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REVIEW

Anti-cancer effects of aloe-emodin: a systematic review

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ABSTRACT

Background: Anthraquinones are a possible treatment option for oncological patients due to their anti-cancer properties. Cancer patients often exhaust a plethora of resources that ultimately fail to provide fully curative measures. Alternative treatments are subsequently sought in the hope of finding a therapeutic remedy. Potential regimens include aloe-emodin and its related derivatives. This review therefore summarizes the effects of aloe-emodin and other aloe components in light of their anti-proliferative and anti-carcinogenic properties.

Methods: A systematic search was performed in PubMed for aloe-emodin and cancer in humans. Sixty abstracts of *in vitro* studies were selected and reviewed with subsequent screening of the full text. Thirty-eight articles were summarized.

Results: Aloe-emodin possesses multiple anti-proliferative and anti-carcinogenic properties in a host of human cancer cell lines, with often multiple vital pathways affected by the same molecule. The most notable effects include inhibition of cell proliferation, migration, and invasion; cycle arrest; induction of cell death; mitochondrial membrane and redox perturbations; and modulation of immune signaling. The effects of aloe-emodin are not ubiquitous across all cell lines but depend on cell type.

Conclusions: On the basis of this systematic review, the multiple consistent effects of aloe-emodin in human-derived cancer cell lines suggest that aloe-emodin is a potential anti-cancer agent that acts on cancer cells in a pleiotropic manner.

Relevance for patients: Cancer patients often utilize alternative therapies as a result of suboptimal efficacy of conventional treatments. Aloe-emodin might become a therapeutic option for cancer patients if the basic research is confirmed in clinical trials.

1. Introduction

Cancer incidence and prevalence are increasing in the United States, placing a heavy burden on affected individuals and caregivers [1]. Conventional cancer treatment, consisting of surgery, chemotherapy, and/or radiation, is commonly associated with significant morbidity, and cure rates for many cancers are suboptimal [2]. It is for those reasons, presumably, that cancer patients show great interest in complementary therapies, such as nutraceuticals, both for symptom reduction and in the post-treatment survivorship period.

Supplementing the diet with nutraceuticals containing concentrated levels of bioactive nutrients, as opposed to obtaining those nutrients solely from food, can be beneficial. Certain anthraquinones, such as aloe-emodin and rhein (Figure 1), are phytochemicals that can be used to restore compromised

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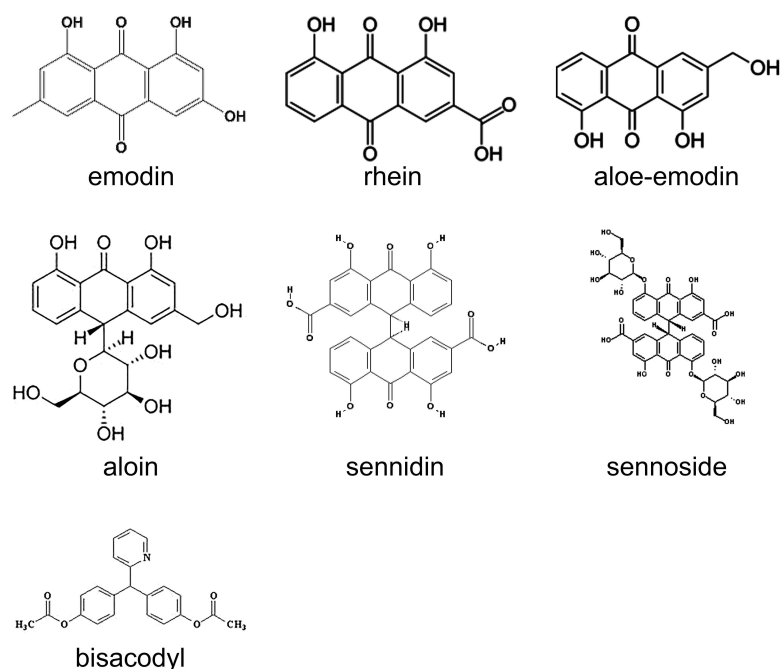Tel: +1 305-243-6227
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Figure 1 Chemical structure of aloe-emodin and structurally related anthraquinones addressed in this paper.

health [3]. Aloe-emodin is one of many bioactive anthraquinone components of aloe vera (*Aloe barbadensis miller*), a perennial cactus-like plant found in tropical climates worldwide. Aloe has been used as a traditional remedy in many cultures for centuries, and it continues to be extremely popular among both cancer and non-cancer patients [4]. Aloe-emodin possesses numerous beneficial biochemical properties. The compound has been used as an anti-inflammatory agent, an immunomodulator, and mediator of wound healing [5]. The most notable effect is that of an antineoplastic agent.

Despite an impressive array of *in vitro* antineoplastic effects, a paucity of clinical research exists on aloe-emodin. Furthermore, aloe vera is used worldwide in an unregulated manner, particularly among cancer patients. Most research has focused on determining the molecular mechanism of current treatments as opposed to creating new therapies. For these reasons, we aim to review the molecular mechanisms of mainly aloe-emodin and structurally related anthraquinones in cancer cells to highlight its oncopharmacological properties. The chemical structure of aloe-emodin has been previously characterized by others [6], but no one has systematically reviewed the anti-cancer effects of aloe-emodin or structurally related anthraquinones in human-derived cancer cell lines.

2. Methods

A systematic search for articles was performed using Pub-Med. Articles published in English between 1989 and 2015 with full text available were searched using the terms "aloe-emodin," "cancer," "aloe vera," and "humans." Inclusion criteria were: (a) *in vitro* study using human-derived cancer cell lines; (b) use of aloe-emodin or structurally related anthraquinone as therapeutic agent; and (c) evaluation of the therapeutic agent on at least one marker of tumor cell proliferation. Two

independent reviewers evaluated the articles for inclusion in this review.

3. Results

The search resulted in 183 articles, of which 60 were identified from title and abstract prior to screening with the inclusion criteria. Full text screening identified the articles that met inclusion criteria. Ultimately, 38 *in vitro* studies of human tumor cells were included in this review; the results of which are listed by origin of cancer and/or cell line (Table 1). The main findings are addressed in the text, while the specific protein/enzyme changes are described in Table 1.

In summary, aloe-emodin exhibits an array of anti-tumor effects in various cancer cell lines, including induction of apoptosis, cell cycle arrest, modulation of immune signaling, and cell mobility alterations. Aloe-emodin reduces cancer cell viability through extrinsic (TNF-α and FASL) and intrinsic (cytochrome c/caspase 9) apoptosis pathways, which coincide with deleterious effects on mitochondrial membrane permeability and/or oxidative stress via exacerbated ROS production. The apoptotic pathways are illustrated in Figure 2. Aloe-emodin further causes cell cycle arrest through cyclin and cyclin-dependent kinase downregulation. The cell cycle pathways and molecular regulators are depicted in Figure 3. Aloe-emodin also decreased transcription factor activity and altered transcriptional expression and/or protein levels of numerous cell signaling proteins important in proliferation and metabolism. In certain cancer cell lines, aloe-emodin induced immune signaling by upregulating, activating, and/or releasing interleukins, GM-CSF, NF-κB, and growth factors. Finally, by altering cell migration, invasion, and adhesion, aloe-emodin negatively affected tumor cell outgrowth propensity.

Table 1. Summary of biochemical effects of aloe-emodin and similar anthraquinones in human-derived cancer cell lines

Author	Cancer type and cell line	Methods	Main results
Lin et al. (2006)	Human bladder cancer (T24)	T24 cells were treated with aloe-emodin and assessed for cell viability, cell cycle, apoptosis, and mitochondria membrane potential. Caspase-3 activity was determine with and without caspase inhibitor (Z-VAD-FMK) pretreatment. Levels of CDK1, cyclin B1, WEE1, CDC25C, FAS, p21, p53, BAX, BCL-2, cytochrome c, and caspase-3 were analyzed with Western blot.	Aloe-emodin significantly decreased cell viability, mitochondrial membrane potential, and induced apoptosis in a time- and dose-dependent manner. Pre-treatment with Z-VAD-FMK attenuated apoptosis. WEE1, CDC25C, p53, p21, BAX, active caspase-3, FAS, and cytochrome c increased, while CDK1, cyclin B1, and BCL-2 decreased.
Guo et al. (2007)	Human cervical cancer (HeLa)	HeLa cells incubated with aloe-emodin were subjected to cell cycle analysis by flow cytometry and ALP activity analysis using a chemical analyzer assay. PKC, c-MYC, cyclins, CDKs, and PCNA were analyzed with Western blot.	Aloe-emodin significantly inhibited HeLa cells in a time- and dose- dependent manner with cell cycle arrest in the G_2/M phase. Aloe-emodin decreased cyclin A, CDK2, and increased cyclin B1 and CDK1. Aloe-emodin significantly increased ALP activity and decreased PKCα and c-MYC.
Schörkhuber et al. (1998)	Human colorectal tumor (SW480 carcinoma, VACO235 adenoma)	SW480, VACO235, and normal colonic epithelial cells were treated with DHA-containing media of varying concentrations including rhein, aloe-emodin, sennidine A/B, sennoside, and diphenylic laxative bisacodyl. Cell number was assessed via neutral red uptake, and cell proliferation by DNA uptake of BrdU, ELISA, or immunocytochemistry. Urokinase secretion was determined by chromogenic substrate spectrozyme urokinase assay.	In SW480 cells, DHAs caused non-specific cellular and nuclear toxicity with a predominant increase in urokinase secretion. Sennoside and bisacodyl did not exhibit a cytotoxic effect. In VACO235 cells, sennidine A/B and aloe-emodin stimulated cell growth with an increase in cell number and DNA synthesis, but did not affect urokinase secretion. VACO235 cells demonstrated sensitivity to sennoside and bisacodyl. DHAs did not have any effect on normal colonic epithelium.
Suboj et al. (2012)	Human colorectal adenocarcinoma (WiDr)	WiDr cells were treated with aloe-emodin, evaluated for migration, invasion, and antibodies against phospho-ERK1/2, phospho-JNK1/2, phospho-p38, RHOB, NF-κB, IκB, histone, β-actin. Promoter activity of MMP-2/9, RHOB, and VEGF was assayed along with DNA binding activity of NF-κB, AP1, and PCR analysis of MMP-2/9, RHOB, and VEGF-A.	Aloe-emodin decreased PMA-induced migration and invasion of WiDr cells, MMP-2/9, RHOB, and VEGF mRNA expression and promoter activity. ERK1/2 phosphorylation and NF-κB-DNA binding was attenuated with no effect on AP1.
Suboj et al. (2012)	Human colorectal adenocarcinoma (WiDr)	Aloe-emodin-treated WiDr cells were assayed for cell viability, cell cycle, cyclin B1 promoter and caspase-3/6/7/9 activity. Cells were probed with antibodies against PARP, procaspase-3/7/9, cleaved caspase-3/6/7/9, cyclin B1, phospho-ERK1/2, SAPK/JNK1/2, p38, MAPK, and β-actin.	Aloe-emodin inhibited proliferation of WiDr cells in a concentration-dependent manner with cell cycle arrest induced in G2/M phase. Cyclin B1 promoter activity was inhibited with a time-dependent increase in p21 expression. Protein expression of p53 and mRNA levels of p21 and p53 were unaffected. Apoptosis was seen with activation of caspase-6/9 and cleavage of PARP with no change in caspase-3/7. Aloe-emodin downregulated phosphorylated ERK and amplified phosphorylation of SAPK/JNK and p38 with a protective effect noted after treatment with JNK and p38 inhibitors. PDTC and N-acetyl cysteine (NAC) did not provide a protective effect against aloe-emodin.
Chen et al. (2007)	Human gastric carcinoma (AGS and NCI-N87)	AGS and NCI-N87 cells were cultured with aloe-emodin, assessed for viability, and mitochondrial proteins were isolated and extracted. Cells were incubated with monoclonal anti-apoptosis-inducing factor (E-1) and monoclonal anticytochrome c (A-8) antibodies. Caspase-3 and casein kinase II activity was assayed.	Aloe-emodin treatment induced apoptosis in a dose- and time-dependent manner, with AGS cells showing a higher sensitivity to aloe-emodin. Caspase-3 activity, release of AIF and cytochrome c significantly increased. An apoptotic pathway was indicated owing to nuclear fragmentation, release of AIF and cytochrome c from mitochondria into the cytosol. Pretreatment with a caspase-3 inhibitor, Ac-DEVD-CHO, inhibited activation in AGS cells. Casein kinase II showed a time-dependent decrease after aloe-emodin exposure, which caused a decrease in Ser61 phosphorylation of BID.
Chihara et al. (2015)	Mouse gastric carcinoma (MKN45)	MKN45 cells from Adenomatous polyposis coli (Apc)-deficient MIN (multiple intestinal neoplasia) mice were treated with aloe-emodin and emodin to assess for viability. Intracellular polyamine levels and DNA fragmentation were also measured.	Both aloe-emodin and emodin inhibited cell proliferation, but emodin did so to a greater degree. The cells were arrested in the G0/G1 phase by aloe-emodin and in G2/M by emodin. The differing levels of polyamine and DNA fragmentation suggest that aloe-emodin and emodin exerted cytotoxicity through different pathways.
Guo et al. (2008)	Human gastric cancer (MGC-803 and SGC-7901)	MGC-803 and SGC-7901 cells treated with varying concentrations of aloe-emodin were assayed for motility, cell cycle, DNA fragmentation, and Western blot.	Dose-dependent inhibition of PKC by aloe-emodin was seen in both cell lines. Cell migration in both cell lines was significantly decreased after treatment with aloe-emodin, with a reduction in gene expression of MMP-2. A dose-dependent increase in G2/M phase distribution of SGC-7901 cells was seen with a decrease in cyclin A and CDK2 and an increase in cyclin B1 and CDK1. Aloe-emodin decreased levels of PKCα and c-MYC in a dose-dependent manner.

Continued

Author	Cancer type and cell line	Methods	Main results
Lin et al. (2011)	Human pharyngeal squamous cell carcinoma (FaDu), human hepatoma (Hep3B), human osteosarcoma (MG-63)	FaDu, Hep3B, and MG-63 cells treated with aloe-emodin were cloned to constitutively express p53 short hairpin RNA (shRNA) or GFP shRNA. Viability was assayed and RT-PCR performed and amplified with primers for regions of p53 and CARP1/2. DNA fragmentation and caspase-3 activity was measured along with p53 and p21 promoters. Cells were assayed by flow cytometry, co-immunoprecipitation, and Western blot using antibodies against CDK2, p-CDK2 (Thr14/Thr15), cyclin A/B1/D/E, p21, PCNA, E2F1 transcription factor, p53, p21, ERK, p-ERK (Tyr202/204), BCL-X$_L$, BCL-2, BAX, BAK, BID, tBID, MCL-1, caspase-3/8/9, cytochrome c, AIF, ENDO G, and cyclooxygenase. Mitochondrial membrane potential, ROS, and cytosolic calcium levels were analyzed by flow cytometry.	Aloe-emodin induced cell death and p21 expression in FaDu, Hep3B, and MG-63 cells in a dose-dependent manner independent of p53 expression. Aloe-emodin increased levels of cyclin A, E2F1 and phosphorylation of CDK2 (Thr14/Thr15). Levels of cyclin B1/D/E were not affected. Binding of cyclin A to CDK2 was induced, which associated with p21. Cyclin A-CDK2 complexes did not associate with PCNA and EF21. Aloe-emodin inhibition occurred with cell cycle arrest in S phase. Aloe-emodin caused phosphorylation of ERK but not activation of p38 MAPK or JNK. BCL-2 expression was unchanged and BCL-X$_L$ expression was significantly inhibited. BAX was upregulated, tBID increased and BAK and MCL-1 levels were unaffected. Aloe-emodin treatment resulted in a decrease in mitochondrial membrane potential due to caspase release, which was inhibited by CsA and caspase-8 inhibitor Z-IETD-FMK. ROS and calcium were increased and also inhibited by Z-IETD-FMA and CsA. Caspase-8 activation resulted in loss of mitochondrial membrane potential, increased ROS and calcium, caspase-9 activation, cytochrome c release, AIF, ENDO G, and ERK phosphorylation.
Acevedo-Duncan et al. (2004)	Transformed glia cell line (SVG) and human glioma (U-373MG)	SVG and U-373MG cells treated with aloe-emodin were evaluated for cell proliferation, cell cycle, viability, and PKC activity.	Aloe-emodin prolonged S phase duration in SVG cells and delayed entry into S phase in U-373MG cells. Both cell lines exhibited increased apoptosis with reduction in survivin, PARP, and cleavage of caspase-7. PKC activity and isozyme levels were reduced.
Ismail et al. (2013)	Human malignant glioma (U87)	U87 glioma cells treated with aloe-emodin were assayed for proliferation, cell cycle, DNA fragmentation, and mitochondrial membrane potential.	Aloe-emodin significantly inhibited cell viability in U87 cells in a time- and dose-dependent manner through a primarily apoptotic pathway with minimal necrosis. Phase contrast microscopy confirmed cytotoxicity. DNA fragmentation occurred in a time-dependent manner, with S phase arrest and disruption of mitochondrial membrane potential.
Tabolacci et al. (2011)	Human monoblastic leukemia cells (U937)	U937 cells treated with aloe-emodin were assayed for viability, cell cycle, and generation of ROS. Nitrite levels were determined as an indicator of nitric oxide production. Transglutaminase activity, intracellular protoporphyrin IX (PpIX) concentration, CD11b, CD14, acidic vesicular organelles, phagocytic activity, migration, attachment, MMP secretion, polyamine concentration, ornithine decarboxylase, and spermine oxidase activity were measured. Protein levels of IL-1Ra (interleukin Ra subunit), IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p70, IL-13, IL-15, IL-17, TNF-α, IFN-γ, MIP-1α, MIP-1β, eotaxin, MCP-1, G-CSF, GM-CSF, bFGF, VEGF, IP-10, RANTES, and PDGFbb were determined.	Aloe-emodin-treated U937 cells had a reduction in proliferation, absence of cell injury, increase in ROS and NO production, active phagocytosis, and cellular acidity. Transglutaminase activity was enhanced, and the concentration of intracellular PpIX significantly increased. Aloe-emodin induced CD11b expression in a dose-dependent manner and same with CD14, both showing a significant increase in fluorescence intensity. Increased cell migration was shown in a dose-dependent manner. MMP-2/9, putrescine, ornithine decarboxylase, and spermine oxidase activity increased, and spermine levels decreased. Polyamine content was not altered after 48 hours. Extracellular IL-12, GM-CSF, IP-10, IL-6, IL-15, and VEGF increased in a dose-dependent manner with a decrease in IL-8.
Lai et al. (2007)	Human lung non-small cell carcinoma (H460)	Aloe-emodin-treated H460 cells were assessed for mitochondrial reductase activity, ATP levels, RNA concentration, and lactate dehydrogenase (LDH) release. RT-PCR was used to evaluate gene expression of HSP60, HSP70, and protein disulfide isomerase (PDI). Levels of HSP60, HSP70, PDI, and 150 kDa oxygen-regulated protein (ORP150), a marker for endoplasmic reticulum (ER) stress, were evaluated with Western blot.	Treatment with aloe-emodin resulted in significantly lower ATP synthase expression, increased mitochondrial damage, and a time-dependent reduction in ATP. HSP60, HSP70, and protein disulfide isomerase increased with no change in mRNA or gene expression. LDH release was not affected by aloe-emodin, suggesting apoptosis rather than necrosis.
Lee et al. (2001)	Human lung squamous cell carcinoma (CH27), human lung non-small cell carcinoma (H460)	CH27 and H460 cells were treated with aloe-emodin or emodin. Cells were analyzed for DNA fragmentation, apoptosis, PKC activity, and probed with antibodies against caspase-3, PARP, PKCα, β, δ, ε, ζ, η, θ, ι, μ, and cytochrome c.	Treatment with aloe-emodin and emodin resulted in time- and dose-dependent apoptosis of CH27 and H460 cells with an increase in nuclear and DNA fragmentation, cytochrome c release, and caspase-3 activation. In CH27 cells, PKCβ, γ, θ were not found and PKCζ was minimal. In H460 cells, PKCβ, γ, ζ, and μ were not found and the isozymes PKCα, δ, ε, ζ, η, θ, and ι were observed. PKCα was increased in aloe-emodin-treated H460 cells and emodin-treated H460 and CH27 cells, but was decreased in aloe-emodin-treated CH27 cells. PKCη and ι showed varying results in both cell lines. PKCδ and ε were significantly decreased in aloe-emodin-and emodin-treated CH27 and H460 cells. Aloe-emodin-treated CH27 cells resulted in increased PKC activity and emodin-treated CH27 cells had

Author	Cancer type and cell line	Methods	Main results
			decreased PKC activity. Aloe-emodin-treated H460 cells had increased PKC with a decrease after emodin treatment. A caspase-3 inhibitor, Ac-DEVD-CHO, reversed the inhibition by emodin on PKC activity but had no effect on aloe-emodin.
Lee et al. (2001)	Human lung squamous cell carcinoma (CH27)	Aloe-emodin-treated CH27 cells were assayed for cell viability, DNA fragmentation, and apoptosis. Samples were exposed to antibodies against BAG-1, BAK, BAX, BCL-X$_L$, caspase-8, and cytochrome c.	CH27 cells underwent irreversible apoptosis in a dose- and time-dependent manner, confirmed by DNA fragmentation analysis with a sub-G$_1$ peak of DNA content. BAK and cytochrome c release increased, with no change in BCL-2 and BAX, and a significant decrease in BCL-X$_L$ and BAG-1. BAK and BAX translocated from cytosol to mitochondria. Activation of caspases-3/8/9 was significant.
Lee et al. (2006)	Human lung non-small cell carcinoma (H460)	Aloe-emodin-treated H460 cells were assessed for single-cell DNA damage along with DNA repair enzyme gene expression of human homocysteine s-methyltransferase 1 (HMHT1), human 8-oxoguanine DNA N-glycosylase (HOGG1), and apurinic endonuclease. Intracellular ROS and SOD dismutase activity was assayed and cells were incubated with antibodies against β-actin, Cu/ZnSOD, and MnSOD.	Aloe-emodin treatment resulted in large DNA aggregates with significantly increased tail migration. Gene expression of DNA repair enzymes HMHT1, HOGG1, and apurinic endonuclease were significantly decreased. Total SOD and MnSOD activity exhibited a biphasic response with an initial increase in enzyme activity. Potassium cyanide, a Cu/ZnSOD inhibitor, did not attenuate this response. Protein levels of MnSOD and Cu/ZnSOD did not change after aloe-emodin treatment, while ROS increased.
Lee et al. (2005)	Human lung non-small cell carcinoma (H460)	H460 cells treated with aloe-emodin were cultured and assayed with antibodies against β-actin and nucleophosmin. Protein activity was analyzed with 2D gel and silver staining.	Aloe-emodin induced the release of nucleophosmin into the cytosol with apoptosis-associated degradation. Preform and fragment nucleophosmin increased, with no change in gene expression.
Lee et al. (2010)	Human lung non-small cell carcinoma (H460)	Aloe-emodin-irradiated H460 cells (400-700 nm wavelength) were assayed for mitochondrial reductase activity, F-actin, α- and β-tubulin visualization, and incubated with antibodies against α-tubulin, α-actinin, HSP27, p38, AIF, BAX, BCL-2, caspase-3/7/8/9, cytochrome c, ERK1, and JNK. Classification of apoptosis vs. necrosis was performed by annexin V/PI staining. Mitochondrial permeability transition pore opening was measured with calcein.	Mitochondrial reductase activity demonstrated photo-activated cytotoxicity and change in cellular morphology not seen with aloe-emodin-only treatment. Cytoskeletal damage to actin microfilaments, microtubules and α-actinin disorganization with nuclear localization of HSP27 was seen. A significant decrease in cytosolic p38 protein occurred and externalization of phosphatidylserine by binding of annexin V revealed an apoptotic mechanism. Cells underwent a rapid apoptosis-to-necrosis switch as confirmed by PI staining. AIF, cytochrome c, and preform caspase-8/9 increased while preform caspase-3/7 decreased. BCL-2 decreased, while BAX and ERK1 increased. Prior to irradiation, ERK1, JNK, and p38 decreased while in the photodynamic light, but no effect on protein expression of while in the dark JNK, ERK1, and p38. This means that these MAP kinases are only affected by the light source and not the aloe-emodin.
Yeh et al. (2003)	Human lung non-small cell carcinoma (H460)	H460 cells treated with aloe-emodin were measured for cyclic AMP via enzyme immunoassay. Caspase-3 activity was determined and Western blot analysis was performed after incubation with antibodies against β-actin, ATF-2, BCL-2, caspase-3, PKCδ, ERK1/ERK2, JNK1, p38, and PKAc.	Continuous exposure to aloe-emodin resulted in a time- and dose-dependent increase in irreversible cell death. Pretreatment with forskolin (adenylate cyclase activator), isobutyl methylxanthine (a phosphodiesterase inhibitor), and chelerythrine (a PKC inhibitor) had no effect on aloe-emodin-induced apoptosis. However, SB202190 (a p38 MAP kinase inhibitor) resulted in significant inhibition of aloe-emodin-induced cell death. Forskolin increased cAMP, and aloe-emodin decreased it along with PKAc concentrations. Forskolin prevented aloe-emodin-induced decrease in PKAc and PKCδ, while pretreatment with chelerythrine and SB202190 had no effect. Forskolin and chelerythrine prevented aloe-emodin-induced downregulation of BCL-2 and SB202190 had no effect. Aloe-emodin induced a biphasic change in caspase-3 activity. Pretreatment with forskolin and chelerythrine prevented caspase-3 cleavage. SB202190 had no effect. Aloe-emodin induced decreases in ERK1/ERK2 and p38 levels, with no effect on JNK1 levels. Pretreatment with forskolin and chelerythrine prevented p38 degradation but did not have as much effect on the induced decrease in p38 as SB202190. SB202190 had a significant effect in aloe-emodin-induced decrease in ATF-2 expression.
Jeon et al. (2012)	Human hepatoma (Huh-7)	Huh-7 cells were treated with aloe-emodin and assessed for cell viability, DNA fragmentation, and ROS. CAPN2 and UBE3A protein levels were analyzed with Western blot after 2-DE proteomic analysis.	Aloe-emodin-inhibited cell proliferation and induced apoptotic changes in a time- and dose-dependent manner with an increase in DNA fragmentation and intracellular ROS. Aloe-emodin decreased CAPN2 and UBE3A.
Lu et al. (2007)	Human hepatocellular carcinoma (HepG2, HCCM, and Hep3B)	HepG2, HCCM, and Hep3B cells treated with aloe-emodin were evaluated for nuclear fragmentation and chromatin condensation. Intracellular glutathione (GSH/GSSG) was analyzed	Aloe-emodin-treated HepG2 cells showed a time-dependent increase in apoptosis through a caspase-dependent pathway that was inhibited by pretreatment with a pan-caspase inhibitor Z-VAD-FMK. Time- and dose-dependent cleavage/activation of caspase-3/9 was noted with cleavage of

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Author	Cancer type and cell line	Methods	Main results
		and production of ROS measured via flow cytometry.	PARP. No effect was seen with cleavage/activation of caspase-8. Cytochrome c translocated from mitochondria to cytosol through intrinsic (mitochondria-dependent) apoptosis. HCCM and Hep3B cells underwent aloe-emodin-induced apoptosis with a time-dependent increase in nuclear condensation, cleavage of caspase-3/9, PARP, but not caspase-8. Aloe-emodin significantly increased oxidation of PRDX and intracellular ROS increased with a depletion of GSH. Aloe-emodin resulted in a delayed, sustained activation of JNK and inhibition of the ERK pathway. Phosphorylation/activation of p38 was unaffected in all three cell lines. SOD1 decreased aloe-emodin-induced JNK phosphorylation/activation and ERK inhibition was unaffected. Aloe-emodin-induced apoptosis was promoted through oxidative stress and sustained JNK activation.
Lin et al. (2010)	Human nasopharyngeal carcinoma (NPC)	NPC cells treated with aloe-emodin were assayed for viability, cell cycle, caspase-3 activity, DNA fragmentation, DAPI staining for changes in nuclear morphology, and comet assay. Cells were treated with antibodies against cyclin A/B1/E, CDC2, BCL-X_L, BCL2, BAX, GRP78, p-PERK (Thr981), BAK, BAD, BID, tBID, ATF6α, ENDO G, AIF, caspase-3/4/8/9, and cytochrome c. Cells were transfected with BAX siRNA or FADD siRNA, and assessed with antibodies against BAX or FADD. Release of cytochrome c, AIF, and ENDO G were assayed. Mitochondrial membrane potential, ROS, and cytosolic calcium were measured.	Aloe-emodin induced apoptosis through caspase-3 activation. Comet assay revealed increased DNA fragmentation, which was inhibited by caspase-3 inhibitor Ac-DEVD-CMK. Cell cycle arrest occurred in the G2/M phase in consequence to cyclin B1 complexing to CDC2. Cyclin A/E were not affected. Aloe-emodin induced binding of cyclin B1 to CDC2. Aloe-emodin inhibited expression of BCL-X_L and BCL-2 was unaffected. BAX was upregulated and BAK and BAD were unaffected. GRP78, PERK, ATF6, and cleavage of caspase-4 were not affected. SiRNA attenuation of BAX suppressed cleavage of PARP and induction of apoptosis. Mitochondrial membrane permeability decreased through a caspase-8-dependent pathway that was blocked by Z-IETD-FMK, a caspase-8 inhibitor. ROS and cytosolic calcium were increased after treatment with aloe-emodin that was attenuated by pretreatment with Z-IETD-FMK or CsA. Aloe-emodin increased BAX, BID cleavage, and tBID translocation into mitochondria, which was inhibited by Z-IETD-FMK. Caspase-8 and aloe-emodin-induced apoptosis were not suppressed by FADD siRNA transfection. Caspase-3/9 cleavage were inhibited by Z-LEHD-FMK. AIF and cytochrome c increased and a low level of ENDO G was detected. These effects were blocked by pretreatment with Z-IETD-FMK.
Lin et al. (2010)	Human nasopharyngeal carcinoma (NPC)	Aloe-emodin-treated NPC cells were assayed for cell viability, proliferation, cell cycle, caspase-3 activity, and annexin-V binding of early apoptotic cells. Cells were incubated with antibodies against cyclin A/B1/D/E, CDK1, MMP-1/2/7/9, VEGF, p-ERK (Tyr 202.204), ERK, p-p38 MAPK (Tyr 180/182), p38 MAPK, p-JNK, JNK, RHOA, COX-2, ROCK-1, IκBα, and NF-κB. MMP-2/9 promoters and NF-κB promoters were assayed.	Aloe-emodin significantly inhibited cell growth with no effect on viability, PS exposure, and caspase-3. Cyclin B1 increased. Cyclins A/E were not affected. Binding of cyclin B1 to CDK1 was induced and attenuation of cyclin B1 suppressed aloe-emodin-induced S and G2/M phase arrest. Aloe-emodin inhibited expression of MMP-2, NF-κB, and VEGF, while MMP-1/7/9 were not affected. Aloe-emodin suppressed phosphorylation of p38 MAPK, JNK, ERK, RHOA. COX-2 and ROCK-1 were not affected.
Pecere et al. (2003)	Human neuroblastoma [SK-N-Be (2c) and SJ-N-KP]	SK-N-Be(2c) and SJ-N-KP cells treated with aloe-emodin were assayed for cell viability, topoisomerase II cleavage, cell cycle, caspase-3/8, and antibodies against p53, p21, BCL-2, BAX, CD95, and PARP.	Aloe-emodin demonstrated dose-dependent cytotoxicity in the order of SJ-N-KP > SK-N-Be(2c). MRNA expression of p53 increased in both cell lines, with an increase in p21, BCL-2, BAX, and CD95 mRNA in SJ-N-KP cells only due to loss of p53 function in SK-N-e(2c) cells. Both cell lines showed a time-dependent increase in p53 levels with induction of p21 and CD95 protein expression in SJ-N-KP. Mitochondrial BAX and BCL-2 increased in both cell lines, along with increased cytochrome c release and caspase-3 activity with no change in caspase-8. Pre-treatment with pifithrin-α decreased apoptosis in SJ-N-KP cells but not SK-N-Be(2c) cells.
Xiao et al. (2007)	Human oral cancer (KB)	KB cells were treated with aloe-emodin and assayed for viability, cell cycle, DNA fragmentation, and ALP activity.	Aloe-emodin resulted in a time- and dose-dependent inhibition of cell proliferation, with an increase in G2/M phase and a decrease in S phase, no DNA fragmentation, and an increase in ALP activity.
He et al. (2008)	Human ovarian carcinoma (HO-8910PM)	Aloe-emodin-treated HO-8910PM cells were assessed for cell viability, migration, and invasive ability. Expression of FAK and FAK mRNA was evaluated with Western blot and RT-PCR.	Aloe-emodin was mildly cytotoxic to cells at high concentrations. Migration, invasion, and adhesion were significantly inhibited. Aloe-emodin reduced FAK levels and downregulated FAK mRNA.
Liu et al. (2012)	Human prostate cancer (PC3)	PC3 cells treated with aloe-emodin were subjected to the MTS assay and Western blot. *In vitro* kinases of mTOR, mTORC2, and PI3-K were assayed along with cell proliferation. Phosphorylation of AKT at Ser473 and Thr308 was analyzed.	Aloe-emodin attenuated Akt phosphorylation at Ser473 and GSK3β at Ser9 in a dose-dependent manner. Phosphorylation of p70S6k at Thr389 was not affected. Aloe-emodin did not affect the MAPK pathway. Aloe-emodin inhibited proliferation of PC3 cells, which was unrelated to cytotoxicity. Aloe-emodin bound to mTORC2, and inhibited mTORC2 kinase activity that resulted in suppression of cell proliferation.

| Author	Cancer type and cell line	Methods	Main results
Chou et al. (2009)	Human premalignant keratinocytes (HaCaT), human skin fibroblasts (Hs68), human epidermoid carcinoma (A431), human head and neck squamous cell carcinoma (SCC25), and skin melanoma (A375)	All cell lines were treated with 5-fluorouracil, aloe-emodin, and 5-fluorouracil + aloe-emodin. A431 and SCC25 cells were assessed for proliferation, cell cycle, and apoptosis. Intracellular ROS and GSH levels were determined with a fluorometric assay. Immunoblot analysis was used to determine protein expression of cleaved caspase-3/8/9.	Aloe-emodin showed dose-dependent cytotoxicity and time-dependent inhibition of cell proliferation in A431 and SCC25 cells, with no significant effect on A375, HaCaT, and Hs68 cells. Apoptosis was induced when higher concentrations were used (increased cell cycle sensitivity during S-G$_2$/M phase). TNF-α and FASL death pathways, p53, cytochrome c, and BAX were upregulated with downregulation of BCL-2. ROS and caspase-3/7/8/9 increased with reduction in GSH. 5-Fluorouracil and aloe-emodin were synergistic in terms of cytotoxicity, and liposomal aloe-emodin demonstrated enhanced drug permeation.
Fenig et al. (2004)	Human Merkel cell carcinoma (MCC)	MCC cells were treated with aloe-emodin, emodin, and aloin and assayed for cell proliferation. Aloe-emodin was evaluated in conjunction with cisplatin, doxorubicin, and 5-fluorouracil.	Aloe-emodin and emodin inhibited cell proliferation in a dose-dependent manner, with a slightly greater effect for aloe-emodin, while aloin had no effect. Aloe-emodin, cisplatin, doxorubicin, 5-fluorouracil, and tyrosine kinase inhibitor STI 571 all had a significant inhibitory effect on MCC cells with an additive synergistic inhibition when each agent was combined at low concentrations with varying doses of aloe-emodin.
Lin et al. (2005)	Human malignant melanoma (A375.S2)	A375.S2 was incubated with 2-aminofluorene (known carcinogen) and varying concentrations of aloe-emodin to evaluate *N*-acetylation. NAT1 and NAT2 mRNA levels were assessed.	NAT1 levels and mRNA expression were inhibited in a dose-dependent manner, resulting in decreased *N*-acetylation of 2-aminofluorene after treatment with aloe-emodin. NAT2 levels and mRNA expression were not affected.
Radovic et al. (2012)	Human malignant melanoma (A375)	Aloe-emodin-treated human A375 cells underwent analysis of cell cycle, apoptosis, caspase (isoform not specified), and ROS. Tyrosinase activity was evaluated through the L-dopa oxidation rate. Immunoblot analysis was used for AKT, p-ERK, p53, cyclin D1 and D3, BCL-2, inducible nitric oxide synthase (iNOS), and IκB. Aloe-emodin was evaluated for a synergistic effect with doxorubicin and paclitaxel via isobologram analysis.	Aloe-emodin decreased mitochondrial respiration in this cell line in a dose-dependent manner, with greater cell cycle arrest in G$_2$/M phase. Apoptosis was pronounced in A375 cells, with a decrease in BCL-2 and no effect on iNOS or pIκB. Aloe-emodin demonstrated a decrease of p-ERK. AKT phosphorylation was significantly increased in A375 cells. Aloe-emodin was cytoprotective in the presence of other toxins as evidenced by an antagonistic effect on doxorubicin and paclitaxel.
Wasserman et al. (2002)	Human Merkel cell carcinoma (MCC)	MCC cells (novel cell line) were verified by immunocytochemical staining with specific antibodies. Proliferation inhibition was tested with exposure to bFGF, transforming growth factor (TGFβ1), nerve growth factor (NGF), epidermal growth factor (EGF), sodium butyrate, DMSO, aloe-emodin, and aloin.	Aloe-emodin showed a significant inhibitory effect on cell number, with no effect by aloin. TGFβ1, bFGF, NGF, and EGF did not stimulate or inhibit proliferation of MCC cells. Sodium butyrate and DMSO showed a concentration-dependent decrease in cell viability.
Chen et al. (2010)	Human tongue squamous cell carcinoma (SCC-4)	SCC-4 cells were treated with emodin, aloe-emodin, and rhein and tested for viability, migration, and Western blot to evaluate the effects on MMP-2/9, urokinase (uPA), TIMP-1, FAK, NF-κB, p65, p-AKT, p-P38, p-JNK, and p-ERK. The activity of MMP-2 and gene expression of MMP-2/7/9 was assayed.	SCC-4 cells exhibited reduced viability after exposure to emodin, aloe-emodin, and rhein in a time- and dose-dependent manner. Emodin, aloe-emodin, and rhein inhibited migration and invasion of SCC-4 cells, with a reduction in MMP-2, uPA, FAK, NF-κB, p65, p-AKT, p-38, p-JNK, and p-ERK and an increase in TIMP-1 and no effect on MMP-9. Activity of MMP-2 was inhibited with no effect on gene expression. MMP-9 mRNA expression was inhibited.
Chen et al. (2010)	Human tongue squamous cell carcinoma (SCC-4)	SCC-4 cells treated with aloe-emodin, emodin, and rhein were assessed for viability, DNA damage, and real-time PCR of ATR, 14-3-3σ, BRCA1, ATM, DNA-PK, and MGMT.	Emodin, aloe-emodin, and rhein induced dose-dependent cytotoxicity in SCC-4 cells with induction of DNA damage, as evidenced by a longer comet tail in the order of emodin > aloe-emodin > rhein. Expression of MGMT, DNA-PK, BRCA1, 14-3-3σ, ATR, and ATM mRNA was significantly inhibited by aloe-emodin, excluding MGMT under 48 hours. MGMT mRNA expression was inhibited by rhein after 48-hour incubation and emodin had no effect. Rhein showed similar effects in ATR but had no effect on ATM.
Chiu et al. (2009)	Human tongue squamous cell carcinoma (SCC-4)	Aloe-emodin-treated SCC-4 cells were assayed for viability, cell cycle, ROS, mitochondrial membrane potential, and calcium levels. Activity of caspase-3/8/9 was analyzed and Western blotting was performed to determine total protein and levels of p53, p21, p27, cyclin A/E, thymidylate synthase, CDK2, CDC25A, FAS, FASL, caspase-3/8/9, BID, cytochrome c, AIF, poly(ADP-ribose), PARP, BAX, BCL-2, ATF-6α, and GRP78.	Aloe-emodin showed dose-dependent inhibition in cell viability and an S phase arrest with sub-G$_1$ phase accumulation and changes in nuclear morphology. ROS production, calcium levels, and caspase-3/8/9 activity increased in a time-dependent manner, with a reduction in mitochondrial membrane potential. Pretreatment with NAC and caspase inhibitors significantly blocked aloe-emodin-triggered apoptosis. Aloe-emodin increased p53, p21, p27, FAS, FASL, caspase-3/8/9, BID, cytochrome c, AIF, ATF-6α, and GRP78. Cyclin A/E, thymidylate synthase, CDK2, CDC25A, PARP, and BCL-2 were decreased.
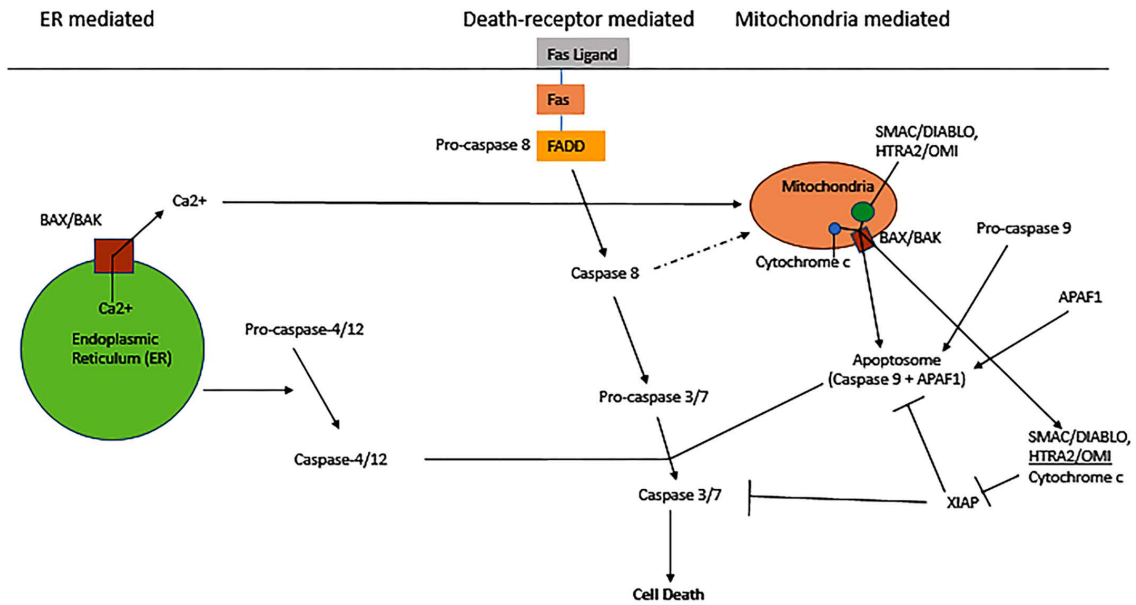
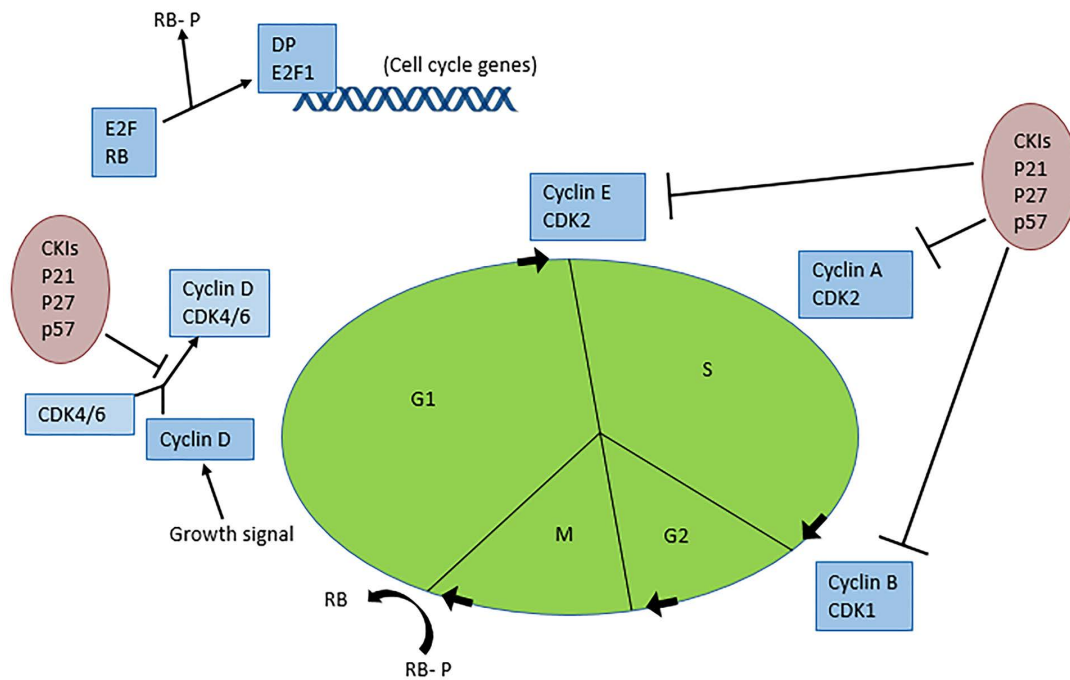**Figure 2.** Apoptotic pathways and their regulation. Adapted from: http://www.nature.com/nrm/journal/v6/n4/images/nrm1618-i2.jpg

Figure 3. The cell cycle and its regulating factors. Adapted from: http://www.nature.com/nrn/journal/v8/n5/box/nrn2124_BX1.html.

3.1. Bladder cancer

In bladder cancer cells (T24), aloe-emodin induced time- and dose-dependent apoptosis [7]. The cell death induction was accompanied by perturbation of mitochondrial membrane potential and reduced levels of cyclin-dependent kinase (CDK) 1, cyclin B1, and BCL-2 after treatment with aloe-emodin.

3.2. Cervical cancer

Cervical cancer cells (HeLa) were treated with aloe-emodin,

which caused cell cycle arrest in the G_2/M phase. The cells showed a decrease in cyclin A and CDK2, which reduces the cell's ability to proliferate, and suppression of protein kinase Cα (PKCα) and c-MYC, signifying that proliferation and differentiation were suppressed [8]. Increases in cyclin B1, CDK1, and alkaline phosphatase (ALP) activity were observed along with inhibition of proliferating cell nuclear antigen (PCNA), showing decreased proliferation.

3.3. Colon cancer

It has been previously shown that 1,8-dihydroxyanthraquinone (DHA) laxatives are associated with colon cancer development [9]. SW480 carcinoma cells, VACO235 adenoma cells, and normal colonic epithelium were treated with various DHA laxatives to determine their effects. SW480 carcinoma cells showed a dose-dependent increase in urokinase secretion (an enzyme that digests extracellular matrix, which could increase tumor cell migration and metastasis, but also causes cell lysis) that caused a reduction in cell numbers by DHA-aglycones. Rhein and aloe-emodin (types of DHA laxatives) increased BrdU (5-bromo-2'-desoxyuridine; a marker of cell proliferation) by 37% and 50%, respectively. In contrast, premalignant VACO235 adenoma cells did not show an increase in urokinase secretion by sennidine A/B and aloe-emodin. However, cell growth and DNA synthesis increased as reflected by elevated BrdU staining. DHA laxatives had no effect on the normal colorectal epithelium [9]. The anti-proliferative effect of aloe-emodin in WiDr cells (colon cancer cell type) was shown by suppression of phorbol-12-myristyl-13-acetate (PMA), which induces tumor migration and invasion [10]. Aloe-emodin downregulated messenger RNA expression and promoter/ gelatinolytic activity of matrix metalloproteinase (MMP)-2/9 and decreased Ras homologue gene family member B (RHOB) expression. Nuclear translocation of and DNA binding by NF-κB were suppressed along with vascular endothelial growth factor (VEGF), demonstrating that aloe-emodin targets multiple molecules necessary for tumorigenesis. Cell cycle arrest in WiDr cells occurred in the G_2/M phase with inhibition of cyclin B1. Another study showed that apoptosis was induced through caspases-6/9, with specific caspase-6 activation by aloe-emodin [11].

3.4. Gastric cancer

Gastric carcinoma (AGS, NCI-N87) cells treated with aloe-emodin demonstrated mitochondrial release of apoptosis-inducing factor (AIF) and cytochrome c-mediated activation of caspase-3[12]. AGS cells showed greater sensitivity to aloe-emodin than NCI-N87 cells. Another study showed that MKN45 cell growth was substantially inhibited by both aloe-emodin and emodin, but more so by emodin [13]. These cells were arrested in the G_0/G_1 and G_2/M phase by aloe-emodin and emodin, respectively. Time- and dose-dependent inhibition was demonstrated in MGC-803 cells, with an increase in S phase and a decrease in ALP activity [8]. Another study showed a cytostatic effect in MGC-803 and SGC-7901 cells, with a significant decrease in cell migration [14]. SGC-7901 cells became arrested in the G_2/M phase in a time and dose-dependent manner, with a decrease in cell cycle-inducing proteins.

3.5. Leukemia

Monoblastic leukemia (U937) cells were treated with aloe-emodin, resulting in reduced proliferation rate. Reactive oxygen species (ROS) and NO production, phagocytosis, and intracellular acidity also increased [15], the significance of which is currently elusive.

3.6. Lung cancer

Researchers in one study on human lung non-small cell carcinoma (H460) treated the cells with aloe-emodin and examined the cells with 2D electrophoresis. They found a time- dependent reduction in ATP, lower ATP synthase expression, and increased mitochondrial damage with unaffected lactate dehydrogenase (LDH) levels, suggesting the induction of apoptosis. HSP60, HSP70, and protein disulfide isomerase increased, which were hypothesized to cause apoptosis by augmenting endoplasmic reticulum stress [16].

Another series of five different studies by Lee et al. evaluated aloe-emodin and emodin in lung squamous carcinoma (CH27) and lung non-small cell carcinoma (H460). The first study demonstrated apoptotic changes through nuclear morphological change, DNA fragmentation, increased the relative abundance of cytochrome c levels, activation of caspase-3, and decreased levels of PKC isozymes generally [17]. The second study showed that CH27 cells underwent apoptotic cell death in an irreversible dose- and time-dependent manner, which coincided with DNA fragmentation. BAK, BAX, and cytochrome c were elevated in the cytosol, consistent with the intrinsic apoptosis pathway [18]. In the third study, aloe-emodin treatment was associated with an increased release of nucleophosmin into the cytoplasm, but no change in its gene expression [19]. Nucleophosmin is a nucleolar phosphoprotein that hyperaccumulates in the nucleoplasm of malignant cells and decreases with drug-induced apoptosis. This study showed that nucleophosmin protein levels were increased, but that the protein predominantly localized to the cytoplasm in its (inactive) proform. It was concluded that this could be a possible mechanism in aloe-emodin-induced apoptosis in cancer cells. In the fourth study, aloe-emodin caused single strand DNA breaks and a decrease in the levels of DNA repair enzymes [20]. The final study supported programmed cell death via anoikis and apoptosis of H460 cells through photo-activated aloe-emodin [21]. Anoikis is a form of programmed cell death whereby the cell separates from its environment and eventually dies because it no longer receives nutrients from its surroundings. In apoptosis, specific cell signals are given to the intact cell to shut down. Increased protein expression of α-actinin and mitogen-activated protein (MAP) kinase members was observed, and apoptosis was mediated through caspase-dependent intrinsic and extrinsic pathways.

In another study, aloe-emodin treatment resulted in time- and dose-dependent irreversible cell death of human lung non-small cell carcinoma (H460) [22]. Aloe-emodin decreased BCL-2, which abrogated the inhibition of pro-apoptotic proteins (such as BAK and BAX) and increased gene expression of p38 and caspase-3 activity, exacerbating apoptosis.

3.7. Liver cancer

Aloe-emodin inhibited cell growth and induced apoptosis in hepatoma (Huh-7) cells in a time- and dose-dependent manner [23]. DNA fragmentation and ROS levels were increased with a reduction in CAPN2 (calpain-2) and UBE3A (ubiquitin protein ligase E3A). These two proteins are involved in protein degradation via proteasomal processing, which enables maintenance of normal cellular activity. With their decrease, cells are unable to function and undergo apoptosis. Another study showed that aloe-emodin-treated hepatocellular carcinoma (HepG2) cells underwent apoptosis through a caspase-dependent pathway with cleavage and activation of caspases-3/9 and cleavage of PARP [24]. Execution of intrinsic apoptosis was supported by translocation of cytochrome c. Hepatocellular carcinoma (HCCM and Hep3B) cells underwent apoptosis via caspases-3/9 and PARP. Activation of p38 was unaffected in all three cell lines. Aloe-emodin-induced apoptosis was seen through oxidative stress and sustained c-JUN N-terminal kinase (JNK) activation.

3.8. Nasopharyngeal cancer

Two studies by Lin et al. showed the effects of aloe-emodin in nasopharyngeal carcinoma (NPC) cells. The first study demonstrated that aloe-emodin induced apoptosis via caspase-3 activation with DNA fragmentation [25]. Cell cycle arrest in the G_2/M phase was associated with increased cyclin B1-CDC2 complex formation. Matrix metalloproteinase-2 was significantly decreased, with an increase in ROS and cytosolic calcium. The second study showed that aloe-emodin significantly inhibited cell growth without affecting viability [26]. Cyclin B1 binding to CDK1 was induced, and aloe-emodin triggered cell cycle arrest in the S and G_2/M phase.

3.9. Neuroectodermal cancer

A study demonstrated dose-dependent cytotoxicity of aloe-emodin in neuroblastoma cells (SJ-N-KP wild-type p53 and SK-N-Be(2c) mutant p53 type) [27]. The SK-N-Be(2c) cells lack transcriptional activity of p53-targeted genes, which allowed studying the effect of aloe-emodin in terms of apoptosis. Expression of p53 mRNA was increased in both cell lines, but only SJ-N-KP cells showed an increase in p21, BCL-2, BAX, and CD95 mRNA due to loss of p53 function in SK- N-Be(2c) cells. Both cell lines had a time-dependent increase in p53 levels, with induction of p21 and CD95 protein expression in SJ-N-KP cells.

In addressing glial tumors, one group of researchers treated a transformed glia cell line (SVG) and a glioma U-373MG cell line with aloe-emodin, which delayed the number of cells entering and exiting the S phase, indicating inhibited S phase progression [28]. Another study showed aloe-emodin-induced apoptosis of U87 malignant glioma cells through disruption of mitochondrial membrane potential, cell cycle arrest in the S phase, and DNA fragmentation in a time-dependent manner

with minimal necrosis [29].

3.10. Oral cancer

A time- and dose-dependent inhibition of cell growth was found in oral cancer (KB) cells treated with aloe-emodin, with cell cycle stalling in the G_2/M phase and a decrease in S phase [30]. ALP activity was increased and no DNA fragmentation was observed.

3.11. Ovarian cancer

HO-8910M ovarian carcinoma cells were evaluated for migration and invasion [31]. Migration, invasion, and adhesion were significantly inhibited by aloe-emodin, with a corresponding decrease in focal adhesion kinase (FAK; involved in cellular mobility, and in this case, metastasis) protein expression and mRNA levels. Aloe-emodin use in these cells attested to its anti-metastatic potential.

3.12. Prostate cancer

Tumor growth suppression was noted in prostate cancer (PC3) cells treated with aloe-emodin. The normal growth of prostate cells is through mTORC2 and its downstream effects. Following treatment with aloe-emodin, mTORC2's downstream enzymes, AKT and PKCα, were inhibited and hence exhibited decreased phosphorylation activity in a dose-dependent manner [32]. Aloe-emodin did not affect MAPK, p38, or c-JNK or phosphorylation of ERKs. Proliferation of PC3 cells was inhibited as a result of aloe-emodin binding to mTORC2, with inhibition of mTORC2 kinase activity.

3.13. Skin cancer

Aloe-emodin had a greater cytotoxic effect in non-melanoma cancer cells (epidermoid carcinoma (A431) cells and head and neck squamous cell carcinoma (SCC25) cells) than non-cancerous skin cells (pre-malignant keratinocytic HaCaT cells and Hs68 fibroblasts) [33]. This occurred through upregulation of tumor necrosis factor-α (TNF-α), FAS ligand, and the associated death domains for TNF-R1 and FAS. Aloe-emodin activated caspases-3/7/8/9 and upregulated p53, cytochrome c, and BAX. Intracellular ROS increased, while intracellular-reduced glutathione (GSH) was depleted and BCL-2 (anti-apoptotic protein) was down-regulated. Further, aloe-emodin inhibited cytosolic *N*-acetyltransferase 1 (NAT1) enzyme activity and mRNA expression in A375.S2 malignant melanoma cells in a dose-dependent manner [34]. NAT1, expressed in many human cancer cell lines, is an enzyme that N-acetylates arylamine carcinogens and drugs (initial metabolism) in A375.S2 cells as well as other cancer cell lines.

Aloe-emodin also sensitizes skin cancer cells to chemotherapy. A combination of aloe-emodin and 5-fluorouracil caused an increase in cell death, as did liposomally delivered aloe-emodin. Another study showed that aloe-emodin and emodin potentiated the therapeutic effects of cisplatin, doxo-

rubicin, 5-fluorouracil, and tyrosine kinase inhibitor STI 571 in Merkel cell carcinoma, which is known to be resistant to antineoplastic agents [35]. Aloe-emodin had a small advantage over emodin with respect to anti-proliferative effects when combined with these chemotherapeutic drugs at low concentrations, while aloin showed no effect.

Radovic et al. found that aloe-emodin caused A375 melanoma cells to undergo apoptosis accompanied by BCL-2 downregulation and caspase-mediated apoptosis [36]. An interesting finding was that aloe-emodin rescued cells from doxorubicin- or paclitaxel-induced death in a dose-dependent manner, exhibiting a cytoprotective effect. Accordingly, caution is warranted when using aloe-emodin with these chemotherapy drugs. Finally, aloe-emodin significantly inhibited Merkel cell carcinoma growth with no effect by aloin [37]. Basic fibroblast growth factor (bFGF), transforming growth factor-β1 (TGFβ1), nerve growth factor (NGF), and epidermal growth factor (EGF) did not affect proliferation of Merkel cell carcinoma cells.

3.14. Tongue cancer

Chen and colleagues investigated the effects of aloe-emodin, emodin, and rhein on SCC-4 tongue squamous cell carcinoma in two studies. The first study revealed a decrease in viability in a time- and dose-dependent manner, with the greatest effect induced by emodin, followed by aloe-emodin, then rhein [38]. Migration and invasion of SCC-4 cells was inhibited, with reductions in MMP-2 and NF-κB, signifying decreased cell mobility. In the second study, cytotoxicity and induction of DNA damage were seen in the same order of magnitude per anti-carcinogenic agent [39]. Expression of DNA-PK, BRCA1, and ATM mRNA (all DNA repair enzymes) was significantly inhibited by aloe-emodin, with varying effects by emodin and rhein. Another study showed that aloe-emodin inhibited SCC-4 cell viability in a dose-dependent manner with S phase arrest and changes in nuclear morphology [40]. Levels of ROS, calcium, and caspases-3/8/9 activity increased in a time-dependent manner, accompanied by a reduction in mitochondrial membrane potential.

3.15. General/other

Aloe-emodin demonstrated p53-independent apoptosis in FaDu (pharyngeal squamous cell carcinoma), Hep3B (hepatoma), and MG-63 (osteosarcoma) cells [41]. This resulted in S phase cell cycle arrest. Caspase-8/10-associated RING protein (CARP) expression was decreased by aloe-emodin. When CARPs were overexpressed, aloe-emodin-induced apoptosis was attenuated. CARPs normally interact with caspase-8/10 by inhibiting their function through ubiquitin-mediated proteolysis. With decreased levels of CARPs, apoptosis is increased.

4. Discussion

This review of *in vitro* data clearly suggests that aloe-emodin, an inexpensive, readily available nutrient from aloe vera with a longstanding history of safe use, has anti-neoplastic and anti-proliferative effects on multiple cancer types and cell lines. A secondary finding from selected studies suggests that aloe-emodin has great potential to serve as an adjunct to conventional chemotherapeutic regimens, as these data demonstrate potential for synergy with selected chemotherapeutic agents, allowing for a reduction in drug dose. Nevertheless, one study showed cytoprotective effect of aloe-emodin on cancer cells, possibly leading to a restricted efficacy of selected chemotherapeutic agents.

Aloe-emodin clearly induced cancer cell apoptosis in multiple studies, involving various types of cancer and different cell lines. Specifically, aloe-emodin's anti-cancer properties *in vitro* emanate mainly from cell death induction and anti-proliferative processes that entail (a) increased levels of pro-apoptotic caspases, cytochrome c, p53 and p21, BAX, and free radicals, and decreased levels of anti-apoptotic BCL-2 and DNA repair enzymes; (b) decreased cyclin A, CDK2, VEGF, and NF-κβ levels; (c) cell cycle arrest in the S and G_2/M phase; and (d) decreased MMP levels and migration. A key *in vitro* finding was the increased release of cytochrome c, the molecular initiator of intrinsic apoptosis in many cancer cell lines, including: non-small cell lung carcinoma (H460) [16,17], squamous cell lung carcinoma (CH27) [18], hepatocellular carcinoma (HepG2, HCCM, and Hep3B) [24], nasopharyngeal carcinoma (NPC) [25,26], human neuroblastoma (SK-N-Be(2c) and SJ-N-KP) [27], premalignant keratinocytic (HaCaT) cells [33], skin fibroblast (Hs68) [33], epidermoid carcinoma (A431) [33], head and neck SCC (SCC25) [33], skin melanoma (A375) [33,34,36], and tongue squamous cell carcinoma (SCC-4) [40].

When co-administered with a variety of anti-neoplastic agents, aloe-emodin served as a chemosensitizer. In Merkel cell carcinoma, aloe-emodin exhibited synergistic effects with cisplatin, doxorubicin, 5-fluorouracil, and the tyrosine kinase inhibitor STI 571 [35]. Conversely, aloe-emodin demonstrated increased protection in malignant melanoma cells against doxorubicin and paclitaxel (A375) [36]. The authors proposed that aloe-emodin protects cells once they have been exposed to toxic molecules and not necessarily against chemotherapy.

Furthermore, the pharmacological efficacy of liposomal aloe-emodin was more profound versus non-encapsulated aloe-emodin, indicating that aloe-emodin may be formulated into nanoparticulate drug delivery systems to increase the distribution of the active ingredient. Increased ROS and caspase-3/7/8/9 with decreased GSH were noted in this study [33]. This particular study also demonstrated similar anti-tumor properties in additional cell lines, including premalignant keratinocytic (HaCaT), skin fibroblast (Hs68), epidermoid carcinoma (A431), head and neck SCC (SCC25), and skin melanoma (A375) cells, attesting to aloe-emodin's efficacy against a wide range of target cell types.

Additional studies are needed to gather clinical knowledge and to investigate the potential use of aloe-emodin and related compounds as adjuvants in conventional cancer treatment. For example, a study by Lissoni et al. showed that *Aloe arborescens* combined with chemotherapy improved solid tumor

regression and survival time in patients with lung, colorectal, gastric, and pancreatic cancer [42]. Insofar as aloe-emodin at higher doses was shown to inhibit chemotherapy in one isolated study, it is particularly important to further investigate the potential for drug-supplement interactions.

Several limitations of this review are noteworthy. With this area of research on aloe-emodin being fairly recent, dysregulation at the transcript and protein level was found, but the mechanisms are poorly understood. This review only focuses on *in vitro* studies, which could prove to have limited translatability to *in vivo* studies. Additionally, multiple studies noted the fact that aloe-emodin could possibly be used prophylactically. As far as we are aware, no studies have yet been undertaken to test this hypothesis. Finally, few studies to date have investigated the effects of aloe-emodin in noncancerous cell lines. Such studies are warranted to determine whether aloe-emodin exerts similar cytotoxic effects in typically slow-proliferating and quiescent cells. Cytotoxicity in cancer cells and no effects in non-malignant cells would be the desired outcome and a rudimentary gauge of a compound's target specificity (i.e., highly proliferative cancer cells).

In summary, aloe-emodin shows great promise as an anti-neoplastic agent with potential use as a synergistic and/or cytoprotective agent as part of conventional cancer treatment. Numerous *in vitro* results support this claim, yet further research is needed to elucidate the molecular mechanisms *in vivo*, as well as to investigate its potential use as a prophylactic agent clinically. Aloe-emodin may ultimately prove to be another phytonutrient with anti-cancer properties.

Disclosure

The authors declare no conflict of interests.

List of abbreviations: 14-3-3σ: regulatory protein (also known as stratifin) expressed in all eukaryotic cells, helps with translocation to cell membrane as well as signal transduction; 2-DE: 2-dimensional gel electrophoresis, used to sort out different proteins based on their size, charge, and other biochemical properties; Ac-DEVD-CHO: specific, potent, and reversible inhibitor of caspase-3; AIF: apoptosis-inducing factor; AKT: also known as protein kinase B, inhibits apoptosis; ALP: alkaline phosphatase, dephosphorylates proteins helping to break cells down; AP1: activator protein-1, a transcription factor; ATF-2: activating transcription factor-2; ATM: ataxia telangiectasia mutated; phosphorylates key regulatory proteins leading to cell cycle arrest, DNA repair, or apoptosis; ATP: adenosine triphosphate, molecule used as energy for the cell to carry out many functions in the body; ATR: ataxia telangiectasia and Rad3-related protein, checks for DNA damage and initiates repair; BAD: BCL-2-associated death promoter protein, helps to initiate apoptosis; BAG-1: BCL-2-associated anthanogene, binds to Bcl-2 and helps to prevent apoptosis; BAK: BCL-2 homologous antagonist/killer; BAX: BCL-2-associated X protein, induces apoptosis; BCL-2: B-cell lymphoma 2, inhibits apoptosis by preventing release of cytochrome c, has many different forms and can also induce apoptosis; BCL-XL: B-cell lymphoma extra-large, inhibits apoptosis by preventing the release of cytochrome c; bFGF: basic fibroblast growth factor, induces cell proliferation; BID: BH3-interacting domain death agonist, works in conjunction with BAX to become pro-apoptotic; BIK: BCL-2-interacting killer, destroys BCL-2 to induce apoptosis; BIM: BCL-2-like protein 11, part of the BCL-2 family but this protein induces apoptosis; BRCA1: breast cancer 1, a tumor suppressor gene that, if mutated, can be the cause of hereditary breast cancers; BrdU: 5-bromo-2'-desoxyuridine, a thymidine analogue used to detect cell proliferation; cAMP: cyclic adenosine monophosphate, derivative of ATP, second messenger involved in cell proliferation; CAPN2: calcium-activated neutral proteases, breaks down protein; CARP1/2: caspase-8/10-associated RING protein, inhibit caspase 8 and 10, decreasing apoptosis; CD11b/14/95: cluster of differentiation, integrin expressed on the surface of leukocytes; CDC25C: cell division cycle 25c, triggers entry into mitosis; CDK1/2: cyclin-dependent kinase 1/2, once phosphorylated it progresses the cell cycle; CDT1: chromatin licensing and DNA replication factor 1, 'licenses' DNA to flag it ready for replication; CKI: cyclin-dependent kinase inhibitors; c-MYC: Myc proto-oncogene; has a role in cell cycle progression, apoptosis, and cell transformation; CsA: cyclosporin A, caspase 8 inhibitor; Cu: copper; DAPI: 4',6-diamidino-2-phenylindole, fluorogenic probe with an affinity for A-T-rich regions of DNA; DHA: dihydroxyanthraquinone, class of molecules to which aloe emodin and structurally related compounds belongs to; DMSO: dimethylsulfoxide, polar aprotic solvent; DNA: deoxyribonucleic acid; DNA-PK: deoxyribonucleic acid-dependent protein kinase, functions during DNA repair; ELISA: enzyme-linked immunosorbent assay, used for the determination of specific proteins; ENDO G: endonuclease G, participates in caspase-independent apoptosis; ERK: extracellular signal-related kinase, part of the MAP kinase pathway, functions to induce cellular proliferation; FADD: FAS-associated protein with death domain, helps to induce formation of the death complex to carry out apoptosis; FAK: focal adhesion kinase, also known as protein tyrosine kinase 2, involved in cell adhesion and spreading; FAS: Fas cell surface death receptor, mediator of extrinsic apoptosis; G2/M: G2 phase and M phase of the cell cycle, mediates growth and mitosis/meiosis, respectively; G-CSF: granulocyte colony-stimulating factor, stimulates growth of granulocytes (white blood cells); GM-CSF: granulocyte macrophage colony-stimulating factor; GRP78: 78-kDa glucose-regulated protein, also known as binding immunoglobulin protein, chaperone protein that helps newly synthesized proteins to be formed and shaped; GSH: reduced glutathione, an endogenous antioxidant; GSSG:glutathione disulfide (oxidized form of GSH); HOGG1: human 8-oxoguanine DNA N-glycosylase 1, a DNA repair enzyme; HSP:heat shock protein, increased in cancer cells and stressed cells, necessary for cancer cell survival; IFN: interferon, a class of molecules used in immunity to confer specific immune signals; IgG: immunoglobulin G, a type of antibody in long-term immunity; IL: interleukin, class of molecules used in immunity to confer specific immune signals; IP-10: interferon gamma-induced protein 10; IκB: inhibitor of kappa B, inhibits NF-κB signal transduction; JNK:c-JUN N-terminal kinase, helps activate pro-apoptotic proteins; JNKK1: Jun N-terminal kinase kinase; MAPK: mitogen-activated protein kinase; MCC: Merkel cell carcinoma; MCL-1: myeloid cell leukemia 1, alternative splicing occurs on this gene, one splice variant induces apoptosis while the other inhibits it; MCM complex; minichromosome maintenance protein complex, helicase protein required for DNA replication; MCP-1: monocyte chemotactic protein-1; MGMT: O-6-methylguanine-DNA methyltransferase, DNA repair enzyme; MIP: macrophage inflammatory protein; MMP: matrix metallo-protease, degrades extracellular matrix, facilitates metastasis; Mn: manganese; mRNA: messenger ribonucleic acid: mTOR: mammalian target of rapamycin, protein involved in cell proliferation: mTORC2:

mTOR complex 2; NAT: N-acetyl transferase, initiates metabolism; NF-κB: nuclear factor kappa B, induces transcription of genes involved in many processes including inflammation, angiogenesis, and cell survival; NO: nitric oxide, causes vasodilation; NOXA: phorbol-12-myristate-13-acetate-induced protein 1, pro-apoptotic member of the BCL-2 family; ORC: origin recognition complex; p21: cyclin-dependent kinase inhibitor 1; p38: class of mitogen-activated protein kinases involved in cell differentiation, apoptosis, and autophagy; p53: 53 kDa protein, conserves DNA stability as a tumor suppressor; p65: REL-associated protein involved in NF-κB function; PARP: poly adenosine diphosphate-ribose polymerase, DNA repair enzyme; PCNA: proliferating cell nuclear antigen; PCR: polymerase chain reaction, used to replicate DNA sequences; PDGFbb: platelet-derived growth factor bb; PDI: protein disulfide isomerase, helps with protein folding; p-ERK: phosphorylated (activated) extracellular signal-related kinase; PI: propidium Iodide, florescent stain used to determine cell viability; PI3-K: phosphoinositide-3 kinase, enzyme involved in cell proliferation; PKA: protein kinase A, intracellular enzyme used in many pathways to sustain cell viability; PKAc: protein kinase A, catalytic subunit; PKC: protein kinase C, involved in cell proliferation; p-PERK:phosphorylated (activated) protein kinase RNA-like endoplasmic reticulum kinase, monitors cell homeostasis; PRDX: peroxiredoxin, a redox regulator; PS: phosphatidylserine, a phospholipid used as a signal for macrophages to engulf the cell during apoptosis; PUMA: P53 upregulated modulator of apoptosis, pro-apoptotic signaling protein; RAF:rapidly accelerating fibrosarcoma, protein kinase related to retroviral oncogenes, part of signal cascade aiding in cell proliferation; RANTES: regulated on activation, normal T cell expressed and secreted , also known as chemokine (C-C motif) ligand 5 (CCL5); RAS:rat sarcoma, protein involved in signal transduction; RHOA: Ras homolog gene family member A, used in actin organization to control cell morphology; RHOB: Ras homolog gene family member B, similar to RHOA; ROCK-1: Rho-associated, coiled coil-containing protein kinase 1, helps with cell morphology and migration; ROS: reactive oxygen species; RT-PCR: reverse transcription polymerase chain reaction, to form DNA from RNA and then replicate it; SAPK: stress-activated protein kinase, homologues to JNKs; SCC: squamous cell carcinoma; siRNA: small interfering ribonucleic acid, blocks protein formation (translation); SOD: superoxide dismutase, removes oxygen free radicals; SPM: spermine, involved in cell metabolism; tBID: truncated BH3-interacting death domain agonist; Thr14/Tyr15: threonine 14/tyrosine 15, ; phosphorylation of these proteins by WEE1 inhibits mitosis; TIMP-1: tissue inhibitor of metalloproteinases 1; TNF: tumor necrosis factor, cytokine causing cell death; UBE3A: ubiquitin protein ligase E3A, assists with ubiquitination or cell death; U-PA: urokinase plasminogen activator, involved in extracellular matrix degradation; VEGF: vascular endothelial growth factor; WEE1: nuclear kinase, inhibits CDK1 and the cell cycle; Z-IETD-FMK: caspase-8 inhibitor, inhibits T cell proliferation and prevents oxyhemoglobin-induced cell death; Zn:zinc; Z-VAD-FMK: carbobenzoxy-valyl-alanyl-aspartyl-[O-methyl]-fluoromethylketone, binds to the protease site of caspases and can inhibits apoptosis.

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