing the release of cytochrome c from the mitochondria into the cytosol. This prevention occurred as the autophagosomes sequestered and trapped the mitochondria making them unavailable for the action of sulforaphane [197]. The simultaneous treatment of sulforaphane and autophagy inhibitors both in vitro using 3-MA inhibitor [197] and in vivo using chloroquine inhibitor [205] enhanced drug cytotoxicity and apoptosis and inhibited tumor cell proliferation. In addition, the sulforaphane-chloroquine combination was found to suppress metastasis by inhibiting epithelial mesenchymal transition and the secretion of the pro-angiogenic cytokine VEGF [205], confirming that autophagy by sulforaphane is a tumor survival mechanism rather than a cell death mechanism.

Necrosis, apoptosis, and mitotic catastrophe by sulforaphane

In addition to causing autophagy and cell cycle arrest, sulforaphane was shown to be involved in initiating several pathways implicated in cell death. Sulforaphane has been associated with both apoptosis and programmed necrosis depending on the concentrations used and drug exposure

time. Different cell types treated with a range of sulforaphane concentrations were found to possess major apoptotic features including caspase activation, elevated Bax expression, PARP cleavage, and nuclear chromatin condensation.

HT29 cells treated with sulforaphane were found to undergo apoptotic cell death dependent on a proteasome degradation pathway, since blocking proteasomal activity by MG132 reduced sulforaphane-induced apoptosis [201]. In these HT29 cells, the retinoblastoma tumor suppressor protein (RB) was found to be highly phosphorylated. The RB protein, an important cell cycle regulator, is known to act as an apoptotic inhibitory factor in its unphosphorylated form. The presence of high amounts of phosphorylated RB upon sulforaphane treatment of cells may be involved in initiating apoptotic cell death in response to sulforaphane in HT29 cells [201].

Apoptosis by sulforaphane may be also a consequence of the same factors that cause cell cycle arrest. Perturbation in microtubule polarization was found to cause aneuploidy followed by apoptotic cell death [199]. In addition, the same inappropriate activation of cdc2 that led to cell cycle arrest at G_2/M phase may be the reason for the observed mitotic catastrophe in F3II cells [198]. Death by mitotic catastrophe was also observed in MCF-7 cells exposed to sulforaphane which was characterized by aberrant mitosis and micronucleation [198].

Sulforaphane also induced the production of ROS in primary hepatocytes [206]. The production of ROS by sulforaphane was mediated by the depletion of the intracellular glutathione pool which ultimately resulted in oxidative stress. This oxidative stress suggests an additional mechanism for sulforaphane-induced apoptosis [207]. The apoptotic effect of sulforaphane in Caco-2 colorectal cancer cells appeared to be dependent on drug exposure time. Apoptotic effects in these cells reached a climax following the fifth hour of treatment with a combination of sulforaphane and the chemotherapeutic drug oxaliplatin, after which a higher number of cells shifted to necrosis. These necrotic cells showed alterations such as osmotic swelling, cell lysis and loss of membrane integrity [208]. Although the mechanisms by which sulforaphane causes a switch from apoptosis to necrosis is still unclear, it seems to be related to both the concentrations used and drug exposure time [208].

In vivo cell death effects of sulforaphane

Sulforaphane treatment in vivo was found to inhibit tumor growth against several cancers, including F3II sarcomatoid mammary carcinoma [198], UM-UC-3 human bladder cancer [209], MM.1S human B lymphoblasts [210], and PC-3 prostate cancer [211]. The mechanism of tumor inhibition ranged from apoptosis which was associated with increased Bax expression [211], caspase 3 cleavage and cytochrome c release [209], as well as the inhibition of HDAC activity and increased acetylation of histones H3 and H4 [212]. Anti-angiogenesis, reduced necrosis, and immune regulation were also detected in treated mice [209]. The range of inhibition of tumor growth in these animal models ranged from 50 to 70 %. Treated mice showed no signs of toxicity [209, 213], indicating that sulforaphane may be considered an interesting non-toxic anticancer drug. In addition, orthotopical implants of human pancreatic CSCs in mice showed a 47 % reduction in the growth of tumors in sulforaphane-treated animals, an effect that was due to the blockage of the hedgehog pathway and the initiation of apoptosis in target cells [213].

Summary and perspectives

Anticancer drugs, including plant-derived ones, mostly exert their cell death effects by inducing apoptosis in tumor cells. Luckily, cancer cells resistant to apoptotic stimuli may be induced to die by other non-apoptotic mechanisms [214]. There is a pressing need for discovering anticancer drugs that induce alternative modes of cell death since apoptosis-resistant cancer cells are selected at advanced stages of tumor formation and metastasis. In fact, 90 % of cancer patients die of metastasis due to the resistance of their cancers to apoptosis inducing drugs [215].

Non-apoptotic mechanisms of key plant-derived anticancer drugs in clinical development are shown in Tables 1, 2, and 3. Several conclusions could be made from the literature presented in these tables. First, it appears that polyphenol as well as alkaloid anticancer drugs are capable of inducing the four different alternative modes of cell death i.e. autophagy, programmed necrosis, mitotic catastrophe, and senescence ultimately causing cell death. Second, the more recently discovered terpenoid and organosulfur anticancer drugs are less studied, and consequently have less reported non-apoptotic mechanisms of cell death. Third, autophagy seems to be the most commonly observed alternative mode of cell death induced by the selected plant-derived anticancer drugs which may also play a pro-survival function depending on the particular stimuli and cellular context. Fourth, few studies elaborated on the mechanism of programmed necrosis by these compounds.

The majority of the studies on non-apoptotic mechanisms of plant-derived anticancer drugs have been documented in in vitro tumor models, while apoptotic mechanisms have been observed in cultured cells as well as in tumor animal models and in human tissues. This raises

the question as to whether these alternative cell death mechanisms do commonly occur in vivo in response to plant-derived anticancer drugs. In fact, only a few of the plant-derived anticancer drugs have been reported to induce alternative pathways of cell death in vivo. For instance, curcumin was shown to trigger autophagy, necrosis, and mitotic catastrophe in animal models while berberine and colchicine induced autophagy and necrosis, respectively. It is essential to determine whether the drug concentrations that trigger the observed non-apoptotic modes of cell death in cultured tumor cells are achievable in vivo and can induce similar cell death mechanisms in humans and animals. Furthermore, discrepancies in modes of cell death are expected to be observed by a particular drug in different experimental settings. For instance, berberine induced programmed necrosis in vitro; however it prevented necrosis in vivo if used in combination treatments and it induced or inhibited senescence in different types of proliferating cells.

Cell death pathways are interrelated and their players may cross-talk resulting in accelerated or reduced cell death [216, 217]. Apoptotic, autophagic, and necrotic pathways that impact normal development, tissue homeostasis, and pathological conditions are interconnected [216–218]. The cross-talk between these mechanisms of cell death is complex. For instance, autophagy may facilitate apoptosis, antagonize apoptosis, or cooperate with apoptotic signaling pathways by a variety of mechanisms. Several players regulate autophagy and apoptosis namely the mTOR kinase pathway, Beclin 1, caspases, DAPK, and p53 [216]. Autophagy and programmed necrosis are also intimately interlinked with variable outcomes ranging from autophagy being an inducer, inhibitor, or with no effect on necrosis [216, 218].

The plant-derived anticancer drugs listed in this review share common cell death signaling pathways (Tables 1, 2, 3). For instance, polyphenols, alkaloids, terpenoids, and organosulfur compounds induce autophagy, through the inhibition of the Akt/mTOR and NF-KB pathways, and ROS generation, whereas senescence is stimulated by ATM/ATR, p53/p21 and Rb/p16 activation, and inhibition of telomerase activity. Interestingly, drugs belonging to the different categories of PSMs activate the Ras/Raf/MAPK signaling and also elevate ROS levels which in turn have a pleotropic effect on the different types of cell death. However, some unique cell death signaling pathways are emerging and may direct the design and the development of more selective and specific small molecules for cancer treatment. For instance, resveratrol-induced autophagy results in estrogen receptor coactivator PELP1 accumulation in autophagosomes and suppresses Wnt/β-catenin signaling whereas genistein-induced autophagy reduces the levels of the glucose transporter Glu1 limiting glucose utilization by tumor cells. Terpenoids-induced autophagy may be due to some unique pathways as parthenolide downregulates translation by depleting the levels of 4E-BP1 in leukemic and cervical cancer cells whereas it induces intracellular calcium level and reduces heat shock proteins in neuroblastoma cells. Senescence may be induced specifically in glioma cells by resveratrol due to the inhibition of H2B ubiquitin ligase RNF20 and by berberine due to the reduction of EGFR levels.

Future research is required to depict the specific route selected by plant-derived anticancer drugs to activate a specific cell death pathway and to identify the mechanisms by which a drug causes a switch from a cell death process into another. This is particularly relevant in the context of autophagy where this pathway may favor cell death or survival depending on the different drugs used, combination treatments, and genetic and epigenetic alterations in tumor cells [219, 220]. In particular, treatment of tumor cells with noscapine, parthenolide, and betulinic acid, induced autophagic cell death or cell survival.

The future of cancer therapeutics lies in exploiting alternative mechanisms of cell death separately or concomitantly to eradicate tumors. We know less about cell death mechanisms in CSCs and future research should focus on whether CSCs rely on alternative non-apoptotic mechanisms of cell death and/or on apoptotic ones. With this knowledge one could successfully target CSCs by using a combination of drugs that induce multiple cell death mechanisms [221]. More so, combination treatments may target the heterogeneous areas of the tumor which possess different genetic and epigenetic signatures [222, 223]. Whether other factors such as the tumor microenvironment [224, 225], alterations in cell metabolism [30], and the immune system [226, 227] affect the cell death response of the heterogeneous cells in a tumor remains to be determined. Future genomic and systems-biology approaches and cell signaling network studies will shed light on whether certain molecular signatures or pathways favor or hinder particular modes of cell death. It is this information that is instrumental for successful cancer therapy.

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References

1. May M (2014) Statistics: attacking an epidemic. Nature 509(7502):S50–S51. doi:10.1038/509S50a
2. Siegel RL, Miller KD, Jemal A (2015) Cancer statistics, 2015. CA Cancer J Clin 65(1):5–29. doi:10.3322/caac.21254
3. Vogelstein B, Kinzler KW (2004) Cancer genes and the pathways they control. Nat Med 10(8):789–799. doi:10.1038/nm1087

4. Millimouno FM, Dong J, Yang L, Li J, Li X (2014) Targeting apoptosis pathways in cancer and perspectives with natural compounds from mother nature. Cancer Prev Res 7(11):1081–1107. doi:10.1158/1940-6207.capr-14-0136
5. Pan S, Zhou S, Gao S, Yu Z, Zhang S, Tang M, Sun J, Ma D, Han Y, Fong W, Ko K (2013) New perspectives on how to discover drugs from herbal medicines: CAM's outstanding contribution to modern therapeutics. Evid Based Complement Alternat Med. doi:10.1155/2013/627375
6. Ansari J, Inamdar N (2010) The promise of traditional medicines. Int J Pharmacol 6(6):808–812. doi:10.3923/ijp.2010.808.812
7. WHO (2010) WHO monographs on medicinal plants commonly used in the Newly Independent States (NIS). WHO, Geneva. ISBN 978 92 4 159772 2
8. Azaizeh H, Saad B, Cooper E, Said O (2010) Traditional arabic and islamic medicine, a re-emerging health aid. Evid Based Complement Alternat Med 4:419–424. doi:10.1093/ecam/nen039
9. Yang B, Xu FY, Sun HJ, Zou Z, Shi XY, Ling CQ, Tang L (2014) Da-cheng-qi decoction, a traditional Chinese herbal formula, for intestinal obstruction: systematic review and meta-analysis. Afr J Tradit Complement Altern Med 11(4):101–119
10. Bodeker G, Kronenberg F (2002) A public health agenda for traditional, complementary, and alternative medicine. Am J Public Health 92(10):1582–1591
11. Werneke U, Earl J, Seydel C, Horn O, Crichton P, Fannon D (2004) Potential health risks of complementary alternative medicines in cancer patients. Br J Cancer 90(2):408–413. doi:10.1038/sj.bjc.6601560
12. Shukla S, Meeran SM, Katiyar SK (2014) Epigenetic regulation by selected dietary phytochemicals in cancer chemoprevention. Cancer Lett 355(1):9–17. doi:10.1016/j.canlet.2014.09.017
13. Dias DA, Urban S, Roessner U (2012) A historical overview of natural products in drug discovery. Metabolites 2(2):303–336. doi:10.3390/metabo2020303
14. Darwiche N, El-Banna S, Gali-Muhtasib H (2007) Cell cycle modulatory and apoptotic effects of plant-derived anticancer drugs in clinical use or development. Expert Opin Drug Discov 2(3):361–379. doi:10.1517/17460441.2.3.361
15. Lee JH, Khor TO, Shu L, Su ZY, Fuentes F, Kong AN (2013) Dietary phytochemicals and cancer prevention: Nrf2 signaling, epigenetics, and cell death mechanisms in blocking cancer initiation and progression. Pharmacol Ther 137(2):153–171. doi:10.1016/j.pharmthera.2012.09.008
16. Chan FK, Luz NF, Moriwaki K (2015) Programmed necrosis in the cross talk of cell death and inflammation. Annu Rev Immunol 33:79–106. doi:10.1146/annurev-immunol-032414-112248
17. Kharb M, Jat RK, Anju G (2012) A review on medicinal plants used as a source of anticancer agents. Int J Drug Res Tech 2(2):177–183
18. Gonzalez-Vallinas M, Gonzalez-Castejon M, Rodriguez-Casado A, Ramirez de Molina A (2013) Dietary phytochemicals in cancer prevention and therapy: a complementary approach with promising perspectives. Nutr Rev 71(9):585–599. doi:10.1111/nure.12051
19. Naasani I, Oh-Hashi F, Oh-Hara T, Feng WY, Johnston J, Chan K, Tsuruo T (2003) Blocking telomerase by dietary polyphenols is a major mechanism for limiting the growth of human cancer cells in vitro and in vivo. Cancer Res 63(4):824–830
20. Arapitsas P (2012) Hydrolyzable tannin analysis in food. Food Chem 135(3):1708–1717. doi:10.1016/j.foodchem.2012.05.096
21. Quideau S, Deffieux D, Douat-Casassus C, Pouysegu L (2011) Plant polyphenols: chemical properties, biological activities, and synthesis. Angew Chem 50(3):586–621. doi:10.1002/anie.201000044
22. Thomasset SC, Berry DP, Garcea G, Marczylo T, Steward WP, Gescher AJ (2007) Dietary polyphenolic phytochemicals-promising cancer chemopreventive agents in humans? A review of their clinical properties. Int J Cancer 3:451–458. doi:10.1002/ijc.22419
23. Block G, Patterson B, Subar A (1992) Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. Nutr Cancer 18(1):1–29
24. Lambert JD, Elias RJ (2010) The antioxidant and pro-oxidant activities of green tea polyphenols: a role in cancer prevention. Arch Biochem Biophys 501(1):65–72. doi:10.1016/j.abb.2010.06.013
25. He Q, Shi B, Yao K, Luo Y, Ma Z (2001) Synthesis of gallotannins. Carbohydr Res 335(4):245–250
26. Leopoldini M, Russo N, Toscano M (2006) Gas and liquid phase acidity of natural antioxidants. J Agric Food Chem 54(8):3078–3085
27. Arakawa H, Maeda M, Okubo S, Shimamura T (2004) Role of hydrogen peroxide in bactericidal action of catechin. Biol Pharm Bull 27(3):277–281
28. Charlton AJ, Baxter NJ, Khan ML, Moir AJ, Haslam E, Davies AP, Williamson MP (2002) Polyphenol/peptide binding and precipitation. J Agric Food Chem 50(6):1593–1601
29. Araujo JR, Goncalves P, Martel F (2011) Chemopreventive effect of dietary polyphenols in colorectal cancer cell lines. Nutr Res 31(2):77–87. doi:10.1016/j.nutres.2011.01.006
30. Feitelson MA, Arzumanyan A, Kulathinal RJ et al (2015) Sustained proliferation in cancer: mechanisms and novel therapeutic targets. Semin Cancer Biol. doi:10.1016/j.semcancer.2015.02.006
31. Hasima N, Ozpolat B (2014) Regulation of autophagy by polyphenolic compounds as a potential therapeutic strategy for cancer. Cell Death Dis 5:e1509. doi:10.1038/cddis.2014.467
32. Phuah NH, Nagoor NH (2014) Regulation of microRNAs by natural agents: new strategies in cancer therapies. Biomed Res Int. doi:10.1155/2014/804510
33. Pozo-Guisado E, Alvarez-Barrientos A, Mulero-Navarro S, Santiago-Josefat B, Fernandez-Salguero PM (2002) The antiproliferative activity of resveratrol results in apoptosis in MCF-7 but not in MDA-MB-231 human breast cancer cells: cell-specific alteration of the cell cycle. Biochem Pharmacol 64(9):1375–1386
34. Michels G, Watjen W, Weber N, Niering P, Chovolou Y, Kampkotter A, Proksch P, Kahl R (2006) Resveratrol induces apoptotic cell death in rat H4IIE hepatoma cells but necrosis in C6 glioma cells. Toxicology 225(2–3):173–182. doi:10.1016/j.tox.2006.05.014
35. Cichewicz RH, Kouzi SA (2002) Resveratrol oligomers: structure, chemistry, and biological activity. In: Atta ur R (ed) Studies in natural products chemistry, vol 26, part G. Elsevier, Amsterdam, pp 507–579. doi:10.1016/S1572-5995(02)80014-X
36. Frazzi R, Tigano M (2014) The multiple mechanisms of cell death triggered by resveratrol in lymphoma and leukemia. Int J Mol Sci 15(3):4977–4993. doi:10.3390/ijms15034977
37. Bhat KP, Lantvit D, Christov K, Mehta RG, Moon RC, Pezzuto JM (2001) Estrogenic and antiestrogenic properties of resveratrol in mammary tumor models. Cancer Res 61(20):7456–7463
38. McCalley AE, Kaja S, Payne AJ, Koulen P (2014) Resveratrol and calcium signaling: molecular mechanisms and clinical relevance. Molecules 19(6):7327–7340. doi:10.3390/molecules19067327
39. Opipari AW Jr, Tan L, Boitano AE, Sorenson DR, Aurora A, Liu JR (2004) Resveratrol-induced autophagocytosis in ovarian cancer cells. Cancer Res 64(2):696–703
40. Tyagi A, Singh RP, Agarwal C, Siriwardana S, Sclafani RA, Agarwal R (2005) Resveratrol causes Cdc2-tyr15

phosphorylation via ATM/ATR-Chk1/2-Cdc25C pathway as a central mechanism for S phase arrest in human ovarian carcinoma Ovcar-3 cells. Carcinogenesis 26(11):1978–1987. doi:10.1093/carcin/bgi165
41. Ohshiro K, Rayala SK, Kondo S, Gaur A, Vadlamudi RK, El-Naggar AK, Kumar R (2007) Identifying the estrogen receptor coactivator PELP1 in autophagosomes. Cancer Res 67(17):8164–8171. doi:10.1158/0008-5472.can-07-0038
42. Scarlatti F, Maffei R, Beau I, Codogno P, Ghidoni R (2008) Role of non-canonical Beclin 1-independent autophagy in cell death induced by resveratrol in human breast cancer cells. Cell Death Differ 15(8):1318–1329. doi:10.1038/cdd.2008.51
43. Kueck A, Opipari AW Jr, Griffith KA, Tan L, Choi M, Huang J, Wahl H, Liu JR (2007) Resveratrol inhibits glucose metabolism in human ovarian cancer cells. Gynecol Oncol 107(3):450–457. doi:10.1016/j.ygyno.2007.07.065
44. Trincheri NF, Follo C, Nicotra G, Peracchio C, Castino R, Isidoro C (2008) Resveratrol-induced apoptosis depends on the lipid kinase activity of Vps34 and on the formation of autophagolysosomes. Carcinogenesis 29(2):381–389. doi:10.1093/carcin/bgm271
45. Miki H, Uehara N, Kimura A, Sasaki T, Yuri T, Yoshizawa K, Tsubura A (2012) Resveratrol induces apoptosis via ROS-triggered autophagy in human colon cancer cells. Int J Oncol 40(4):1020–1028. doi:10.3892/ijo.2012.1325
46. Puissant A, Robert G, Fenouille N, Luciano F, Cassuto JP, Raynaud S, Auberger P (2010) Resveratrol promotes autophagic cell death in chronic myelogenous leukemia cells via JNK-mediated p62/SQSTM1 expression and AMPK activation. Cancer Res 70(3):1042–1052. doi:10.1158/0008-5472.can-09-3537
47. Yamamoto M, Suzuki SO, Himeno M (2010) Resveratrol-induced autophagy in human U373 glioma cells. Oncol Lett 1(3):489–493. doi:10.3892/ol_00000086
48. Back JH, Zhu Y, Calabro A, Queenan C, Kim AS, Arbesman J, Kim AL (2012) Resveratrol-mediated downregulation of Rictor attenuates autophagic process and suppresses UV-induced skin carcinogenesis. Photochem Photobiol 88(5):1165–1172. doi:10.1111/j.1751-1097.2012.01097.x
49. Alayev A, Doubleday PF, Berger SM, Ballif BA, Holz MK (2014) Phosphoproteomics reveals resveratrol-dependent inhibition of Akt/mTORC1/S6K1 signaling. J Proteome Res 13(12):5734–5742. doi:10.1021/pr500714a
50. Signorelli P, Munoz-Olaya JM, Gagliostro V, Casas J, Ghidoni R, Fabrias G (2009) Dihydroceramide intracellular increase in response to resveratrol treatment mediates autophagy in gastric cancer cells. Cancer Lett 282(2):238–243. doi:10.1016/j.canlet.2009.03.020
51. Fu Y, Chang H, Peng X, Bai Q, Yi L, Zhou Y, Zhu J, Mi M (2014) Resveratrol inhibits breast cancer stem-like cells and induces autophagy via suppressing Wnt/beta-catenin signaling pathway. PLoS ONE 9(7):e102535. doi:10.1371/journal.pone.0102535
52. Siedlecka-Kroplewska K, Jozwik A, Boguslawski W, Wozniak M, Zauszkiewicz-Pawlak A, Spodnik JH, Rychlowski M, Kmiec Z (2013) Pterostilbene induces accumulation of autophagic vacuoles followed by cell death in HL60 human leukemia cells. J Physiol Pharmacol 64(5):545–556
53. Heiss EH, Schilder YD, Dirsch VM (2007) Chronic treatment with resveratrol induces redox stress- and ataxia telangiectasia-mutated (ATM)-dependent senescence in p53-positive cancer cells. J Biol Chem 282(37):26759–26766. doi:10.1074/jbc.M703229200
54. Luo H, Yang A, Schulte BA, Wargovich MJ, Wang GY (2013) Resveratrol induces premature senescence in lung cancer cells via ROS-mediated DNA damage. PLoS ONE 8(3):e60065. doi:10.1371/journal.pone.0060065
55. Gao Z, Xu MS, Barnett TL, Xu CW (2011) Resveratrol induces cellular senescence with attenuated mono-ubiquitination of histone H2B in glioma cells. Biochem Biophys Res Commun 407(2):271–276. doi:10.1016/j.bbrc.2011.02.008
56. Yang Q, Wang B, Zang W, Wang X, Liu Z, Li W, Jia J (2013) Resveratrol inhibits the growth of gastric cancer by inducing G1 phase arrest and senescence in a Sirt1-dependent manner. PLoS ONE 8(11):e70627. doi:10.1371/journal.pone.0070627
57. Carter LG, D'Orazio JA, Pearson KJ (2014) Resveratrol and cancer: focus on in vivo evidence. Endocr Relat Cancer 21(3):R209–R225. doi:10.1530/ERC-13-0171
58. Guo LY, Peng Y, Li YL, Yao JP, Wang J, Zhang GM, Chen J, Sui LH (2010) Mechanisms of resveratrol bovine serum albumin nanoparticle-induced cell death in human ovarian cancer SKOV3 cells. Nan Fang Yi Ke Da Xue Xue Bao. 30(11):2440–2442
59. Tome-Carneiro J, Larrosa M, Gonzalez-Sarrias A, Tomas-Barberan FA, Garcia-Conesa MT, Espin JC (2013) Resveratrol and clinical trials: the crossroad from in vitro studies to human evidence. Curr Pharm Des 19(34):6064–6093
60. Mohan A, Narayanan S, Sethuraman S, Krishnan UM (2014) Novel resveratrol and 5-fluorouracil coencapsulated in PEGylated nanoliposomes improve chemotherapeutic efficacy of combination against head and neck squamous cell carcinoma. BioMed Res Intern 2014:424239. doi:10.1155/2014/424239
61. Patel KR, Brown VA, Jones DJ, Britton RG, Hemingway D, Miller AS, West KP, Booth TD, Perloff M, Crowell JA, Brenner DE, Steward WP, Gescher AJ, Brown K (2010) Clinical pharmacology of resveratrol and its metabolites in colorectal cancer patients. Cancer Res 70(19):7392–7399. doi:10.1158/0008-5472.can-10-2027
62. Sordillo PP, Helson L (2015) Curcumin and cancer stem cells: curcumin has asymmetrical effects on cancer and normal stem cells. Anticancer Res 35(2):599–614
63. Li Y, Zhang T (2014) Targeting cancer stem cells by curcumin and clinical applications. Cancer Lett 346(2):197–205. doi:10.1016/j.canlet.2014.01.012
64. Zang S, Liu T, Shi J, Qiao L (2014) Curcumin: a promising agent targeting cancer stem cells. Anticancer Agents Med Chem 14(6):787–792
65. Vallianou NG, Evangelopoulos A, Schizas N, Kazazis C (2015) Potential anticancer properties and mechanisms of action of curcumin. Anticancer Res 35(2):645–651
66. Tuorkey MJ (2014) Curcumin a potent cancer preventive agent: mechanisms of cancer cell killing. Interv Med Appl Sci 6(4):139–146. doi:10.1556/imas.6.2014.4.1
67. Aoki H, Takada Y, Kondo S, Sawaya R, Aggarwal BB, Kondo Y (2007) Evidence that curcumin suppresses the growth of malignant gliomas in vitro and in vivo through induction of autophagy: role of Akt and extracellular signal-regulated kinase signaling pathways. Mol Pharmacol 72(1):29–39. doi:10.1124/mol.106.033167
68. Mosieniak G, Adamowicz M, Alster O, Jaskowiak H, Szczepankiewicz AA, Wilczynski GM, Ciechomska IA, Sikora E (2012) Curcumin induces permanent growth arrest of human colon cancer cells: link between senescence and autophagy. Mech Ageing Dev 133(6):444–455. doi:10.1016/j.mad.2012.05.004
69. Kim JY, Cho TJ, Woo BH, Choi KU, Lee CH, Ryu MH, Park HR (2012) Curcumin-induced autophagy contributes to the decreased survival of oral cancer cells. Arch Oral Biol 57(8):1018–1025. doi:10.1016/j.archoralbio.2012.04.005
70. Yamauchi Y, Izumi Y, Asakura K, Hayashi Y, Nomori H (2012) Curcumin induces autophagy in ACC-MESO-1 cells. Phytother Res 26(12):1779–1783. doi:10.1002/ptr.4645
71. Jia YL, Li J, Qin ZH, Liang ZQ (2009) Autophagic and apoptotic mechanisms of curcumin-induced death in K562 cells.

J Asian Nat Prod Res 11(11):918–928. doi:10.1080/10286020903264077
72. Lee YJ, Kim NY, Suh YA, Lee C (2011) Involvement of ROS in curcumin-induced autophagic cell death. Korean J Physiol Pharmacol 15(1):1–7. doi:10.4196/kjpp.2011.15.1.1
73. Lee SJ, Krauthauser C, Maduskuie V, Fawcett PT, Olson JM, Rajasekaran SA (2011) Curcumin-induced HDAC inhibition and attenuation of medulloblastoma growth in vitro and in vivo. BMC Cancer 11:144. doi:10.1186/1471-2407-11-144
74. O'Sullivan-Coyne G, O'Sullivan GC, O'Donovan TR, Piwocka K, McKenna SL (2009) Curcumin induces apoptosis-independent death in oesophageal cancer cells. Br J Cancer 101(9):1585–1595. doi:10.1038/sj.bjc.6605308
75. Pietrocola F, Lachkar S, Enot DP, Niso-Santano M, Bravo-San Pedro JM, Sica V, Izzo V, Maiuri MC, Madeo F, Marino G, Kroemer G (2015) Spermidine induces autophagy by inhibiting the acetyltransferase EP300. Cell Death Differ 22(3):509–516. doi:10.1038/cdd.2014.215
76. Thongrakard V, Titone R, Follo C, Morani F, Suksamrarn A, Tencomnao T, Isidoro C (2014) Turmeric toxicity in A431 epidermoid cancer cells associates with autophagy degradation of anti-apoptotic and anti-autophagic p53 mutant. Phytother Res 28(12):1761–1769. doi:10.1002/ptr.5196
77. Saleh EM, El-awady RA, Eissa NA, Abdel-Rahman WM (2012) Antagonism between curcumin and the topoisomerase II inhibitor etoposide: a study of DNA damage, cell cycle regulation and death pathways. Cancer Biol Ther 13(11):1058–1071. doi:10.4161/cbt.21078
78. Basile V, Belluti S, Ferrari E, Gozzoli C, Ganassi S, Quaglino D, Saladini M, Imbriano C (2013) bis-Dehydroxy-Curcumin triggers mitochondrial-associated cell death in human colon cancer cells through ER-stress induced autophagy. PLoS ONE 8(1):e53664. doi:10.1371/journal.pone.0053664
79. Qu W, Xiao J, Zhang H, Chen Q, Wang Z, Shi H, Gong L, Chen J, Liu Y, Cao R, Lv J (2013) B19, a novel monocarbonyl analogue of curcumin, induces human ovarian cancer cell apoptosis via activation of endoplasmic reticulum stress and the autophagy signaling pathway. Intern J Biol Sci 9(8):766–777. doi:10.7150/ijbs.5711
80. Zhou GZ, Xu SL, Sun GC, Chen XB (2014) Novel curcumin analogue IHCH exhibits potent antiproliferative effects by inducing autophagy in A549 lung cancer cells. Mol Med Rep 10(1):441–446. doi:10.3892/mmr.2014.2183
81. Wang G, Wang JJ, Yang GY, Du SM, Zeng N, Li DS, Li RM, Chen JY, Feng JB, Yuan SH, Ye F (2012) Effects of quercetin nanoliposomes on C6 glioma cells through induction of type III programmed cell death. Int J Nanomed 7:271–280. doi:10.2147/ijn.s26935
82. Kang D, Park W, Lee S, Kim JH, Song JJ (2013) Crosstalk from survival to necrotic death coexists in DU-145 cells by curcumin treatment. Cell Signal 25(5):1288–1300. doi:10.1016/j.cellsig.2013.01.014
83. Watanabe FT, Chade DC, Reis ST, Piantino C, Dall' Oglio MF, Srougi M, Leite KR (2011) Curcumin, but not Prima-1, decreased tumor cell proliferation in the syngeneic murine orthotopic bladder tumor model. Clinics 66(12):2121–2124
84. Jiang Z, Jin S, Yalowich JC, Brown KD, Rajasekaran B (2010) The mismatch repair system modulates curcumin sensitivity through induction of DNA strand breaks and activation of G2-M checkpoint. Mol Cancer Ther 9(3):558–568. doi:10.1158/1535-7163.mct-09-0627
85. Westermann S, Weber K (2003) Post-translational modifications regulate microtubule function. Nat Rev Mol Cell Biol 4(12):938–947. doi:10.1038/nrm1260
86. Subramaniam D, Ramalingam S, Linehan DC, Dieckgraefe BK, Postier RG, Houchen CW, Jensen RA, Anant S (2011) RNA binding protein CUGBP2/CELF2 mediates curcumin-induced mitotic catastrophe of pancreatic cancer cells. PLoS ONE 6(2):e16958. doi:10.1371/journal.pone.0016958
87. Ogiwara H, Ui A, Shiotani B, Zou L, Yasui A, Kohno T (2013) Curcumin suppresses multiple DNA damage response pathways and has potency as a sensitizer to PARP inhibitor. Carcinogenesis 34(11):2486–2497. doi:10.1093/carcin/bgt240
88. Hendrayani SF, Al-Khalaf HH, Aboussekhra A (2013) Curcumin triggers p16-dependent senescence in active breast cancer-associated fibroblasts and suppresses their paracrine procarcinogenic effects. Neoplasia 15(6):631–640
89. Li Y, Gao J, Zhong Z, Hoi P, Lee S, Wang Y (2013) Bisdemethoxycurcumin suppresses MCF-7 cells proliferation by inducing ROS accumulation and modulating senescence-related pathways. Pharmacol Rep 65(3):700–709
90. Zhuang W, Long L, Zheng B, Ji W, Yang N, Zhang Q, Liang Z (2012) Curcumin promotes differentiation of glioma-initiating cells by inducing autophagy. Cancer Sci 103(4):684–690. doi:10.1111/j.1349-7006.2011.02198.x
91. Gossner G, Choi M, Tan L, Fogoros S, Griffith KA, Kuenker M, Liu JR (2007) Genistein-induced apoptosis and autophagocytosis in ovarian cancer cells. Gynecol Oncol 105(1):23–30. doi:10.1016/j.ygyno.2006.11.009
92. Tominaga Y, Wang A, Wang RH, Wang X, Cao L, Deng CX (2007) Genistein inhibits Brca1 mutant tumor growth through activation of DNA damage checkpoints, cell cycle arrest, and mitotic catastrophe. Cell Death Differ 14(3):472–479. doi:10.1038/sj.cdd.4402037
93. Xie X, Wang SS, Wong TC, Fung MC (2013) Genistein promotes cell death of ethanol-stressed HeLa cells through the continuation of apoptosis or secondary necrosis. Cancer Cell Intern 13(1):63. doi:10.1186/1475-2867-13-63
94. Bao B, Azmi AS, Li Y, Ahmad A, Ali S, Banerjee S, Kong D, Sarkar FH (2014) Targeting CSCs in tumor microenvironment: the potential role of ROS-associated miRNAs in tumor aggressiveness. Curr Stem Cell Res Ther 9(1):22–35
95. Banerjee S, Li Y, Wang Z, Sarkar FH (2008) Multi-targeted therapy of cancer by genistein. Cancer Lett 269(2):226–242. doi:10.1016/j.canlet.2008.03.052
96. Dixon RA, Ferreira D (2002) Genistein. Phytochemistry 60(3):205–211
97. Singletary K, Milner J (2008) Diet, autophagy, and cancer: a review. Cancer Epidemiol Biomark Prev 17(7):1596–1610. doi:10.1158/1055-9965.epi-07-2917
98. Sprouse AA, Steding CE, Herbert BS (2012) Pharmaceutical regulation of telomerase and its clinical potential. J Cell Mol Med 16(1):1–7. doi:10.1111/j.1582-4934.2011.01460.x
99. Ajay G, Genistein AB (2009) Genistein: a multipurpose isoflavone. Int J Green Pharmacy 3(3):176–183
100. Kim SH, Kim CW, Jeon SY, Go RE, Hwang KA, Choi KC (2014) Chemopreventive and chemotherapeutic effects of genistein, a soy isoflavone, upon cancer development and progression in preclinical animal models. Lab Anim Res 30(4):143–150. doi:10.5625/lar.2014.30.4.143
101. Uckun FM, Evans WE, Forsyth CJ, Waddick KG, Ahlgren LT, Chelstrom LM, Burkhardt A, Bolen J, Myers DE (1995) Biotherapy of B-cell precursor leukemia by targeting genistein to CD19-associated tyrosine kinases. Science 267(5199):886–891
102. Kalfalah FM, Mielke C, Christensen MO, Baechler S, Marko D, Boege F (2011) Genotoxicity of dietary, environmental and therapeutic topoisomerase II poisons is uniformly correlated to prolongation of enzyme DNA residence. Mol Nutr Food Res 55(Suppl 1):S127–142. doi:10.1002/mnfr.201000509
103. Sarkar FH, Li Y (2002) Mechanisms of cancer chemoprevention by soy isoflavone genistein. Cancer Metastasis Rev 21(3–4):265–280

104. Lu JJ, Bao JL, Chen XP, Huang M, Wang YT (2012) Alkaloids isolated from natural herbs as the anticancer agents. Evid Based Complement Alternat Med. doi:10.1155/2012/485042
105. Manske R (1965) The alkaloids: chemistry and physiology. In: Manske RHF (ed) The alkaloids: chemistry and physiology, vol 8. Academic Press, New York, pp 47–53. doi:10.1016/S1876-0813(08)60042-1
106. McNaught AD, Wilkinson A (1997) Compendium of chemical terminology. http://old.iupac.org/publications/compendium/
107. Meyers R (2001) Encyclopedia of physical science and technology, 3rd edn. Academic Press, San Diego
108. Tohme R, Darwiche N, Gali-Muhtasib H (2011) A journey under the sea: the quest for marine anti-cancer alkaloids. Molecules 16(11):9665–9696. doi:10.3390/molecules16119665
109. Ortiz LM, Lombardi P, Tillhon M, Scovassi AI (2014) Berberine, an epiphany against cancer. Molecules 19(8):12349–12367. doi:10.3390/molecules190812349
110. Tillhon M, Guaman Ortiz LM, Lombardi P, Scovassi AI (2012) Berberine: new perspectives for old remedies. Biochem Pharmacol 84(10):1260–1267. doi:10.1016/j.bcp.2012.07.018
111. Li S, Zhang B (2013) Traditional Chinese medicine network pharmacology: theory, methodology and application. Chin J Nat Med 11(2):110–120
112. Sun Y, Xun K, Wang Y, Chen X (2009) A systematic review of the anticancer properties of berberine, a natural product from Chinese herbs. Anticancer Drugs 20(9):757–769. doi:10.1097/CAD.0b013e328330d95b
113. Jabbarzadeh Kaboli P, Rahmat A, Ismail P, Ling KH (2014) Targets and mechanisms of berberine, a natural drug with potential to treat cancer with special focus on breast cancer. Eur J Pharmacol 740:584–595. doi:10.1016/j.ejphar.2014.06.025
114. Li XL, Hu YJ, Wang H, Yu BQ, Yue HL (2012) Molecular spectroscopy evidence of berberine binding to DNA: comparative binding and thermodynamic profile of intercalation. Biomacromolecules 13(3):873–880. doi:10.1021/bm2017959
115. Peng PL, Kuo WH, Tseng HC, Chou FP (2008) Synergistic tumor-killing effect of radiation and berberine combined treatment in lung cancer: the contribution of autophagic cell death. Int J Radiat Oncol Biol Phys 70(2):529–542. doi:10.1016/j.ijrobp.2007.08.034
116. Wang N, Feng Y, Zhu M, Tsang CM, Man K, Tong Y, Tsao SW (2010) Berberine induces autophagic cell death and mitochondrial apoptosis in liver cancer cells: the cellular mechanism. J Cell Biochem 111(6):1426–1436. doi:10.1002/jcb.22869
117. Hou Q, Tang X, Liu H, Tang J, Yang Y, Jing X, Xiao Q, Wang W, Gou X, Wang Z (2011) Berberine induces cell death in human hepatoma cells in vitro by downregulating CD147. Cancer Sci 102(7):1287–1292. doi:10.1111/j.1349-7006.2011.01933.x
118. Yu R, Zhang ZQ, Wang B, Jiang HX, Cheng L, Shen LM (2014) Berberine-induced apoptotic and autophagic death of HepG2 cells requires AMPK activation. Cancer Cell Intern 14:49. doi:10.1186/1475-2867-14-49
119. Letasiova S, Jantova S, Cipak L, Muckova M (2006) Berberine-antiproliferative activity in vitro and induction of apoptosis/necrosis of the U937 and B16 cells. Cancer Lett 239(2):254–262. doi:10.1016/j.canlet.2005.08.024
120. Zhao X, Tong N (2012) Protective effects of berberine on doxorubicin-induced nephrotoxicity in mice. J Transl Med 10(Suppl 2):A66–A66. doi:10.1186/1479-5876-10-S2-A66
121. Wang Y, Liu Q, Liu Z, Li B, Sun Z, Zhou H, Zhang X, Gong Y, Shao C (2012) Berberine, a genotoxic alkaloid, induces ATM-Chk1 mediated G2 arrest in prostate cancer cells. Mutat Res 734(1–2):20–29. doi:10.1016/j.mrfmmm.2012.04.005
122. Vitale I, Galluzzi L, Castedo M, Kroemer G (2011) Mitotic catastrophe: a mechanism for avoiding genomic instability. Nat Rev Mol Cell Biol 12(6):385–392. doi:10.1038/nrm3115
123. Naasani I, Seimiya H, Yamori T, Tsuruo T (1999) FJ5002: a potent telomerase inhibitor identified by exploiting the disease-oriented screening program with COMPARE analysis. Cancer Res 59(16):4004–4011
124. Pierpaoli E, Arcamone AG, Buzzetti F, Lombardi P, Salvatore C, Provinciali M (2013) Antitumor effect of novel berberine derivatives in breast cancer cells. BioFactors 39(6):672–679. doi:10.1002/biof.1131
125. Elzi DJ, Lai Y, Song M, Hakala K, Weintraub ST, Shiio Y (2012) Plasminogen activator inhibitor 1-insulin-like growth factor binding protein 3 cascade regulates stress-induced senescence. Proc Natl Acad Sci USA 109(30):12052–12057. doi:10.1073/pnas.1120437109
126. Liu Q, Xu X, Zhao M, Wei Z, Li X, Zhang X, Liu Z, Gong Y, Shao C (2015) Berberine induces senescence of human glioblastoma cells by downregulating the EGFR-MEK-ERK signaling pathway. Mol Cancer Ther 14(2):355–363. doi:10.1158/1535-7163.mct-14-0634
127. Halicka HD, Zhao H, Li J, Lee YS, Hsieh TC, Wu JM, Darzynkiewicz Z (2012) Potential anti-aging agents suppress the level of constitutive mTOR- and DNA damage- signaling. Aging 4(12):952–965
128. Zhao H, Halicka HD, Li J, Darzynkiewicz Z (2013) Berberine suppresses gero-conversion from cell cycle arrest to senescence. Aging 5(8):623–636
129. Anis K, Kuttan G, Kuttan R (1999) Role of berberine as an adjuvant response modifier during tumour therapy in mice. Pharm Pharmacol Commun 5(12):697–700. doi:10.1211/146080899128734415
130. Nishino H, Kitagawa K, Fujiki H, Iwashima A (1986) Berberine sulfate inhibits tumor-promoting activity of teleocidin in two-stage carcinogenesis on mouse skin. Oncology 43(2):131–134
131. Mahmoudian M, Rahimi-Moghaddam P (2009) The anti-cancer activity of noscapine: a review. Recent Pat AntiCancer Drug Discov 4(1):92–97
132. Karna P, Zughaier S, Pannu V, Simmons R, Narayan S, Aneja R (2010) Induction of reactive oxygen species-mediated autophagy by a novel microtubule-modulating agent. J Biol Chem 285(24):18737–18748. doi:10.1074/jbc.M109.091694
133. Jaiswal AS, Aneja R, Connors SK, Joshi HC, Multani AS, Pathak S, Narayan S (2009) 9-bromonoscapine-induced mitotic arrest of cigarette smoke condensate-transformed breast epithelial cells. J Cell Biochem 106(6):1146–1156. doi:10.1002/jcb.22099
134. Pannu V, Rida PC, Ogden A, Clewley R, Cheng A, Karna P, Lopus M, Mishra RC, Zhou J, Aneja R (2012) Induction of robust de novo centrosome amplification, high-grade spindle multipolarity and metaphase catastrophe: a novel chemotherapeutic approach. Cell Death Dis 3:e346. doi:10.1038/cddis.2012.82
135. Ye K, Ke Y, Keshava N, Shanks J, Kapp JA, Tekmal RR, Petros J, Joshi HC (1998) Opium alkaloid noscapine is an antitumor agent that arrests metaphase and induces apoptosis in dividing cells. Proc Natl Acad Sci USA 95(4):1601–1606
136. Landen JW, Hau V, Wang M, Davis T, Ciliax B, Wainer BH, Van Meir EG, Glass JD, Joshi HC, Archer DR (2004) Noscapine crosses the blood-brain barrier and inhibits glioblastoma growth. Clin Cancer Res 10(15):5187–5201. doi:10.1158/1078-0432.ccr-04-0360
137. Roubille F, Kritikou E, Busseuil D, Barrere-Lemaire S, Tardif JC (2013) Colchicine: an old wine in a new bottle? AntiInflamm Antiallergy Agents Med Chem 12(1):14–23
138. Terkeltaub RA (2009) Colchicine update: 2008. Semin Arthritis Rheum 38(6):411–419. doi:10.1016/j.semarthrit.2008.08.006
139. Sivakumar G (2013) Colchicine semisynthetics: chemotherapeutics for cancer? Curr Med Chem 20(7):892–898

140. Atkinson JM, Falconer RA, Edwards DR, Pennington CJ, Siller CS, Shnyder SD, Bibby MC, Patterson LH, Loadman PM, Gill JH (2010) Development of a novel tumor-targeted vascular disrupting agent activated by membrane-type matrix metalloproteinases. Cancer Res 70(17):6902–6912. doi:10.1158/0008-5472.can-10-1440
141. Arthur CR, Gupton JT, Kellogg GE, Yeudall WA, Cabot MC, Newsham IF, Gewirtz DA (2007) Autophagic cell death, polyploidy and senescence induced in breast tumor cells by the substituted pyrrole JG-03-14, a novel microtubule poison. Biochem Pharmacol 74(7):981–991. doi:10.1016/j.bcp.2007.07.003
142. Biggers JW, Nguyen T, Di X, Gupton JT, Henderson SC, Emery SM, Alotaibi M, White KL Jr, Brown R, Almenara J, Gewirtz DA (2013) Autophagy, cell death and sustained senescence arrest in B16/F10 melanoma cells and HCT-116 colon carcinoma cells in response to the novel microtubule poison, JG-03-14. Cancer Chemother Pharmacol 71(2):441–455. doi:10.1007/s00280-012-2024-6
143. Larocque K, Ovadje P, Djurdjevic S, Mehdi M, Green J, Pandey S (2014) Novel analogue of colchicine induces selective pro-death autophagy and necrosis in human cancer cells. PLoS ONE 9(1):e87064. doi:10.1371/journal.pone.0087064
144. Blakey DC, Westwood FR, Walker M, Hughes GD, Davis PD, Ashton SE, Ryan AJ (2002) Antitumor activity of the novel vascular targeting agent ZD6126 in a panel of tumor models. Clin Cancer Res 8(6):1974–1983
145. LoRusso PM, Gadgeel SM, Wozniak A, Barge AJ, Jones HK, DelProposto ZS, DeLuca PA, Evelhoch JL, Boerner SA, Wheeler C (2008) Phase I clinical evaluation of ZD6126, a novel vascular-targeting agent, in patients with solid tumors. Invest New Drugs 26(2):159–167. doi:10.1007/s10637-008-9112-9
146. Tozer GM, Kanthou C, Baguley BC (2005) Disrupting tumour blood vessels. Nat Rev Cancer 5(6):423–435. doi:10.1038/nrc1628
147. Quatrale AE, Porcelli L, Gnoni A, Numico G, Paradiso A, Azzariti A (2014) New vascular disrupting agents in upper gastrointestinal malignancies. Curr Med Chem 21(8):1039–1049
148. Crielaard BJ, van der Wal S, Lammers T, Le HT, Hennink WE, Schiffelers RM, Storm G, Fens MH (2011) A polymeric colchicinoid prodrug with reduced toxicity and improved efficacy for vascular disruption in cancer therapy. Int J Nanomed 6:2697–2703. doi:10.2147/ijn.s24450
149. Huang M, Lu JJ, Huang MQ, Bao JL, Chen XP, Wang YT (2012) Terpenoids: natural products for cancer therapy. Expert Opin Investig Drugs 21(12):1801–1818. doi:10.1517/13543784.2012.727395
150. Ghantous A, Gali-Muhtasib H, Vuorela H, Saliba NA, Darwiche N (2010) What made sesquiterpene lactones reach cancer clinical trials? Drug Discov Today 15(15–16):668–678. doi:10.1016/j.drudis.2010.06.002
151. Yang H, Dou QP (2010) Targeting apoptosis pathway with natural terpenoids: implications for treatment of breast and prostate cancer. Curr Drug Targets 11(6):733–744
152. Merfort I (2011) Perspectives on sesquiterpene lactones in inflammation and cancer. Curr Drug Targets 12(11):1560–1573
153. Kreuger MR, Grootjans S, Biavatti MW, Vandenabeele P, D'Herde K (2012) Sesquiterpene lactones as drugs with multiple targets in cancer treatment: focus on parthenolide. Anticancer Drugs 23(9):883–896. doi:10.1097/CAD.0b013e328356cad9
154. Ghantous A, Sinjab A, Herceg Z, Darwiche N (2013) Parthenolide: from plant shoots to cancer roots. Drug Discov Today 18(17–18):894–905. doi:10.1016/j.drudis.2013.05.005
155. Schneider-Stock R, Ghantous A, Bajbouj K, Saikali M, Darwiche N (2012) Epigenetic mechanisms of plant-derived anticancer drugs. Front Biosci 17:129–173
156. Guzman ML, Rossi RM, Neelakantan S, Li X, Corbett CA, Hassane DC, Becker MW, Bennett JM, Sullivan E, Lachowicz JL, Vaughan A, Sweeney CJ, Matthews W, Carroll M, Liesveld JL, Crooks PA, Jordan CT (2007) An orally bioavailable parthenolide analog selectively eradicates acute myelogenous leukemia stem and progenitor cells. Blood 110(13):4427–4435. doi:10.1182/blood-2007-05-090621
157. D'Anneo A, Carlisi D, Lauricella M, Puleio R, Martinez R, Di Bella S, Di Marco P, Emanuele S, Di Fiore R, Guercio A, Vento R, Tesoriere G (2013) Parthenolide generates reactive oxygen species and autophagy in MDA-MB231 cells. A soluble parthenolide analogue inhibits tumour growth and metastasis in a xenograft model of breast cancer. Cell Death Disease 4:e891. doi:10.1038/cddis.2013.415
158. Lu C, Wang W, Jia Y, Liu X, Tong Z, Li B (2014) Inhibition of AMPK/autophagy potentiates parthenolide-induced apoptosis in human breast cancer cells. J Cell Biochem 115(8):1458–1466. doi:10.1002/jcb.24808
159. Sun J, Zhang C, Bao YL, Wu Y, Chen ZL, Yu CL, Huang YX, Sun Y, Zheng LH, Wang X, Li YX (2014) Parthenolide-induced apoptosis, autophagy and suppression of proliferation in HepG2 cells. Asian Pac J Cancer Prev 15(12):4897–4902
160. Lan B, Wan YJ, Pan S, Wang Y, Yang Y, Leng QL, Jia H, Liu YH, Zhang CZ, Cao Y (2015) Parthenolide induces autophagy via the depletion of 4E-BP1. Biochem Biophys Res Commun 456(1):434–439. doi:10.1016/j.bbrc.2014.11.102
161. Pozarowski P, Halicka DH, Darzynkiewicz Z (2003) Cell cycle effects and caspase-dependent and independent death of HL-60 and Jurkat cells treated with the inhibitor of NF-kappaB parthenolide. Cell Cycle 2(4):377–383
162. Zhou W, Xu X (1994) Total synthesis of the antimalarial sesquiterpene peroxide Qinghaosu and Yingzhaosu A. Acc Chem Res 27(7):211–216. doi:10.1021/ar00043a005
163. Ofengeim D, Yuan J (2013) Regulation of RIP1 kinase signalling at the crossroads of inflammation and cell death. Nat Rev Mol Cell Biol 14(11):727–736. doi:10.1038/nrm3683
164. Wang C, Lu J, Wang Y, Bai S, Wang Y, Wang L, Sheng G (2012) Combined effects of FLT3 and NF-kappaB selective inhibitors on acute myeloid leukemia in vivo. J Biochem Mol Toxicol 26(1):35–43. doi:10.1002/jbt.20411
165. Li XJ, Jiang ZZ, Zhang LY (2014) Triptolide: progress on research in pharmacodynamics and toxicology. J Ethnopharmacol 155(1):67–79. doi:10.1016/j.jep.2014.06.006
166. Chen L, Liu Q, Huang Z, Wu F, Li Z, Chen X, Lin T (2012) Tripchlorolide induces cell death in lung cancer cells by autophagy. Int J Oncol 40(4):1066–1070. doi:10.3892/ijo.2011.1278
167. McCallum C, Kwon S, Leavitt P, Shen DM, Liu W, Gurnett A (2007) Triptolide binds covalently to a 90 kDa nuclear protein. Role of epoxides in binding and activity. Immunobiology 212(7):549–556. doi:10.1016/j.imbio.2007.02.002
168. Wang Y, Lu JJ, He L, Yu Q (2011) Triptolide (TPL) inhibits global transcription by inducing proteasome-dependent degradation of RNA polymerase II (Pol II). PLoS ONE 6(9):e23993. doi:10.1371/journal.pone.0023993
169. Yang S, Chen J, Guo Z, Xu XM, Wang L, Pei XF, Yang J, Underhill CB, Zhang L (2003) Triptolide inhibits the growth and metastasis of solid tumors. Mol Cancer Ther 2(1):65–72
170. Phillips PA, Dudeja V, McCarroll JA, Borja-Cacho D, Dawra RK, Grizzle WE, Vickers SM, Saluja AK (2007) Triptolide induces pancreatic cancer cell death via inhibition of heat shock protein 70. Cancer Res 67(19):9407–9416. doi:10.1158/0008-5472.can-07-1077
171. Mujumdar N, Mackenzie TN, Dudeja V, Chugh R, Antonoff MB, Borja-Cacho D, Sangwan V, Dawra R, Vickers SM, Saluja AK (2010) Triptolide induces cell death in pancreatic cancer

cells by apoptotic and autophagic pathways. Gastroenterology 139(2):598–608. doi:10.1053/j.gastro.2010.04.046
172. Mujumdar N, Banerjee S, Chen Z, Sangwan V, Chugh R, Dudeja V, Yamamoto M, Vickers SM, Saluja AK (2014) Triptolide activates unfolded protein response leading to chronic ER stress in pancreatic cancer cells. Am J Physiol Gastrointest Liver Physiol 306(11):G1011–1020. doi:10.1152/ajpgi.00466.2013
173. Krosch TC, Sangwan V, Banerjee S, Mujumdar N, Dudeja V, Saluja AK, Vickers SM (2013) Triptolide-mediated cell death in neuroblastoma occurs by both apoptosis and autophagy pathways and results in inhibition of nuclear factor-kappa B activity. Am J Surg 205(4):387–396. doi:10.1016/j.amjsurg.2013.01.008
174. Jia W, Pua HH, Li QJ, He YW (2011) Autophagy regulates endoplasmic reticulum homeostasis and calcium mobilization in T lymphocytes. J Immunol 186(3):1564–1574. doi:10.4049/jimmunol.1001822
175. Kiviharju TM, Lecane PS, Sellers RG, Peehl DM (2002) Antiproliferative and proapoptotic activities of triptolide (PG490), a natural product entering clinical trials, on primary cultures of human prostatic epithelial cells. Clin Cancer Res 8(8):2666–2674
176. Tengchaisri T, Chawengkirttikul R, Rachaphaew N, Reutrakul V, Sangsuwan R, Sirisinha S (1998) Antitumor activity of triptolide against cholangiocarcinoma growth in vitro and in hamsters. Cancer Lett 133(2):169–175
177. Antonoff MB, Chugh R, Borja-Cacho D, Dudeja V, Clawson KA, Skube SJ, Sorenson BS, Saltzman DA, Vickers SM, Saluja AK (2009) Triptolide therapy for neuroblastoma decreases cell viability in vitro and inhibits tumor growth in vivo. Surgery 146(2):282–290. doi:10.1016/j.surg.2009.04.023
178. Fulda S (2008) Betulinic acid for cancer treatment and prevention. Int J Mol Sci 9(6):1096–1107. doi:10.3390/ijms9061096
179. Gheorgheosu D, Duicu O, Dehelean C, Soica C, Muntean D (2014) Betulinic acid as a potent and complex antitumor phytochemical: a minireview. AntiCancer Agents Med Chem 14(7):936–945
180. Csuk R (2014) Betulinic acid and its derivatives: a patent review (2008-2013). Expert Opin Ther Pat 24(8):913–923. doi:10.1517/13543776.2014.927441
181. Xu T, Pang Q, Zhou D, Zhang A, Luo S, Wang Y, Yan X (2014) Proteomic investigation into betulinic acid-induced apoptosis of human cervical cancer HeLa cells. PLoS ONE 9(8):e105768. doi:10.1371/journal.pone.0105768
182. Chadalapaka G, Jutooru I, Burghardt R, Safe S (2010) Drugs that target specificity proteins downregulate epidermal growth factor receptor in bladder cancer cells. Mol Cancer Res 8(5):739–750. doi:10.1158/1541-7786.MCR-09-0493
183. Gonzalez P, Mader I, Tchoghandjian A, Enzenmuller S, Cristofanon S, Basit F, Debatin KM, Fulda S (2012) Impairment of lysosomal integrity by B10, a glycosylated derivative of betulinic acid, leads to lysosomal cell death and converts autophagy into a detrimental process. Cell Death Differ 19(8):1337–1346. doi:10.1038/cdd.2012.10
184. Yang LJ, Chen Y, He J, Yi S, Wen L, Zhao J, Zhang BP, Cui GH (2012) Betulinic acid inhibits autophagic flux and induces apoptosis in human multiple myeloma cells in vitro. Acta Pharmacol Sin 33(12):1542–1548. doi:10.1038/aps.2012.102
185. Potze L, Mullauer FB, Colak S, Kessler JH, Medema JP (2014) Betulinic acid-induced mitochondria-dependent cell death is counterbalanced by an autophagic salvage response. Cell Death Dis 5:e1169. doi:10.1038/cddis.2014.139
186. Yasukawa K, Takido M, Matsumoto T, Takeuchi M, Nakagawa S (1991) Sterol and triterpene derivatives from plants inhibit the effects of a tumor promoter, and sitosterol and betulinic acid inhibit tumor formation in mouse skin two-stage carcinogenesis. Oncology 48(1):72–76
187. Pisha E, Chai H, Lee IS, Chagwedera TE, Farnsworth NR, Cordell GA, Beecher CW, Fong HH, Kinghorn AD, Brown DM et al (1995) Discovery of betulinic acid as a selective inhibitor of human melanoma that functions by induction of apoptosis. Nat Med 1(10):1046–1051
188. Sawada N, Kataoka K, Kondo K, Arimochi H, Fujino H, Takahashi Y, Miyoshi T, Kuwahara T, Monden Y, Ohnishi Y (2004) Betulinic acid augments the inhibitory effects of vincristine on growth and lung metastasis of B16F10 melanoma cells in mice. Br J Cancer 90(8):1672–1678. doi:10.1038/sj.bjc.6601746
189. Zuco V, Supino R, Righetti SC, Cleris L, Marchesi E, Gambacorti-Passerini C, Formelli F (2002) Selective cytotoxicity of betulinic acid on tumor cell lines, but not on normal cells. Cancer Lett 175(1):17–25
190. Wang P, Li Q, Li K, Zhang X, Han Z, Wang J, Gao D, Li J (2012) Betulinic acid exerts immunoregulation and anti-tumor effect on cervical carcinoma (U14) tumor-bearing mice. Pharmazie 67(8):733–739
191. Safe S, Abdelrahim M (2005) Sp transcription factor family and its role in cancer. Eur J Cancer 41(16):2438–2448. doi:10.1016/j.ejca.2005.08.006
192. Chintharlapalli S, Papineni S, Lei P, Pathi S, Safe S (2011) Betulinic acid inhibits colon cancer cell and tumor growth and induces proteasome-dependent and -independent downregulation of specificity proteins (Sp) transcription factors. BMC Cancer 11:371. doi:10.1186/1471-2407-11-371
193. Chintharlapalli S, Papineni S, Ramaiah SK, Safe S (2007) Betulinic acid inhibits prostate cancer growth through inhibition of specificity protein transcription factors. Cancer Res 67(6):2816–2823. doi:10.1158/0008-5472.can-06-3735
194. Mertens-Talcott SU, Noratto GD, Li X, Angel-Morales G, Bertoldi MC, Safe S (2013) Betulinic acid decreases ER-negative breast cancer cell growth in vitro and in vivo: role of Sp transcription factors and microRNA-27a:ZBTB10. Mol Carcinog 52(8):591–602. doi:10.1002/mc.21893
195. Rajendran P, Jaggi M, Singh MK, Mukherjee R, Burman AC (2008) Pharmacological evaluation of C-3 modified betulinic acid derivatives with potent anticancer activity. Invest New Drugs 26(1):25–34. doi:10.1007/s10637-007-9081-4
196. Fahey JW, Talalay P (1999) Antioxidant functions of sulforaphane: a potent inducer of phase II detoxication enzymes. Food Chem Toxicol 37(9–10):973–979
197. Herman-Antosiewicz A, Johnson DE, Singh SV (2006) Sulforaphane causes autophagy to inhibit release of cytochrome C and apoptosis in human prostate cancer cells. Cancer Res 66(11):5828–5835. doi:10.1158/0008-5472.can-06-0139
198. Jackson SJ, Singletary KW (2004) Sulforaphane inhibits human MCF-7 mammary cancer cell mitotic progression and tubulin polymerization. J Nutr 134(9):2229–2236
199. Azarenko O, Okouneva T, Singletary KW, Jordan MA, Wilson L (2008) Suppression of microtubule dynamic instability and turnover in MCF7 breast cancer cells by sulforaphane. Carcinogenesis 29(12):2360–2368. doi:10.1093/carcin/bgn241
200. Mi L, Xiao Z, Hood BL, Dakshanamurthy S, Wang X, Govind S, Conrads TP, Veenstra TD, Chung FL (2008) Covalent binding to tubulin by isothiocyanates. A mechanism of cell growth arrest and apoptosis. J Biol Chem 283(32):22136–22146. doi:10.1074/jbc.M802330200
201. Parnaud G, Li P, Cassar G, Rouimi P, Tulliez J, Combaret L, Gamet-Payrastre L (2004) Mechanism of sulforaphane-induced cell cycle arrest and apoptosis in human colon cancer cells. Nutr Cancer 48(2):198–206. doi:10.1207/s15327914nc4802_10

202. Marquez RT, Xu L (2012) Bcl-2: beclin 1 complex: multiple, mechanisms regulating autophagy/apoptosis toggle switch. Am J Cancer Res 2(2):214–221
203. Levine B, Klionsky DJ (2004) Development by self-digestion: molecular mechanisms and biological functions of autophagy. Dev Cell 6(4):463–477
204. Pawlik A, Wiczk A, Kaczynska A, Antosiewicz J, Herman-Antosiewicz A (2013) Sulforaphane inhibits growth of phenotypically different breast cancer cells. Eur J Nutr 52(8):1949–1958. doi:10.1007/s00394-013-0499-5
205. Vyas AR, Hahm ER, Arlotti JA, Watkins S, Stolz DB, Desai D, Amin S, Singh SV (2013) Chemoprevention of prostate cancer by d, l-sulforaphane is augmented by pharmacological inhibition of autophagy. Cancer Res 73(19):5985–5995. doi:10.1158/0008-5472.can-13-0755
206. Payen L, Courtois A, Loewert M, Guillouzo A, Fardel O (2001) Reactive oxygen species-related induction of multidrug resistance-associated protein 2 expression in primary hepatocytes exposed to sulforaphane. Biochem Biophys Res Commun 282(1):257–263. doi:10.1006/bbrc.2001.4531
207. Fimognari C, Nusse M, Cesari R, Iori R, Cantelli-Forti G, Hrelia P (2002) Growth inhibition, cell-cycle arrest and apoptosis in human T-cell leukemia by the isothiocyanate sulforaphane. Carcinogenesis 23(4):581–586
208. Kaminski BM, Weigert A, Brune B, Schumacher M, Wenzel U, Steinhilber D, Stein J, Ulrich S (2011) Sulforaphane potentiates oxaliplatin-induced cell growth inhibition in colorectal cancer cells via induction of different modes of cell death. Cancer Chemother Pharmacol 67(5):1167–1178. doi:10.1007/s00280-010-1413-y
209. Wang F, Shan Y (2012) Sulforaphane retards the growth of UM-UC-3 xenographs, induces apoptosis, and reduces survivin in athymic mice. Nutr Res 32(5):374–380. doi:10.1016/j.nutres.2012.03.014
210. Jakubikova J, Cervi D, Ooi M et al (2011) Anti-tumor activity and signaling events triggered by the isothiocyanates, sulforaphane and phenethyl isothiocyanate, in multiple myeloma. Haematologica 96(8):1170–1179. doi:10.3324/haematol.2010.029363
211. Singh AV, Xiao D, Lew KL, Dhir R, Singh SV (2004) Sulforaphane induces caspase-mediated apoptosis in cultured PC-3 human prostate cancer cells and retards growth of PC-3 xenografts in vivo. Carcinogenesis 25(1):83–90. doi:10.1093/carcin/bgg178
212. Myzak MC, Tong P, Dashwood WM, Dashwood RH, Ho E (2007) Sulforaphane retards the growth of human PC-3 xenografts and inhibits HDAC activity in human subjects. Exp Biol Med 232(2):227–234
213. Li SH, Fu J, Watkins DN, Srivastava RK, Shankar S (2013) Sulforaphane regulates self-renewal of pancreatic cancer stem cells through the modulation of Sonic hedgehog-GLI pathway. Mol Cell Biochem 373(1–2):217–227. doi:10.1007/s11010-012-1493-6
214. Tait SW, Ichim G, Green DR (2014) Die another way—non-apoptotic mechanisms of cell death. J Cell Sci 127(Pt 10):2135–2144. doi:10.1242/jcs.093575
215. Wilson TR, Johnston PG, Longley DB (2009) Anti-apoptotic mechanisms of drug resistance in cancer. Curr Cancer Drug Targets 9(3):307–319
216. Nikoletopoulou V, Markaki M, Palikaras K, Tavernarakis N (1833) Crosstalk between apoptosis, necrosis and autophagy. Biochim Biophys Acta 12:3448–3459. doi:10.1016/j.bbamcr.2013.06.001
217. Marino G, Niso-Santano M, Baehrecke EH, Kroemer G (2014) Self-consumption: the interplay of autophagy and apoptosis. Nat Rev Mol Cell Biol 15(2):81–94. doi:10.1038/nrm3735
218. Jain MV, Paczulla AM, Klonisch T, Dimgba FN, Rao SB, Roberg K, Schweizer F, Lengerke C, Davoodpour P, Palicharla VR, Maddika S, Los M (2013) Interconnections between apoptotic, autophagic and necrotic pathways: implications for cancer therapy development. J Cell Mol Med 17(1):12–29. doi:10.1111/jcmm.12001
219. Baehrecke EH (2005) Autophagy: dual roles in life and death? Nat Rev Mol Cell Biol 6(6):505–510. doi:10.1038/nrm1666
220. White E (2012) Deconvoluting the context-dependent role for autophagy in cancer. Nat Rev Cancer 12(6):401–410. doi:10.1038/nrc3262
221. Vidal SJ, Rodriguez-Bravo V, Galsky M, Cordon-Cardo C, Domingo-Domenech J (2014) Targeting cancer stem cells to suppress acquired chemotherapy resistance. Oncogene 33(36):4451–4463. doi:10.1038/onc.2013.411
222. Janku F (2014) Tumor heterogeneity in the clinic: is it a real problem? Therap Adv Med Oncol 6(2):43–51. doi:10.1177/1758834013517414
223. Easwaran H, Tsai HC, Baylin SB (2014) Cancer epigenetics: tumor heterogeneity, plasticity of stem-like states, and drug resistance. Mol Cell 54(5):716–727. doi:10.1016/j.molcel.2014.05.015
224. Goubran HA, Kotb RR, Stakiw J, Emara ME, Burnouf T (2014) Regulation of tumor growth and metastasis: the role of tumor microenvironment. Cancer Growth Metastasis 7:9–18. doi:10.4137/cgm.s11285
225. Pistollato F, Giampieri F, Battino M (2015) The use of plant-derived bioactive compounds to target cancer stem cells and modulate tumor microenvironment. Food Chem Toxicol 75:58–70. doi:10.1016/j.fct.2014.11.004
226. Kroemer G, Galluzzi L, Kepp O, Zitvogel L (2013) Immunogenic cell death in cancer therapy. Annu Rev Immunol 31:51–72. doi:10.1146/annurev-immunol-032712-100008
227. Bracci L, Schiavoni G, Sistigu A, Belardelli F (2014) Immune-based mechanisms of cytotoxic chemotherapy: implications for the design of novel and rationale-based combined treatments against cancer. Cell Death Differ 21(1):15–25. doi:10.1038/cdd.2013.67