Therapeutic potential of esculetin in various cancer types (Review)

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Abstract. Esculetin (Esc), a coumarin derivative and herbal medicinal compound used in traditional Chinese medicine, is extracted from *Fraxinus chinensis*. Esc has shown notable potential in the inhibition of proliferation, metastasis and cell cycle arrest in various cancer cell lines. The present review is based on research articles regarding Esc in the field of carcinoma, published between 2009 and 2023. These studies have unanimously demonstrated that Esc can effectively inhibit cancer cell proliferation through diverse mechanisms and modulate multiple signaling pathways, such as Wnt/β-catenin, PI3K/Akt, MAPK and janus kinase/signal transducer and activator of transcription-3. In addition, the safety profile of Esc has been demonstrated in credible animal experiments, which has indicated Esc as an effective compound. Furthermore, the combination therapy of Esc with commonly used chemotherapeutic drugs holds great promise. The aim of the present review was to encourage further studies and applications of Esc in cancer therapy.

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Abbreviations: Esc, esculetin; IC_{50}, half-maximal inhibitory concentration; ROS, reactive oxygen species; CypD, cyclophilin D; ER, endoplasmic reticulum; IRE1, inositol-requiring enzyme 1; GRP-78, glucose regulatory protein 78; ERK, extracellular signal-regulated kinase; JAK, janus kinase; STAT3, signal transducer and activator of transcription-3; NFE2L2, NFE2-like BZIP transcription factor 2; GPX4, glutathione peroxidase 4; HO-1, heme oxygenase-1; LC3II, light chain 3 phosphatidylethanolamine conjugate; NCOA4, nuclear receptor coactivator 4; FTH1, ferritin heavy chain 1; Nrf2, nuclear factor erythroid 2-related factor 2; MMP, matrix metalloproteinase; NF-κB, nuclear factor-κB; Sp1, specificity protein 1; DR, death receptor; MEK, mitogen-activated protein kinase kinase; Tcf, T-cell factor

Key words: Esc, cancer, signaling pathways, apoptosis, traditional Chinese medicine

Contents

1. Introduction

Esculetin (also termed 6,7-dihydroxy-2-chromenoe or aesculetin; Esc) is a naturally occurring coumarin derivative (1). A notable source of Esc is Cortex Fraxini (2), a Traditional Chinese Medicine (TCM) herb recognized for its wide range of health benefits. Esc can also be derived from a variety of other plants, including *Artemisia capillaris* (3), *Citrus limonia* (4), *Viola yedoensis* (5) and *Cichorium intybus* (6).

Despite notable medical breakthroughs, cancer progression is still among the foremost causes of global morbidity and mortality (7). In 2023, cancer was the second leading cause of death globally, after heart disease. Cancer has some notable features: Self-sufficiency in growth signals, insensitivity to antigrowth signals, evasion of apoptosis, limitless replicative potential and sustained angiogenesis (8).

Esc is an active ingredient with significant medicinal value. Esc has gained attention for its therapeutic implications as an antioxidant and in diabetes, anti-inflammation and oncology (9). Studies on TCM primarily focus on the anti-inflammation and analgesic effects of Esc (10,11). However, as an increasing number of anticancer effects of Esc have been reported, future clinical research is essential. The role of Esc in modulating various cancer cell pathways and inducing apoptosis offers potential as a natural remedy against cancer. In the present review, the role of Esc in cancer is summarized.

2. In vitro anticancer properties of Esc

IC_{50} of Esc in cancer cell lines. The IC_{50} of Esc shows some differences in various cancer types. In laryngeal cancer, the

IC_{50} of Esc treatment for 72 h was 1.958 μM in Hep-2 cells (a HeLa contaminated cell line) (12). In addition, in colorectal cancer, the IC_{50} for 48 h in HT-29 cells was 55 μM (13), and the IC_{50} for 24 h in HCT116 cells was 100 μM (13). Furthermore, the IC_{50} of the designed compound in breast cancer, Esc-NO-DEAC ternary hybrid A11, was 8 nM for 48 h in MDA-MB-231 cells (14). Table I shows the different IC_{50} values for Esc treatment in various cell lines.

Colorectal cancer (CRC). In the world, CRC ranks as the third most lethal malignancy, following breast and lung cancer in mortality rates (7). Esc has shown notable anticancer properties against CRC cells, as evidenced by its ability to inhibit cell proliferation, induce apoptosis, arrest the cell cycle in the G_1 phase, reduce metastatic potential and decrease reactive oxygen species (ROS) production (15-17). Furthermore, in Esc-treated cell lines such as HCT116, HT-29 and DLD-1, inhibition of proliferation in these cell lines in a dose- and time-dependent manner was consistently observed (9-14). Choi *et al* (16) reported that Esc selectively targets cancer cells, sparing normal colonic epithelial cells. The results also showed that Esc notably suppressed anchorage-independent cell proliferation in a dose-dependent manner (16). Choi *et al* (16) and Lee *et al* (18) performed colony formation assays to visually demonstrate the inhibition of cell proliferation. In a study conducted by Li *et al* (19), the BrdU test was used, which revealed that the Esc-treated group exhibited a significantly lower number of $BrdU^+$ cells than the control group. Additionally, the induction of cellular apoptosis was also evidenced by the formation of apoptotic bodies, an increase in the percentage of cells in the sub-G_1 phase and DNA fragmentation. Esc induces ROS formation; however, treatment with an antioxidant reduced Esc-induced apoptotic cell death (19). Moreover, Esc increases mitochondrial membrane depolarization, resulting in the release of cytochrome c into the cytoplasm (17). In the study by Choi *et al* (16), annexin V/PI staining revealed that Esc promoted apoptosis in the LoVo cell line in a dose-dependent manner. Further supporting these findings, western blotting analyses indicated elevated levels of pro-apoptotic proteins, including Bax, cleaved caspase-3, -7 and -9, and poly (ADP-ribose) polymerase (PARP) in Esc-treated cells, whereas the levels of anti-apoptotic protein, Bcl-2, was decreased (16,17). Concurrently, findings such as endoplasmic reticulum (ER) staining, mitochondrial calcium overload and the upregulation of ER stress-related proteins, as mentioned in the study by Kim *et al* (20), suggest that the ER stress response is instrumental in targeting human colon cancer cells.

In addition, the antimetastatic effects of Esc on HCT116 cells have been assessed using wound healing and invasion assays. Increased E-cadherin promoter activity by Esc has been linked to reduced cell migration and invasion, with concentration-dependent inhibition of wound closure and cell invasion (15).

Using PI-stained flow cytometry, in HCT116, HT-29 and LoVo cells, Esc was shown to increase the population of cells in the G_1 phase compared with the control, indicating G_1 phase cell cycle arrest (13,16,20). Park *et al* (13) emphasized the non-altering effects of Esc on p21WAF1 and p53 levels in HCT116 cells, but a marked increase in p27KIP expression was observed, which led to the inhibition of CDK/cyclin complexes, resulting in G_1 phase cell cycle arrest. This study pioneered the discovery of crucial roles of p27KIP and cyclin/CDK in Esc-induced cell cycle arrest in cancer cell lines. Western blotting results also demonstrated increased levels of p53, p27 and p21 and decreased levels of cyclin D1 and specificity protein 1 (Sp1), which is the transcription factor of p53, p27, p21, cyclin D1 and Sp1 proteins, in a dose-dependent manner, suggesting that Esc modulates cell cycle-related proteins (13). Cell cycle arrest has also been reported in HT-29 and LoVo cell lines (HT-29 cells were treated with 55 μM Esc for 24 h; LoVo cells were treated with 300 and 600 μM Esc for 24 h) (16,20).

Gastric cancer. Gastric cancer ranks fifth for incidence and fourth for mortality among all types of cancer worldwide (7). Esc inhibits tumor cell proliferation, and only a small percentage of cell death was found in normal gastric cells, GES-1 cells, indicating its potential as a selective and effective anticancer agent (21). Triciribine (an inhibitor of Akt) and LY294002 (an inhibitor of PI3K), enhanced the pro-apoptotic effects of Esc, indicating the involvement of the PI3K/Akt pathway in Esc-induced proliferation inhibition and pro-apoptosis (22). This was consistent with the results of a previous study that observed the inhibitory effects of Esc on PI3K/Akt activation (23). Esc was also shown to exhibit dose- and time-dependent antiproliferative effects on SGC-7901 gastric cancer cells as the clonogenic potential of SGC-7901 cells was significantly reduced following exposure to Esc. The apoptosis induced by Esc treatment at concentrations of 0, 12, 48 and 96 μM for 24 h in SGC-7901 cells was confirmed using AO/EB and Annexin V/PI staining assays. Moreover, it was also demonstrated that Esc induced cell cycle arrest specifically in the G_2/M phase (21).

Liver and pancreatic cancer. Liver cancer was the third leading cause of cancer-related death worldwide in 2020, while pancreatic cancer has a poor prognosis and is the seventh leading cause of cancer-related death in both sexes (7).

Corroborating research has demonstrated that oxidative stress is closely related to ferroptosis, and ferroptosis is characterized by detrimental lipid peroxidation. Ferritinophagy increases intracellular Fe^{2+} levels, which initiates the Fenton reaction to produce ROS (H_2O_2), leading to an increase in oxidative stress and glutathione (GSH) levels, resulting in ferroptosis (24-26). The results of a study indicated that Esc elevated ROS (H_2O_2) production in HUH7 and HCCLM3 liver cancer cells in a dose-dependent manner, simultaneously diminishing intracellular free radical scavenging and lowering antioxidant activity (27). Esc treatment also led to significant proliferation inhibition, G_1-phase cell cycle arrest and mitochondrial-dependent apoptosis activation in PANC-1, MIA PaCa-2 and AsPC-1 pancreatic cancer cell lines via caspase 3, 8, and 9. Additionally, Esc treatment resulted in decreased intracellular ROS and p65-nuclear factor-κB (NF-κB) protein levels in PANC-1 cells (28). Esc also inhibits hepatitis B virus (HBV) both *in vitro* and *in vivo* (29). Therefore, Esc has been hypothesized to suppress hepatocellular carcinoma by affecting HBV DNA replication, consequently leading to cell death.

In studies on liver and pancreatic cancer, the changes in ROS levels in cancer cells treated with Esc showed pronounced contrasts. This suggests that there are two distinct

Table I. Different IC_{50} values for Esc treatment in various cell lines.

First author/s, year	Compound/agent	Cancer type	Cell line	IC_{50} for 24 h	IC_{50} for 48 h	IC_{50} for 72 h	(Refs.)
Wen *et al*, 2023	Esc-NO-DEAC ternary hybrid A11	Breast cancer	MDA-MB-231	-	8.000 nM	-	(14)
Park *et* al, 2011	Esc	Colon cancer	HT-29	-	55.000 μM	-	(13)
Park *et al*, 2011	Esc	Colon cancer	HCT116	100.000 μM	-	-	(13)
Yin *et al*, 2023	Esc	Ovarian cancer	SKOV3	223.810 μM	86.900 μM	-	(35)
Yin *et al*, 2023	Esc	Ovarian cancer	ID8	192.740 μM	86.870 μM	-	(35)
Jiang *et al*, 2021	Esc	Endometrial cancer	Ishikawa	-	95.000 μM	-	(36)
Jiang *et al*, 2021	Esc	Endometrial cancer	HEC-1B	-	142.500 μM	-	(36)
Yang *et al*, 2010	Esc	Cervical cancer	HeLa	-	-	37.800 μM	(37)
Zhang *et al*, 2019	Esc	Laryngeal cancer	Hep-2 (a HeLa contaminated cell line)	-	-	1.958 μM	(12)
Arora *et al*, 2016	Esc	Pancreatic cancer	PANC-1	-	100.000 μM	-	(28)

Esc, Esculetin; IC_{50}, half-maximal inhibitory concentration.

modes of apoptosis in these cell types. In a study conducted by Arora *et al* (28), Esc treatment decreased ROS levels, leading to mitochondrial-dependent apoptosis. Conversely, in a study by Xiu *et al* (27), Esc inflicted mitochondrial damage, which subsequently accounted for elevated ROS levels.

Breast cancer. Breast cancer is the most commonly diagnosed cancer and the leading cause of cancer-related mortality in women worldwide (7,30). The results of two studies showed that Esc and Esc-Furoxan-DEAC ternary hybrids could induce mitochondrial apoptosis through the calcium and ROS accumulation pathways, respectively, thus promoting the apoptosis of breast cancer cells (14,31). In a study by Chang *et al* (31), the first to explore the effects of Esc on Ca^{2+} in ZR-75-1 human breast cancer cells, Esc was found to induce a dose-dependent Ca^{2+} increase in these cells. The increase in Ca^{2+} was attributed to both Ca^{2+} entry and Ca^{2+} release, which were partially inhibited by the removal of extracellular Ca^{2+}. However, Esc did not induce Ca^{2+} increase in MCF-7 and MDA-MB-231 breast cancer cells. In ZR-75-1 cells, Esc decreased the mitochondrial membrane potential, increased the release of cytochrome c and activated caspase-9 and -3, indicating its involvement in the Ca^{2+}-associated mitochondrial apoptosis pathway. Esc also induced G_2/M cell cycle arrest, potentially through the upregulation of p53 and p21 and the downregulation of CDK1 and cyclin B1 proteins in ZR-75-1 cells (31).

A total of 12 new hybrid Esc compounds have been synthesized, which involved the integration of a furoxan-based NO donor and/or a mitochondria-targeting group onto the 6,7-dihydroxy structure of Esc (14). *In vitro* findings demonstrated that Esc-Furoxan-DEAC ternary hybrids can induce MDA-MB-231 cell apoptosis and halt mitosis at the G_2/M phase, with its effectiveness varying in relation to the dose. This outcome suggested that Esc-Furoxan-DEAC ternary hybrids triggers cell apoptosis via the mitochondrial pathway and that Esc has a crucial role in the Esc-Furoxan-DEAC ternary hybrid-driven enhancement of CypD levels (14).

Ovarian, endometrial and cervical cancer. Ovarian cancer ranks seventh in terms of incidence of malignant tumors and eighth in terms of the cause of cancer-related death in women worldwide (32,33), while endometrial cancer is the sixth most common cancer in women (34), and cervical cancer is the fourth most frequently diagnosed cancer and the fourth leading cause of cancer-related death in women globally (7). Despite advancements in screening and vaccination, these cancers collectively account for a substantial number of cancer-related deaths among women worldwide, highlighting the need for urgent research on prevention, early detection and therapeutic strategies.

Studies conducted by Yin *et al* (35), Jiang *et al* (36) and Yang *et al* (37) revealed that Esc inhibited the proliferation of SKOV3 ovarian cancer cells, Ishikawa and HEC-1B endometrial cancer cells, and HeLa cervical adenocarcinoma cells in a time- and dose-dependent manner, but Esc showed low cytotoxicity towards human L02 normal hepatocyte cells (35). In addition to inhibiting cell proliferation, a single cell colony formation assay showed that Esc exerted an inhibiting effect on the colony forming ability of SKOV3 ovarian cancer cells. Esc also delayed wound healing in these cells, indicating

its migration-inhibitory properties. Transwell assays also demonstrated that Esc suppressed the migration and invasion of SKOV3 cells (35,37). Furthermore, flow cytometry and western blotting analysis revealed that Esc treatment decreased the expression of cyclin D1, CDK4, CDK2 and cyclin E, suggesting its role in regulating the cell cycle in SKOV3 and HeLa cells (35). As also shown in HeLa cells with Esc treatment at 0 mM (control), or 50 mM for 12, 24 or 48 h in flow cytometry analysis, the subG_1 population, which indicates apoptotic cells, increased in a time-dependent manner. These results indicated that Esc induced cell cycle arrest in the G_2/M phase and the apoptosis of HeLa cells in a time-dependent manner (37). Esc also exhibits antimitogenic effects on ovarian, endometrial and cervical cancer cells. Treatment with Esc led to an increase in the ratio of red to green fluorescence, indicating a reduction in the mitochondrial membrane potential by Mitochondrial Membrane Potential Detection Kit (JC-10) (35). Western blotting analysis confirmed that Esc induced apoptosis, as concentration-dependent increase of apoptosis-related proteins and a decrease of anti-apoptosis proteins was induced in SKOV3 ovarian cancer cells, Ishikawa and HEC-1B endometrial cancer cells by causing mitochondrial membrane potential collapse. Additionally, Esc induced ROS (O_2^-) generation as demonstrated by flow cytometry and fluorescence microscopy (35,36). This resulted in an early increase in mitochondrial ROS (O_2^-) production, leading to the opening of the mitochondrial permeability transition pore and a subsequent drop in mitochondrial membrane potential (35,37). These observations demonstrated that the overproduction of ROS (O_2^-) triggers mitochondrial permeability transition pore opening, leading to decreased membrane potential and a subsequent release of cytochrome c from the intermembrane space into the cytosol, resulting in activation of the caspase cascade and apoptotic cell death. These findings suggest that Esc has potential anticancer effects and may be used as a treatment for cancer.

Lung cancer. Lung cancer is the leading cause of cancer-related mortality worldwide for both sexes and is frequently diagnosed in the advanced stages when treatment alternatives are limited (7). In total, two pivotal research articles on lung cancer have studied the antiproliferative effects of Esc and its role in attenuating cell invasion and epithelial-mesenchymal transition (EMT) using the cell lines, Lewis lung carcinoma (LLC) and A549 (a non-small cell lung cancer line) (38,39). These studies observed that Esc suppressed the viability of LLC and A549 cells in a dose- and time- dependent manner. In addition, 40 and 80 μM Esc led to reduced colony formation of LLC cells in soft agar (38). In the study by Li *et al* (39), the treatment of A549 cells with Esc significantly and dose-dependently reduced cell invasive capabilities. Analyses using reverse transcription-quantitative (RT-qPCR and western blotting) in Esc (5 and 20 μM)-treated A549 cells incubated for 24 h indicated an upregulation of E-cadherin mRNA and protein levels, along with a downregulation of vimentin and Snail, which regulate the EMT of A549 cells. However, Zhu *et al* (38) did not report any changes in the invasion capabilities of LLC cells treated with Esc. This may be due to the differential effects of Esc in different cell lines. Further research is still required to elucidate the detailed mechanisms.

Oral and laryngeal cancer. Oral squamous cell carcinoma (OSCC) is ranked eighteenth in terms of cancer incidence and is the most common head and neck squamous cell carcinoma worldwide (7). Laryngeal cancer is the most common malignancy in otolaryngology and comprises 30-40% of head and neck malignancies worldwide (40).

In studies by Cho *et al* (41) and Kok *et al* (42), the viabilities of both the HN22 and HSC4 OSCC cell lines and the SAS human oral cancer cell line were inhibited following Esc treatment in a time- and dose-dependent manner, as demonstrated by MTT assay. Furthermore, fluorescence-activated cell sorting analysis and 4',6-diamidino-2-phenylindole staining of HN22 and HSC4 cells treated with Esc at concentrations of 5, 10 and 20 μM indicated that Esc hindered cell proliferation by inducing cell cycle arrest in the G_0/G_1 phase and promoting apoptosis. Additionally, an Annexin V assay suggested early apoptosis was induced by Esc in these cell lines (41). Western blotting and enzyme-linked immunosorbent assays were performed to examine the apoptotic pathways activated by Esc, which revealed that Esc did not trigger the mitochondria-mediated apoptotic pathway in SAS cells, but instead triggered the death receptor (DR) pathway (42).

In a cell proliferation assay, as a positive control, the IC_{50} of cisplatin against laryngeal cancer cells was 2.15 μM for Hep-2 cells (a HeLa contaminated cell line), while Esc was 1.958 μM. In Hep-2 laryngeal cancer cells, 72 h Esc treatment inhibited cell proliferation, migration and invasion, alongside ROS accumulation and G_1/S cell cycle arrest (12).

Prostate and bladder cancer. Prostate cancer is the fourth most common cancer worldwide, while bladder cancer is the tenth most commonly diagnosed cancer worldwide (7). In studies by Turkekul *et al* (43) and Han *et al* (44), it was demonstrated that cancer cell survival was inhibited after 48 h of treatment with Esc at concentrations of 0, 100 and 200 μM. In addition, the induction of apoptosis, G_1 phase cell cycle arrest and metastasis can be summarized from the experimental results. The detailed molecular mechanisms underlying Esc-induced apoptosis in PC3 cells were investigated using RT-qPCR. Post-Esc treatment resulted in a significant increase in the mRNA levels of TNF-receptor 1, caspase-8, caspase-3, Bax and cytochrome c, alongside a notable reduction in Bcl-2 mRNA. Additionally, western blotting analysis demonstrated a dose-dependent increase in cytochrome c protein levels in PC3 cells treated with Esc (43). Consistently, Han *et al* (44) confirmed that Esc induced the mitochondria-dependent apoptosis of 5637 cells. Esc-treatment caused a loss of mitochondrial membrane potential, the release of cytochrome c and the activation of caspase-9 and -3.

Human acute myeloid leukemia. Human acute myeloid leukemia remains a rare malignancy, but it occupies close to one-third of all diagnosed leukemia (45). Although a number of treatment modalities are currently available for leukemia, numerous malignancies lack efficient treatment owing to multidrug resistance (46). Gong *et al* (47) investigated the anticancer properties of Esc in human acute myeloid leukemia cancer cells, including peripheral blood mononuclear cells as a normal cell line. Transmission electron microscopy and western blotting demonstrated the increase of apoptosis induction, the

reduction of the Bcl-2/Bax ratio and the suppression of cancer cell migration after Esc treatment.

3. In vivo anticancer properties of Esc

To the best of our knowledge, 8 articles pertaining to *in vivo* cancer experiments involving Esc, covering 7 types of cancer have been published (12,15,18,22,27,35,36,38). Table II shows the function of Esc in *in vivo* experiments. All articles found an effective tumorigenesis and metastasis inhibition by Esc with minimal toxicity to normal cells, which is a hallmark advantage of TCM. In addition, the combination of Esc with paclitaxel demonstrated potent anticancer properties. Among the 8 studies, only 1 focused on orthotopic mouse tumors in xenograft models (15).

A total of two *in vivo* experiments (15,19) with distinct designs examined Esc in colon cancer but noted the effects on different molecular mechanisms: The Wnt/β-catenin/T-cell factor (Tcf) and Axin2/Snail1/E-cadherin pathways. In one study, HCT-116 cell xenografts in female athymic nude mice were used, while the other study involved orthotopic transplantation into the mouse ceca. Following surgical resection, isolation and homogenization of the primary culture, L-2 cells were reintroduced orthotopically. Treatments were administered intraperitoneally and the mice were euthanized after 1 week for tumor analysis. Biochemical analysis showed a decrease in Axin2, c-Myc, cyclin D1 and Ki-67 expression and an increase in E-cadherin expression, indicating the antitumor and antimetastatic properties of Esc (15).

In a study by Lee *et al* (18), *Xenopus* embryos were used to demonstrate that Esc inhibits the β-catenin/Tcf-dependent signaling pathway, which was supported by a previous study. In an LLC lung cancer investigation using 80 μM Esc treatment fro 24 h, it was demonstrated that the mechanism involved downregulating Wnt targeted genes and suppressing NF-κB. Although *in vivo* experiments did not show changes in related molecules, Esc was found to reduce lung cancer tumor size and weight (38).

Furthermore, during the examination of laryngeal cancer, Esc not only significantly inhibited tumor proliferation by 80%, but also impeded the JAK/STAT3 signaling pathway *in vivo*, as evidenced by western blotting analysis showing reduced levels of phosphorylated (p-)JAK1, p-JAK2 and p-STAT3.

An animal model experiment using an MGC-803 cell (a HeLa contaminated cell line) xenograft nude mouse model for gastric cancer research showed increased caspase-3 expression and decreased Bcl-2, Ki-67, IGF-1, p-PI3K and p-Akt expression following treatment with Esc, suggesting the induction of apoptosis in gastric cancer cells via the IGF-1/PI3K/Akt signaling pathway. Furthermore, Esc induces lipid peroxidation and iron accumulation in tumor tissue. In hepatocellular carcinoma cells, Esc suppresses tumor proliferation, reduces antioxidant levels, increases ferritinophagy-related protein levels and activates ferritinophagy to promote ferroptosis. Esc treatment downregulates the export of Bcl-xl and X-linked inhibitor of apoptosis protein (XIAP) mRNA from the nucleus to the cytoplasm, as confirmed by nuclear RNA and cytoplasmic RNA isolation experiments. Esc and paclitaxel together inhibit endometrial cancer cell proliferation *in vivo* and have potential as a clinical treatment (22).

4. Mechanism of Esc in different cancer types

Since Esc is a plant-derived compound, its apoptotic effects on cancer cell lines have been extensively studied. Studies have verified its role in mitochondrial apoptosis across multiple cancer types including colon (17), gastric (22,48), ovarian (35), cervical (37) and bladder cancers (44), and its capacity to induce ER stress (20) and ferritinophagy (27).

The role of Esc in cancer cells was revealed to be related to apoptosis (Fig. 1). Apoptosis is a form of programmed cell death that is triggered by DRs and the mitochondrial pathway (49). Table III shows the different apoptotic pathway changes following Esc treatment in cancer cells. A total of 6 studies (17,22,35,37,44,48) on mitochondrial apoptosis have all reported increased levels of pro-apoptotic proteins (including Bax and Bak) and decreased levels of anti-apoptotic proteins (including Bcl-2 and Bcl-xl) in Esc-treated cancer cells. In addition, transfer of cytochrome c from the mitochondria to the cytoplasm, upregulation of related proteases, such as caspase-3, caspase-9 and PARP, and increased ROS production were observed. These results indicate that Esc-induced apoptosis is mediated by the mitochondrial pathway in various cell lines (17,22,35,37,44,48).

The ER is essential for protein synthesis and folding, and calcium homeostasis (50). Esc treatment resulted in mitochondrial Ca^{2+} accumulation in HT-29 cells, suggesting that Esc triggered Ca^{2+} mobilization from the ER. Malfunctioning ER leads to the accumulation of unfolded proteins, triggering the unfolded protein response (UPR). During this process, glucose regulatory protein 78 (GRP-78) activates three key transmembrane signaling molecules: PERK, inositol-requiring enzyme 1 (IRE1) and activating transcription factor 6 (ATF6). As demonstrated through western blotting and qPCR analyses, with the Esc treatment at a concentration of 55 μM, GRP-78, p-PERK, p-IRE1 and cleaved ATF6 were upregulated in HT-29 cells (17). Prolonged ER stress leads to ATF6 relocation to the Golgi apparatus for processing by Sp1 and Sp2 proteases. Cleaved ATF6 then migrates to the nucleus, enhancing the expression of UPR target genes (51). Furthermore, X-box binding protein 1 (XBP1) is spliced by IRE1α, removing a 26-nucleotide intron from XBP1 mRNA, which results in a potent transcription activator that boosts the expression of UPR genes and activates the ER-associated degradation pathway (52). During UPR, the continued synthesis of proteins becomes unsustainable; hence, protein kinase R phosphorylates eIF2α to inhibit protein translation. In HT-29 cells, 55 μM Esc treatment has been shown to cause a time-dependent increase in the expression of spliced eIF2α, XBP1 and cleaved ATF6. The induction of IRE1, PERK and ATF6 during ER stress upregulates expression of the transcription factor, CHOP (53). In addition, caspase-12 is the key initiator of ER stress-induced apoptosis (54). Esc-treatment of colon cancer cells increases the expression of both CHOP and caspase-12 (20).

In terms of ferritinophagy, it was demonstrated in a study by Xiu *et al* (27) that Esc enhanced the production of ROS (H_2O_2) in HUH7 and HCCLM3 cells in a dose-related manner, while simultaneously reducing intracellular free radical scavenging and antioxidant activities. NFE2-like BZIP transcription factor 2 (NFE2L2), a known regulator of ferroptosis through its regulation of glutathione peroxidase 4 (GPX4), ferritin

Table II. Function of Esc in *in vivo* experiments.

First author/s, year	Cancer type	Targets/regulators and signaling pathways	Cell line	Animal model	Routes of administration	Outcome	(Refs.)
Lee *et al*, 2013	Colorectal cancer	Wnt-β/catenin/Tcf	HCT-116 -	Xenograft in Female athymic nude mice (6 to 7-week-old) Embryo of Xenopus	Intraperitoneally -	↓Ki-67, cyclin D1 and c-Myc Confirmed the Wnt-β-catenin-Tcf pathway-not tumor related	(18)
Kim *et al*, 2018	Colorectal cancer	Axin2/Snail1/E-cadherin pathway	HCT-116	Orthotopically inoculated in Male nude mice (BALB/c-nu), 4-6 weeks old,	Intraperitoneally	↓Axin2, c-Myc and cyclin D1; ↑E-cadherin; ↓Ki-67.	(15)
Wang *et al*, 2017	Gastric cancer	IGF-1/PI3K/Akt	MGC-803	Xenograft in nude mice model	Gavage	↑caspase-3; ↓Bcl-2 and Ki-67; ↓IGF-1, p-PI3K and p-Akt	(22)
Xiu *et al*, 2023	Liver cancer	NCOA4 pathway-mediation ferritinophagy	HUHT	Xenograft in nude mice (BALB/c)	Unspecified	↑Fe^{2+} and MDA level in serum; ↓Ki67; ↓NFE2L2, GPX4 and HO-1; ↑LC3II and NCOA4; ↓FTH1	(27)
Yin *et a*l, 2023	Ovarian cancer	JAK2/STAT3	ID8	Xenograft in BALB/c nude mice	Unspecified	↓Ki67	(35)
Jiang *et al*, 2021	Endometrial cancer	via hnRNPA1 to downregulate Bcl-xl and XIAP	HEC-1B	Xenograft in BALB/c nude mice	Intra-tumoral	↓hnRNPA1, Bcl-xl and XIAP	(36)
Zhu *et al*, 2018	Lung cancer	Downregulating Wnt-targeted genes and suppressing NF-κB	LLC	Xenograft in BALB/c nude mice (6 to 8-week-old female)	Subcutaneously	No significant change in body weight was observed between the two groups.	(38)
Zhang *et al*, 2019	Laryngeal cancer	JAK/STAT3 activation	Hep-2 (a HeLa contaminated cell line)	0.2 ml Hep-2 cell (concentration: $6x10^5$/ml) Suspension was subcutaneously injected into the axilla of BALB/c male nude mice	Oral	↓p-JAK1, p-JAK2 and p-STAT3	(12)

Esc, esculetin; ERK1/2, extracellular signal-regulated kinase 1/2; MDA, malondialdehyde; JAK, janus kinase; STAT3, signal transducer and activator of transcription-3; NFE2L2, NFE2-like BZIP transcription factor 2; GPX4, glutathione peroxidase 4; LC3II, light chain 3 phosphatidylethanolamine conjugate; HO-1, heme oxygenase-1; NCOA4, nuclear receptor coactivator 4; FTH1, ferritin heavy chain 1; NF-κB, nuclear factor-κB; Tcf, T-cell factor; ↑, upregulate; ↓, downregulate.

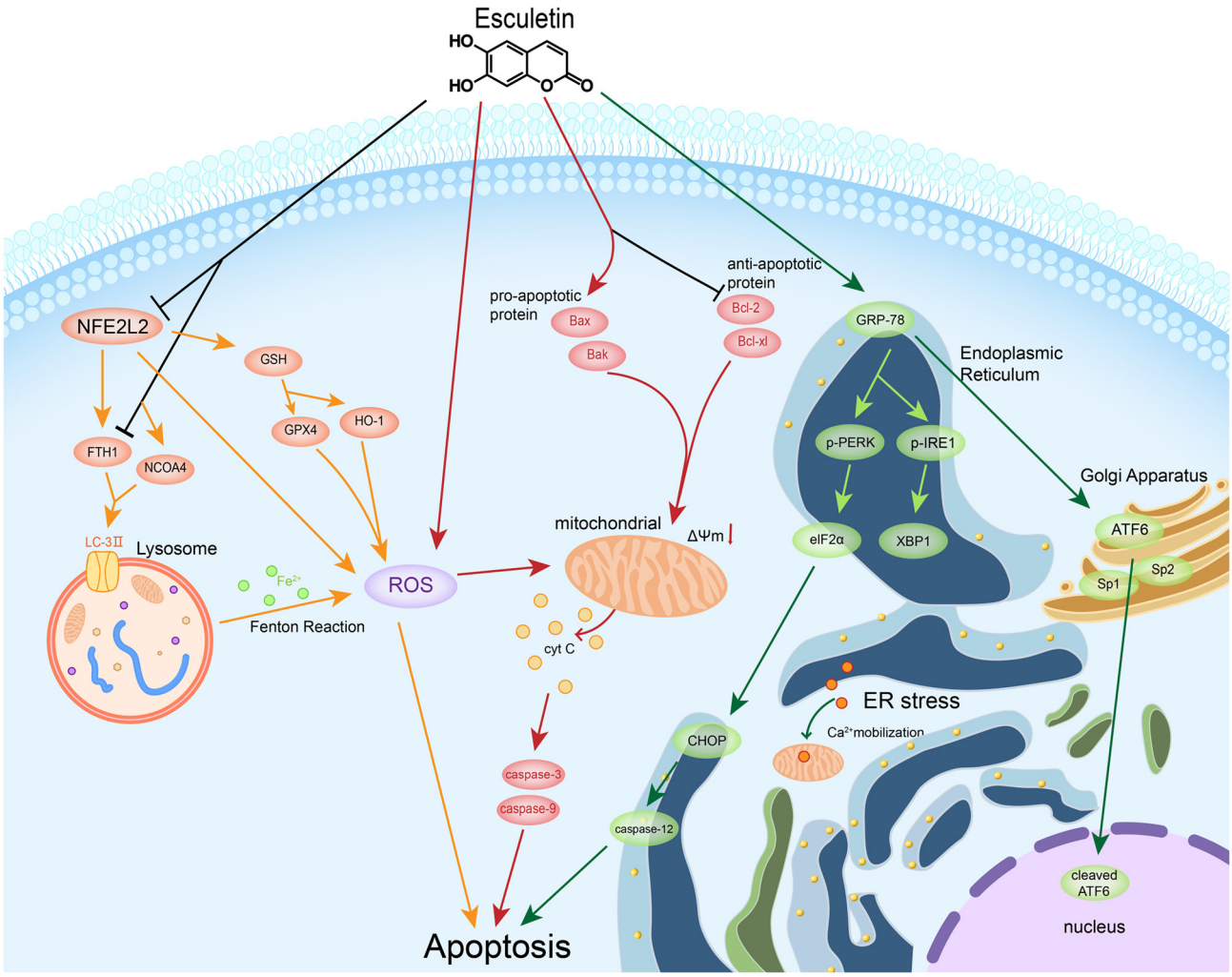

Figure 1. The role of Esc in cancer cells was revealed to be related to apoptosis, which is governed by the mitochondrial pathway, ER stress and ferritinophagy. Esc induces mitochondrial apoptosis by stimulating cyt C release, activating caspase-3 and caspase-9 and enhancing ROS generation across various cell lines. ROS further modulate the mitochondrial membrane potential and amplify pro-apoptotic molecules in the cytosol. ER stress, which is vital for cellular processes, responds to Esc by increasing mitochondrial Ca^{2+} levels in cancer cells. ER stress activates the UPR, activating signaling molecules (such as PERK, IRE1 and ATF6) and transcription factors (such as XBP1 and CHOP) that regulate UPR genes and trigger ER stress-induced apoptosis, particularly caspase-12 activation. Esc elevates ROS levels, suppresses antioxidants and downregulates NFE2L2, fostering ferroptosis in liver cancer cells by promoting NCOA4/LC3II/FTH1-mediated ferritinophagy. Esc-induced ferritinophagy enhances intracellular iron levels and inhibits liver cancer cell proliferation. Esc, Esculetin; ER, endoplasmic reticulum; ROS, reactive oxygen species; UPR, unfolded protein response; IRE1, inositol-requiring enzyme 1; ATF6, activating transcription factor 6; NCOA4, nuclear receptor coactivator 4; FTH1, ferritin heavy chain 1; GSH, glutathione; GPX4, glutathione peroxidase 4; HO-1, heme oxygenase-1; GRP-78, glucose regulatory protein 78; XBP1, X-box binding protein 1; p-, phosphorylated; cyt C, cytochrome c; NFE2L2, NFE2-like BZIP transcription factor 2; Sp1/2, specificity protein 1/2.

heavy chain 1 (FTH1) and heme oxygenase-1 (HO-1), was found to be downregulated by Esc both *in vivo* and *in vitro*, which led to suppressed levels of HO-1, GPX4 and GSH, and the ability to scavenge hydroxyl radicals in serum, thereby altering the antioxidant equilibrium in tumor tissues (55). There is an increase in Fe^{2+} levels, lipid peroxidation and iron deposition in tumor tissues, which are indicative of ferroptosis. Nuclear receptor coactivator 4 (NCOA4), known to bind to FTH1 and associate with LC3 protein on the autophagosome membrane, targets ferritin complexes for autolysis, a key process in ferroptosis. The inhibition of NCOA4 expression or autophagy prevents ferritinophagy and ferroptosis (56). A previous study indicated that Esc increases NCOA4, LC3-II and lysosome levels, while decreasing FTH1 expression, thus promoting ferritinophagy (57). Furthermore, using gene silencing and overexpression techniques, it was demonstrated that Esc suppressed FTH1 expression, and increased NCOA4 and LC3II expression post-silencing. Esc also enhanced the co-expression of NCOA4 and LC3II, facilitating ferritinophagy. Therefore, by activating ferritinophagy, Esc increases intracellular free iron levels, suggesting its potential to suppress liver cancer cell proliferation by inducing ferritinophagy via the NCOA4/LC3II/FTH1 signaling pathway (57). Although experiments have shown that Esc activates ferritinophagy to inhibit the proliferation of HUH7 and HCCLM3 cells, the potential of Esc beyond liver cancer cells and the involvement of other pathways in promoting ferritinophagy remain uncertain. Further research is needed to determine whether Esc can induce ferritinophagy through alternative pathways, and whether it has inhibitory effects on other types of cancer.

Table IV shows different signaling pathway changes following Esc treatment in cancer cells. Esc, a compound with

Table III. Different apoptotic pathway changes following Esc treatment in cancer cells.

First author/s, year	Type of apoptosis	Cancer	Cell line	Molecular change	(Refs.)
Kim *et al*, 2015	Mitochondrial apoptosis	Colon cancer	HT-29	↑pro-apoptotic protein, Bax; ↓anti-apoptotic protein, Bcl-2 ↑ROS; ↑active (cleaved) caspase-9, caspase-3 and cleaved PARP (a target protein of caspase-3)	(17)
Pan *et al*, 2015	Mitochondrial apoptosis	Gastric cancer	MGC-803	↑caspase-9 and caspase-3; ↑cytochrome c in the cytosol; ↑Bax and Bak (pro-apoptotic regulatory proteins); ↓Bcl-2 and Bcl-xl (anti-apoptotic regulatory proteins); ↑ROS	(48)
Wang *et al*, 2017	Mitochondrial apoptosis	Gastric cancer	MGC-803	↑The release of cytochrome c from mitochondria into cytoplasm; ↓the ratio of Bcl-2/Bax, caspase-9 and caspase-3 activity; ↓IGF-1, p-PI3K and p-Akt; ↓cell apoptotic ratio and caspase-3 and caspase-9 activity; ↑Bcl-2/Bax ratio; ↓cytochrome c from mitochondria to cytoplasm	(22)
Yin *et al*, 2023	Mitochondrial apoptosis	Ovarian cancer	SKOV3	↓Mitochondrial membrane potential; ↑Bax, cytochrome c, cleaved caspase 9 and cleaved caspase 3; ↓Bcl-2; ↑Bax/Bcl-2 ratio; ↑ROS	(35)
Yang *et al*, 2010	Mitochondrial apoptosis	Cervical cancer	HeLa	The loss of mitochondrial membrane potential; ↑caspase-3/7/9; ↑the cytosolic level of cytochrome c; ↑ROS	(37)
Han *et al*, 2023	Mitochondrial apoptosis	Bladder cancer	5637	↓In the mitochondrial membrane potential; ↑cytochrome c release to the cytoplasm and the activation of caspase-9 and caspase-3; ↑the ratio of BAX/Bcl-2 proteins	(44)
Kim *et al*, 2015	Endoplasmic reticulum stress	Colon cancer	HT-29	↑GRP-78, p-PERK and p-IRE1 and cleaved ATF6; PERK → ↑p-eIF2α; IRE1α→ ↑ XBP1; ↑cleaved ATF6; ↑spliced XBP1 mRNA; ↑CHOP and cleaved caspase-12	(20)
Xiu *et al*, 2023	Ferritinophagy	Liver cancer	HUH7 and HCCLM3	↓Antioxidant proteins (NFE2L2, GPX4 and HO-1); ↑ROS; ferrostatin-1, a ferroptosis inhibitor, bafilomycin and 3-MA could inhibit esculetin-mediated cell death; ↑Fe^{2+}; ↑NCOA4 and LC3-II in HUH7 and HCCLM3 cells, but ↓FTH1 ↓ p62 expression; Bafilomycin (Baf) and Esc co-treatment, ↑ p62 expression; ↓LC3-II expression, suggested Esc can promote autophagy flux	(27)

Esc, esculetin; ROS, reactive oxygen species; IRE1, Inositol-requiring enzyme 1; GRP-78, glucose regulatory protein 78; ERK1/2, extracellular signal-regulated kinase 1/2; MDA, malondialdehyde; JAK, janus kinase; STAT3, signal transducer and activator of transcription-3; NFE2L2, NFE2-like BZIP transcription factor 2; GPX4, glutathione peroxidase 4; LC3II, light chain 3 phosphatidylethanolamine conjugate; HO-1, heme oxygenase-1; NCOA4, nuclear receptor coactivator 4; FTH1, ferritin heavy chain 1; p-, phosphorylated; ↑, upregulate; ↓, downregulate.

potential anticancer properties, promotes apoptosis and inhibits the proliferation of tumor cells (Fig. 2). The Wnt/β-catenin signaling pathway, frequently cited in studies regarding Esc, stands out due to its crucial role in the mechanisms affected by Esc. Finding small compounds that block Wnt signaling is therefore a possible cancer prevention or therapy approach. In the colon cancer cell lines, HCT116 and SW480, as well as in the LLC lung cancer cell line, post-treatment with Esc led to a downregulation of proteins such as c-Myc and cyclin D1. Specifically, in the HCT116 cell line, Esc showed a direct binding affinity with β-catenin (18), and in both HCT116 and SW480 cells, a downregulation of the β-catenin/Tcf complex

Table IV. Different signaling pathway changes following Esc treatment in cancer cells.

First author/s, year	Cancer type	Cell line	Signaling Pathways	Molecules altered	(Refs.)
Park *et al*, 2011	Colorectal cancer	HCT116	Ras/ERK1/2	↑Ras, ERK1/2, p27KIP	(13)
Lee *et al*, 2013	Colorectal cancer	HCT116, HCT15 and DLD-1	β-catenin/Tcf	Esc directly binds to β-catenin; ↓β-catenin/Tcf complex, c-Myc and cyclinD1	(18)
Kim *et al*, 2015	Colorectal cancer	HT-29	JNK, p38 MAPK, and ERK	↑p-JNK, p-p38 MAPK and p-ERK	(17)
Li *et al*, 2018	Colorectal cancer	SW480	Wnt/β-catenin	↓β-catenin/Tcf complex, c-Myc and cyclin D1	(19)
Kim *et al*, 2018	Colorectal cancer	HEK293, CT116, SW480, LS174T and HCT15	Axin2/ E-cadherin	Formation of E-cadherin/β-catenin complexes; ↑E-cadherin; ↓Snail1 (a transcriptional repressor of the E-cadherin promoter); ↑microRNA-34a expression (a known regulator of EMT and negative regulator of Axin2 post-transcription)	(15)
Zhang *et al*, 2021	Gastric cancer	MGC-803	PI3K/Akt/ mTOR	Activity of mTOR and Akt remained almost unchanged; ↓p-mTOR, p-AKT, p-PI3K and PI3K	(21)
Wang et al, 2017	Gastric cancer	MGC-803	IGF-1/PI3K/ Akt	↓IGF-1, p-PI3K and p-Akt; ↓cell apoptotic ratio and caspase-3 and caspase-9 activity; ↑Bcl-2/Bax ratio; ↓cytochrome c from mitochondria to cytoplasm; combination treatment with Esc and triciribine or LY294002 could further enhance the apoptotic effect of Esc	(22)
Xiu *et al*, 2023	Liver cancer	HUH7 and HCCLM3	NCOA4/ LC3II/FTH1	↓Antioxidant proteins (NFE2L2, GPX4 and HO-1); ↑ROS; ferrostatin-1, a ferroptosis inhibitor, bafilomycin and 3-MA could inhibit Esc-mediated cell death; ↑Fe^{2+}; ↑NCOA4 and LC3-II in HUH7 and HCCLM3 cells, but ↓FTH1 ↓ p62 expression; Bafilomycin (Baf) and ESCM co-treatment, notably ↑ p62 expression; ↓LC3-II expression, suggested Esc could promote autophagy flux	(27)
Arora *et al*, 2016	Pancreatic cancer	PANC-1	KEAP1/Nrf2	↑Nuclear accumulation of Nrf2; ↑NQO1, a direct target of Nrf2	(28)
Yin *et al*, 2023	Ovarian cancer	SKOV3	JAK2/STAT3	↓p-JAK2 and STAT3; ↓MMP2 and MMP9	(35)
Jiang *et al*, 2021	Endometrial cancer	Ishikawa	hnRNPA1/ Bcl-xl and XIAP	↓cellular concentration of hnRNPA1; ↓p-Akt, and anti-apoptotic proteins, Bcl-xl and XIAP; ↑nucleoplasmic RNA ratio of Bcl-xl and XIAP mRNA after si-hnRNPA1 transfection	(36)
Zhu *et al*, 2018	Lung cancer	Murine LLC	Wnt and NF-κB	↓c-Myc and cyclin D1; ↓NF-κB	(38)

Table IV. Continued.

First author/s, year	Cancer type	Cell line	Signaling Pathways	Molecules altered	(Refs.)
Cho *et al*, 2015	Oral squamous cell carcinoma	HN22 and HSC4	Sp1	↓Sp1; ↑cell cycle arrest-related proteins such as p27 and p21; ↓cyclin D1, Mcl-1 and survivin (proteins related to cell proliferation and survival); ↓apoptosis-related proteins, BID and PARP; ↑Bax, cleaved caspase-3 and cleaved PARP; ↓anti-apoptotic protein, Bcl-xl	(41)
Kok *et al*, 2009	Oral oncology	SAS	DR5	↑DR5	(42)
Zhang *et al*, 2019	Laryngeal cancer	Hep-2 (a HeLa contaminated cell line)	JAK/STAT3	↓p-STAT3, pJAK1 and pJAK2	(12)
Han *et al*, 2023	Bladder cancer	5637	MEK/ERK	↓p-ERK and p-MEK; no change in the ERK expression	(44)

Esc, esculetin; ROS, reactive oxygen species; IRE1, inositol-requiring enzyme 1; GRP-78, glucose regulatory protein 78; ERK1/2, extracellular signal-regulated kinase 1/2; MDA, malondialdehyde; JAK, janus kinase; STAT3, signal transducer and activator of transcription-3; NFE2L2, NFE2-like BZIP transcription factor 2; GPX4, glutathione peroxidase 4; LC3II, light chain 3 phosphatidylethanolamine conjugate; HO-1, heme oxygenase-1; NCOA4, nuclear receptor coactivator 4; FTH1, ferritin heavy chain 1; Nrf2, nuclear factor erythroid 2-related factor 2; MMP, matrix metalloproteinase; NF-κB nuclear factor-κB; Sp1, specificity protein 1; DR5, death receptor 5; MEK, mitogen-activated protein kinase kinase; ↑, upregulate; ↓, downregulate.

was observed (18,19). Notably, in LLC lung cancer cells, in addition to the downregulation of cell cycle proteins, the downregulation of NF-κB was also regulated by Esc (38). In a study by Kim *et al* (15), it was revealed that Esc impeded the transcriptional activity of β-catenin by preventing its nuclear translocation. In CRC cells, Esc regulates E-cadherin expression by attenuating Snail1 levels, and also modulates the expression of Snail1 via a nuclear GSK3β-dependent degradation pathway, thereby influencing EMT processes. This modulation is achieved by altering Axin2 and miRNA-34a levels (15).

The MAPK family, which includes p38 MAPK, extracellular-regulated protein kinase (ERK) and c-Jun-N-terminal kinase (JNK), are essential mediators of signal transmission from the cell membrane to the nucleus and are triggered by a variety of external stimuli. Many physiological processes, such as cell proliferation, differentiation and apoptosis, are regulated by MAPKs (58). In HCT116 cells, Esc achieves G_1 phase cell cycle arrest by upregulating Ras, ERK1/2 and p27KIP8 (13), while in HT-29 and 5637 cell lines, involvement of the MAPK signaling pathway is evident (17,44). In colon cancer, apoptosis is induced by Esc through MAPK activation (17), whereas in bladder cancer, its antitumor effects depend on the mitogen-activated protein kinase kinase (MEK)/ERK pathway (44). In contrast to the HT-29 cell line, where Esc treatment activates JNK, p38 MAPK and ERK, leading to the upregulation of p-ERK, the response of bladder cancer cells to Esc exposure is characterized by the downregulation of p-MEK and p-ERK.

Separate studies have highlighted the regulatory effect of Esc on the JAK/STAT3 signaling pathway in ovarian and laryngeal cancers. Yin *et al* (35) observed a reduction in p-JAK2 and p-STAT3 by Esc, leading to decreased matrix metalloproteinase (MMP)2 and MMP9 levels and the promotion of matrix breakdown in SKOV3 cells. While in the Hep-2 cells (a HeLa contaminated cell line), Zhang *et al* (12) showed that Esc decreased the expression of p-STAT3, p-JAK, and p-JAK2.

Moreover, other studies have investigated other signaling pathways that are influenced by Esc. In an article by Arora *et* al (28), it was demonstrated that Esc activated the antioxidant responsive element (ARE) pathway in pancreatic cancer cells by disrupting the nuclear factor erythroid 2-related factor 2 (Nrf2)-kelch-like ECH-associated protein 1 (KEAP1) interaction. Esc activates the Nrf2/ARE pathway by binding to the KEAP1 protein, leading to the suppression of cancer cell proliferation, which is mediated through the ROS-sensitive transcription factor, NF-κB, emphasizing the potential efficacy of Esc in the targeted treatment of pancreatic cancer. Additionally, Jiang *et al* (36) showed that Esc directly binds to heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1). This interaction is crucial for modulating key cellular components, including the expression of p-Akt and anti-apoptotic proteins such as BCL-xl and XIAP. In endometrial cancer (36), Esc has been shown to increase the nucleocytoplasmic RNA of BCL-xl and XIAP mRNA through binding to hnRNPA1, as observed both *in vitro* and *in vivo*. In HN22 and HSC4 OSCC cell lines, Esc treatment decreased

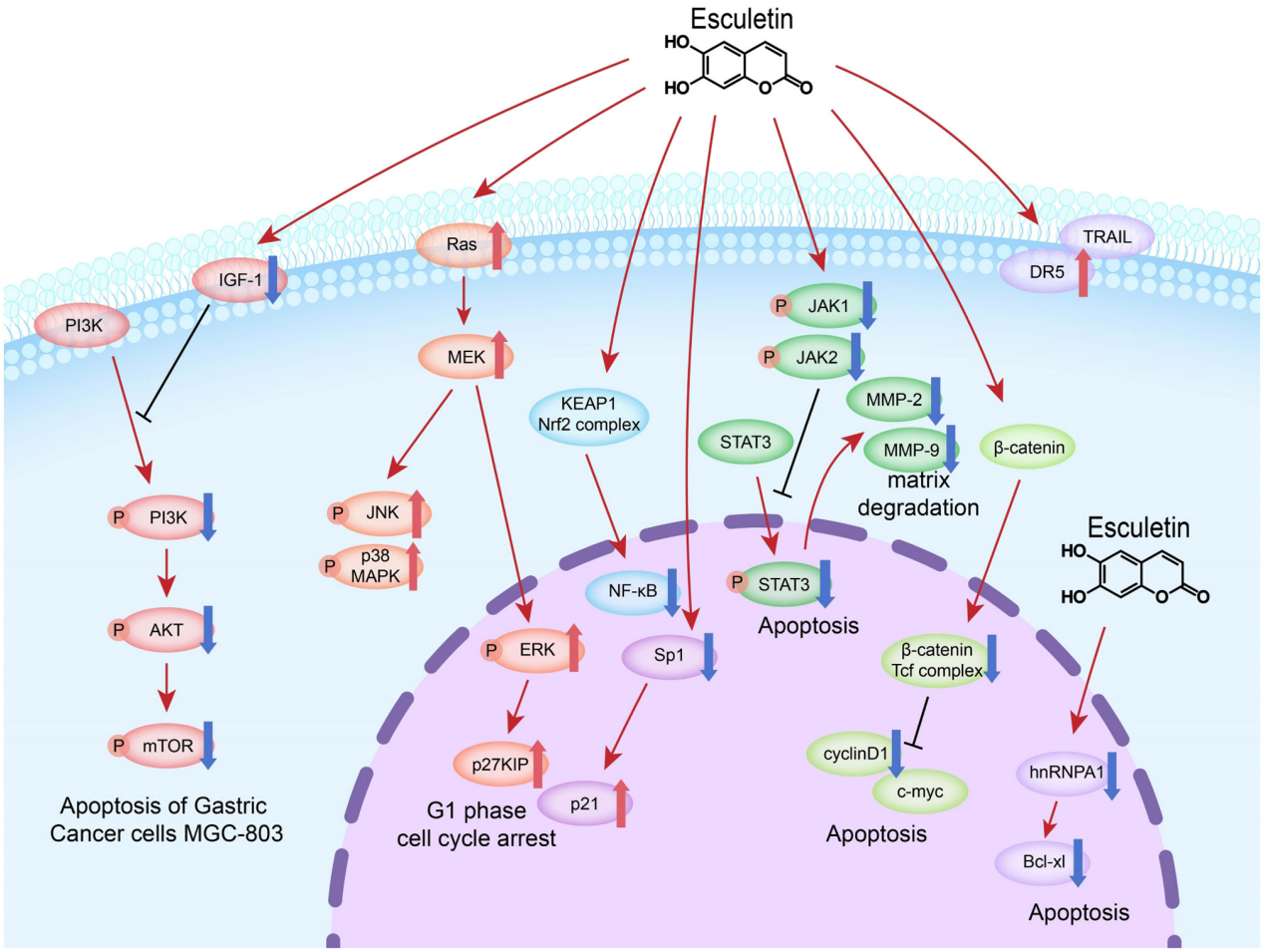

Figure 2. Esc, a compound with potential anticancer properties, promotes apoptosis and inhibits the proliferation of tumor cells. These effects are mediated by various molecular mechanisms involving different signaling pathways. In the PI3K/Akt pathway, Esc modulates the upstream molecule, IGF-1, to regulate the PI3K/Akt pathway in gastric cancer cells. Esc also targets downstream mTOR molecules to induce apoptosis and cell cycle arrest. Esc induces G_1 phase cell cycle arrest by upregulating Ras, ERK1/2 and p27 KIP8, and activates MAPK signaling to induce apoptosis in colon and bladder cancer cells. Esc also regulates NF-κB in lung cancer cells. Esc activates the ARE pathway in pancreatic cancer cells by disrupting the Nrf2-KEAP1 interaction. Esc decreased Sp1 expression in oral squamous cell carcinoma cells and enhances TRAIL-induced apoptosis in oral cancer cells by upregulating DR5. Esc regulates the JAK/STAT3 pathway in ovarian and laryngeal cancers, leading to decreased expression of MMP2 and MMP9. In the Wnt/β-catenin pathway, Esc downregulates proteins such as c-Myc and cyclin D1, binds directly to β-catenin and inhibits the β-catenin/Tcf complex. Esc binds to hnRNPA1 in endometrial cancer, affecting Bcl-xl expression. These pathways highlight the diverse mechanisms through which Esc inhibits cancer proliferation and suggest its potential as a targeted therapy for various cancer types. Esc, esculetin; ERK, extracellular signal-regulated kinase; JAK, janus kinase; STAT3, signal transducer and activator of transcription-3; Nrf2, nuclear factor erythroid 2-related factor 2; MMP, matrix metalloproteinase; NF-κB, nuclear factor-κB; Sp1, specificity protein 1; DR, death receptor; MEK, mitogen-activated protein kinase kinase; hnRNPA1, heterogeneous nuclear ribonucleoprotein A1.

Sp1 expression, leading to proliferation arrest and apoptosis of these cells (41). By contrast, within the context of oral cancer, Esc increased TRAIL-induced apoptosis in the SAS human oral cancer cell line. This enhancement was achieved through the upregulation of DR5 (42), highlighting the differential mechanisms of action across the various cellular environments related to oral cancer.

5. Discussion and future perspectives

Unlike widely used chemotherapeutic agents, Esc is a TCM compound extracted from natural agents and possesses numerous advantages, including low cytotoxicity, the capability to affect various oncogenic pathways and novel bioactive structures. In some studies, chemotherapeutic drugs such as cisplatin, 5-fluorouracil and irinotecan, were used as positive controls. Studies involving *in vivo* experiments have suggested that administration of Esc has no significant effect on the weight of mice and does not exhibit hepatotoxicity or nephrotoxicity (15,18,38).

The present review summarized 10 articles that determined the IC_{50} values of Esc in cancer cells. A novel compound, Esc-NO-DEAC ternary hybrid A11, designed for triple-negative breast cancer has an IC_{50} as low as 8 nM. However, the effects of Esc on other cancer cells were in the micromolar range, demonstrating its efficacy. Additionally, Esc showed its best effect in Hep-2 laryngeal cancer cells (a HeLa contaminated cell line) with a 72-h IC_{50} value as low as 1.958 μM, which is notably different from other cancer types, indicating the need for more research on the ability of Esc to inhibit laryngeal cancer cells effectively. Kim *et al* (15) found that the IC_{50} values of Esc and cisplatin differed by ~20 times in a number of colon cancer cell lines, suggesting that Esc, as a compound, is still far from clinical application

and requires more experimental studies to explore its mechanisms in cancer cells or in combination with paclitaxel in endometrial cancer (36). Therefore, further studies on the resistance, sensitivity and toxicity of Esc in combination with existing chemotherapy drugs are crucial. Xenograft animal models are mainly used for *in vivo* experiments, but one relevant study utilized orthotopic transplantation to better simulate the *in vivo* tumor environment (15). In the HCT116 colon cancer cell line, tumor inhibition reached 64%, whereas in the Hep-2 laryngeal cancer cell line (a HeLa contaminated cell line), the inhibition rate was as high as 80% (12,18). Due to the different administration methods (subcutaneous injection and gavage) used in the two experiments, the comparison was not sufficiently rigorous, reflecting differences between the various cancer types, similar to the reported IC_{50} values. Further *in vivo* experiments are required to explore the antitumor properties of Esc.

In the reviewed studies, assessment of the biological behavior focused on the ability of Esc to inhibit cancer cell proliferation and promote cancer cell apoptosis, with some studies mentioning G_1 phase cell cycle arrest. Only one study has examined the role of Esc in promoting ferroptosis in liver cancer cells (27). Meanwhile, Esc has been shown to affect multiple signaling pathways in cancer cells, the most common being the Wnt/β-catenin, PI3K/Akt, MAPK and JAK/STAT3 pathways. Furthermore, topics such as the tumor microenvironment, cellular autophagy and pyroptosis still have research potential in Esc-related studies. In the field of oncology, research on the mechanisms of Esc through micro (mi)RNA or exosomes remains unexplored. Therefore, further exploration of the role of ESC in these areas is warranted.

Although Esc has shown promising anticancer effects in many tumor studies, its absence as a frontline cancer drug worldwide raises questions. There are concerns regarding issues such as dosage and the fact that its positive effects have only been observed in cell experiments and xenograft models, with only one study involving orthotopically transplanted tumors (15). To the best of our knowledge, no studies have used a patient-derived xenograft (PDX) model to study Esc, and ethical concerns have hindered clinical trials. Therefore, while Esc has demonstrated potential in *in vitro* and *in vivo* experiments using cancer cell lines, further research is needed to prove its efficacy in studies closer to human tumors.

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Availability of data and materials

Not applicable.

Authors' contributions

ML, JW, YH, XY and MW were responsible for writing the original draft. YS and FG helped to write the manuscript and were responsible for visualization of mechanism diagrams. SZ reviewed and edited the manuscript. PL conceptualized the manuscript and was responsible for supervision. Data authentication is not applicable. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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