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Use of coumarins as complementary medicine with an integrative approach against cervical cancer: background and mechanisms of action

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Abstract. – Cervical cancer is characterized by the cellular transformation caused by Human Papillomavirus (HPV), favoring cell proliferation, migration, invasion, and metastasis. Cervical cancer is conventionally treated with radiation therapy, and chemotherapy focused on the destruction of tumor cells. However, chemoresistance and low selectivity between tumor and non-tumor cells have been reported, causing side effects in patients. Metabolites of natural origin have shown selectivity against tumor cells, suggesting their use for reducing the side effects caused by drugs used in conventional therapy. Among these compounds, several natural coumarins stand out, such as auraptene, scopoletin, osthole, and praeruptorin, of which antiproliferative, anti-migratory, and anti-invasive activity have been reported. Auraptene, scopoletin, osthole, and praeruptorin show a cytotoxic or antiproliferative effect on cervical tumor cells, arresting the cell cycle by inducing the overexpression of negative regulators of the cell cycle, or inducing cell death by increasing the expression of pro-apoptotic proteins and decreasing that of anti-apoptotic proteins. On the other hand, auraptene, scopoletin, and praeruptorin inhibit the capacity for migration, invasion, and metastasis of cervical tumor cells, mainly by inhibiting the expression and activity of matrix metalloproteinase-2 and -9. The PI3K/Akt signal pathway appears to be central to the anti-tumor activity of the coumarins analyzed in this review. In addition, auraptene, osthole, and praeruptorin are useful in sensitizing tumor cells to radiotherapy or chemotherapeutic molecules, such as FOLFOX, cisplatin, or DOX. Coumarins offer an excellent possibility for developing new drugs as complementary medicine with an integrative approach against cervical cancer.

Key Words:
Cervical cancer, Coumarins, Auraptene, Scopoletin, Osthole, Praeruptorin.

Abbreviations

AIF: apoptosis-inducing factor; Apaf-1: apoptosis protease-activating factor-1; Bcl-2: B-cell lymphoma-2; Bcl-xL: B-cell lymphoma-extra-large; DOX: doxorubicin; ECM: extracellular matrix; ERK: extracellular-signal-regulated kinase; FOXO: forkhead box O; GSDME: gasdermin E; GSK3: glycogen synthase kinase-3; HO-1: heme oxygenase-1; IKKα: inhibitory-kappaB kinase alpha; LC3-II: light chain 3-II; LDH: lactate dehydrogenase; NF-κβ: nuclear factor-kappa β; MDA: malondialdehyde; MMP: matrix metalloproteinase; NQO1: NAD(P)H quinone oxidoreductase 1; Nrf-2: nuclear factor erythroid 2-related factor 2; PARP1: poly(ADP-ribose) polymerase 1; PI3K: phosphatidylinositol 3-kinase; PINK1: PTEN-induced putative kinase 1; ROS: reactive oxygen species; SKP2: S-phase kinase-associated protein 2; TPA: 12-*O*-tetradecanoylphorbol-13-acetate.

Introduction

Cervical cancer is the fourth most common female malignancy worldwide. There are 569,847 new cases of cervical cancer and approximately 311,365 deaths annually[1]. This pathology initially develops as premalignant lesions, caused mainly

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by the human papillomavirus (HPV); high-risk HPV (HR-HPV) promotes cell transformation, favoring the proliferation, migration, invasion, and metastasis of tumor cells[2,3].

Currently, the conventional therapy against cervical cancer continues to be chemotherapy and radiotherapy, in addition to surgery and cryotreatments, depending on the stage of the disease[4,5]. Chemotherapy uses anti-tumor cell drugs such as cisplatin, carboplatin, and doxorubicin (DOX), to prevent cell division and proliferation. In contrast, radiotherapy uses high-energy electromagnetic waves to alter DNA and cause tumor cell death[6]. However, these treatments fail in most patients due to tumor resistance and the life expectancy of patients is not always good. In addition to being nonselective for tumor cells and affecting nontumor cells, such treatments cause side effects such as immunosuppression, hair loss, fatigue, diarrhea, and vomiting, affecting the health of patients[7,8]. Therefore, it is necessary to search for complementary therapies against cervical cancer.

Studies have shown that some compounds of natural origin are pharmacologically active molecules with anti-tumor activity and a low incidence of side effects, which is why they can be the basis of new cancer therapies. The anti-tumor activity of metabolites has been reported in extracts of various plant species, particularly against cervical cancer[9-12]. These compounds include several natural coumarins recognized for their anti-tumor activity in different types of cancer[13,14], without being cytotoxic nontumor cells[15,16], thus, they may be a good complement to cancer therapy.

This review compiles information regarding the anti-tumor activity of the most studied coumarins in cervical cancer, to analyze and describe the mechanism of action reported and the potential use of coumarins as complementary therapy against cervical cancer. Information was collected from original publications or reviews up to 20 years old (2001-2021), using keywords such as coumarins, cancer, cervical cancer, and anti-tumor therapy **(Supplementary Figure 1)**. Only scientific articles in which the analysis of results showed statistically significant differences (p <0.05) were selected; coumarins showed an anti-tumor effect in experimental models of cervical cancer such as in cell lines and *in vivo* models.

Anti-Tumor Activity of Coumarins

Coumarins (2H-1-benzopyran-2-one) are secondary metabolites present in edible and medicinal plants in a free state or in the form of glycosides[17]. These metabolites are substances derived from 1,2-benzopyrones and comprise a benzene ring attached to a pyrone ring; their basic chemical structure is 2H-1-Benzopyran-2-one (Figure 1)[18]. Coumarins have been reported to possess different biological activities, including antioxidants[19], antidiabetic[19], antimicrobial[20], anti-inflammatory[21,22], and cardiac protection[23]. Moreover, several coumarins have been recognized for their anti-tumor activity in different types of cancer, including cervical cancer[24-27]. Table I presents characteristics of the most studied coumarins with activity against cervical cancer, such as auraptene, scopoletin, osthole (OST), and praeruptorin.

Auraptene

Auraptene (7-geranyloxycoumarin) is a prenyloxycoumarin extracted by distilling various plant species such as orange blossom, *Citrus* genus, and Rutaceae family[28-30], which has been reported to have anti-inflammatory, antibacterial, antioxidant, and anticancer effects[31,32]. Auraptene has inhibitory and chemo-preventive effects on the proliferation, tumorigenesis, and growth of several cancer cell lines, targeting different signaling pathways such as cytokines, genes modulating cellular proliferation, growth factors, transcription factors, and apoptosis[33]. Auraptene at doses of 500 and 1000 mg/kg for two weeks in mice has been shown to prevent the proliferation of lung cancer cells[34], with a significantly decreased viability in HT29 colon cancer cells at a concentration of 20 µg/mL for 72 h[16]; likewise, at a concentration of 2.5-40 µM for 72 h, it inhibited the growth of HCT-116 cells resistant to FOLFOX, combined with chemotherapy with folinic acid, fluorouracil, and oxaliplatin, while in MCF-7 and MDA-MB-231 breast cancer cells, auraptene presented an IC_{50} of 70 µM and 60 µM, respectively[15]. The most relevant finding of these studies was that auraptene caused cytotoxic damage in tumor cells without altering the viability of nontumor cells[15,16], suggesting that auraptene is a

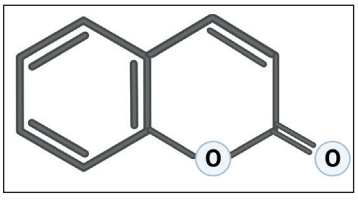
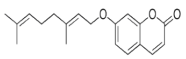
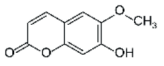
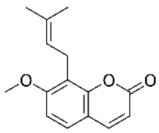
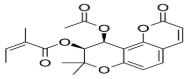


Figure 1. Chemical structure of coumarins. Coumarins are organic heterocycles, and their nucleus is represented by benzo-α-pyrone (2*H*-1-benzopyran-2-one).

Table I. Characteristics and biological activity of the most studied coumarins in cervical cancer.

Coumarin	Class	Subclass	Structure and molecular formula	Biological activity	Authors/Year
Auraptene	Simple	Prenyloxycoumarin	 $C_{19}H_{22}O_3$	Cytotoxic Anti-migration Anti-invasion Anti-metastasis	Lee et al[37] Tanaka et al[38] Jamialahmadi et al[35]
Scopoletin	Simple	Methoxylated	 $C_{10}H_8O_4$	Cytotoxic Antiproliferative Anti-invasion Anti-metastasis	Cai et al[47] Tian et al[27] Sheng et al[49]
Osthole	Simple	Prenyloxycoumarin	 $C_{15}H_{16}O_3$	Cytotoxic Antiproliferative Anti-migration Anti-invasive Anti-chemoresistance and radiotherapy sensitizer	Huangfu et al[72] Huangfu et al[57] Liang et al[61] Lu et al[63] Wang et al[58] Su et al[26] Che et al[60]
Praeruptorin	Complex	Pyranocoumarin	 $C_{21}H_{22}O_7$	Cytotoxic Antiproliferative Anti-migration Anti-invasive Anti-metastasis	Liang et al[89] Lin et al[90] Liu et al[91] Wu et al[92]

good complement against cancer without causing alterations in nontumor cells.

Regarding cervical cancer, auraptene has been shown to have an anti-tumor effect on HeLa cells. In one study, after 24 h of exposure, it significantly reduced cell viability, with an IC_{50} of 47.93 μM[35]. In another study, auraptene cytotoxicity was reported in HeLa cells with an IC_{50} values of 13.33 μg/mL and 13.87 μg/mL at 24 h and 48 h of exposure, respectively[36]. Until now, the cytotoxic mechanism of auraptene in cervical cancer has not been reported. The results in HeLa cells are similar to those found in several tumor cell lines, including A2780 ovarian cancer, Jurkat T cell leukemia, MCF-7 breast cancer, KYSE-30 esophageal carcinoma, PC3, and DU145 prostate cancer, and SNU-1 gastric cancer. The toxicity of auraptene is due to its pro-apoptotic effect by activating pro-apoptotic proteins p53 and Bax and caspases-8, -9, and -3, and due to the decrease in the expression of anti-apoptotic proteins Bcl-2, Bcl-XL; the depolarization of the mitochondrial membrane, and the proteolytic cleavage of poly (ADP-ribose) polymerase 1 (PARP1), a DNA repair protein[14,36,37]. In B16BL6 mouse models with melanoma, dietary supplementation with auraptene suppressed lung metastasis in a dose-dependent manner[38]. Antimetastatic activity was associated with the antioxidant activity of auraptene, which reduces the plasmatic levels of lipid peroxidation products, including malondialdehyde (MDA), as well as the immunostimulatory action of auraptene, which improves macrophage and lymphocyte functions in mice[38,39]. Similar mechanisms are likely to develop in cervical cancer cells; however, further studies are required to elucidate the mechanisms involved in the cytotoxic activity of auraptene in cervical cancer models.

On the other hand, in HeLa cells, auraptene at concentrations of 50-100 μM for 6-24 h reduced migration and invasion capacity[35]. Decreased migration and invasion of HeLa cells exposed to auraptene were related to a concentration-dependent inhibition of the expression and activity of matrix metalloproteinases (MMP) -2 and -9. Likewise, in studies investigating other tumor lines, it has been shown that auraptene represses the expression of pro-MMP-2 and pro-MMP-9 (Figure 2)[35,40]. The MMP family, including pro-MMPs, are enzymes necessary in extracellular matrix (ECM) degradation that promote cell invasion and migration through the epithelial-mesenchymal transition[41]. Therefore, auraptene could be a promising chemotherapeutic metabolite for preventing the invasiveness of cervical cancer cells.

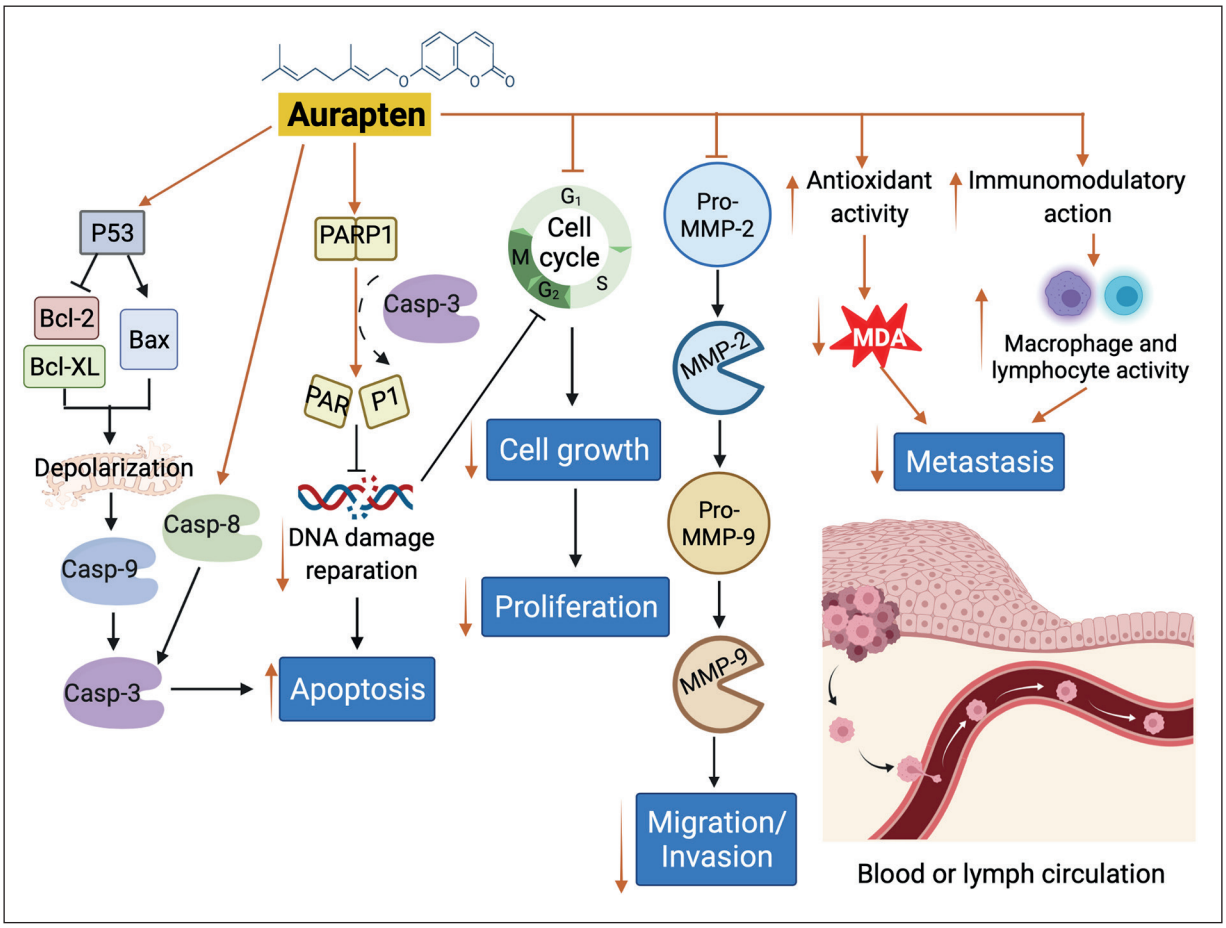


Figure 2. Mechanisms of action of auraptene on proliferation and invasiveness in cervical cancer cells. Auraptene induces cell cycle arrest in the G1 phase, which inhibits cell growth and proliferation. Auraptene induces apoptosis by activating p53, which represses the expression of the anti-apoptotic proteins Bcl-2 and Bcl-XL and promotes the activity of the pro-apoptotic protein Bax, causing depolarization of the mitochondrial membrane. Consequently, the release of cytochrome c and endonucleases favor the activation of caspase-8, -9, and -3 that, in the end, cause nuclear fragmentation and cell death. Auraptene induces an increase in the proteolytic cleavage of PARP1, probably by caspase-3, decreasing DNA reparation, which is associated with cell cycle arrest or apoptosis. On the other hand, auraptene inhibits the expression and activity of MMP-2 and -9, reducing the capacity for migration, invasion, and metastasis of cervical tumor cells. Orange lines and arrows indicate the effect of auraptene on molecules or cellular processes: up arrow—increase; down arrow—repression.

Scopoletin

Scopoletin (6-methoxy-7-hydroxycoumarin) is a coumarin phytoalexin biosynthesized by several medicinal plants in the *Scopolia* genus of the Solanaceae family and the *Artemisia, Brunfelsia, Solanum,* and *Mallotus* species. It is found abundantly in a variety of plants like noni, carrot, grapefruit, and sweet potato. Some studies reported anti-inflammatory, antidiabetic, antioxidant, and cytotoxic activity in different breast, lung, and cervical cancer cell lines[42-46].

Scopoletin derivatives showed cytotoxicity and antiproliferative activity with IC_{50} of 16.5-24.2 μM in breast adenocarcinoma cells (MDA-MB -231), showing no effect on nontumor cells[47]. Another study reported that hybrid compounds of scopoletin and cinnamic acid showed antiproliferative activity in tumor cell lines MCF-7, MDA-MB-231, A549, HCT-116, and HeLa. One of the compounds showed even better activity than doxorubicin (DOX), a chemotherapeutic drug suppressing lung tumor growth without causing weight loss in female C57BL/6 mice, which were used as study models[48]. The anti-tumor activity observed in scopoletin hybrids suggests a good panorama for their use in therapy against cancer, including cervical cancer, based on the design and synthesis of new coumarin-based molecules, with other molecules such as cinnamic acid.

Further, scopoletin also inhibits the proliferation of various cervical cancer cell lines such as DoTc2,

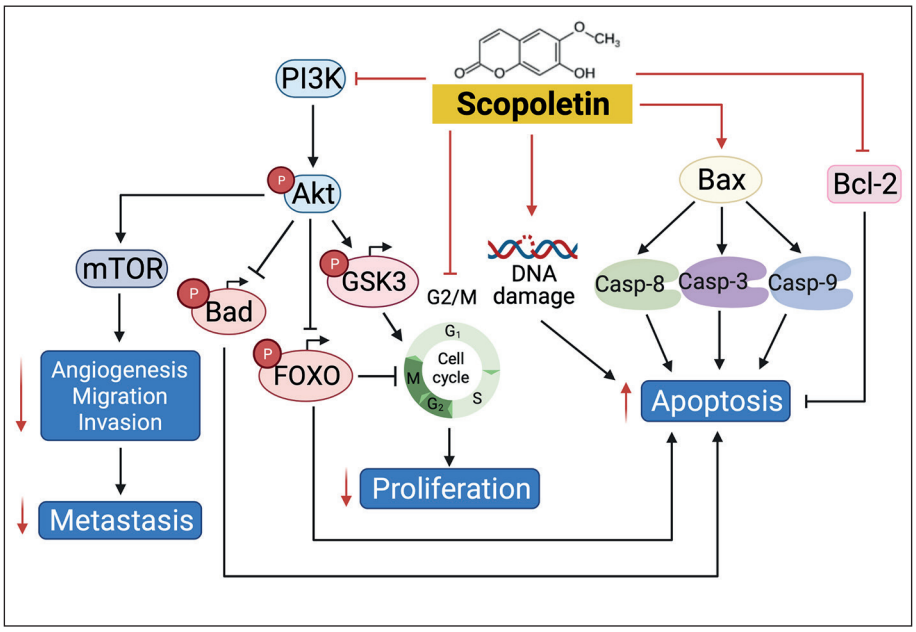


Figure 3. Mechanism of anti-tumor action of scopoletin in cervical cancer cells. The anti-tumor and chemoresistance sensitizing activity of scopoletin is due to the inhibition of the PI3K/Akt pathway, which alters the expression of regulatory proteins such as Bad, FOXO, and GSK3, thus repressing cell survival and proliferation. Inhibition of the PI3K/Akt pathway also suppresses mTOR, reducing the invasiveness of tumor cells. Scopoletin further causes DNA damage in tumoral cells and regulates the expression of pro-apoptotic (Bax and caspases -3, -8, and -9) and anti-apoptotic (Bcl-2) proteins, triggering apoptosis. Red lines and arrows indicate the effect of scopoletin on molecules or cellular processes: up arrow—increase; down arrow—repression.

SiHa, HeLa, and C33A with an IC_{50} of 7.5-25 µM, and inhibits the migration capacity of HeLa cells at concentrations of 7.5, 15, and 30 µM[27]. Another study[49] observed that scopoletin has cytotoxic activity in these cells with an IC_{50} of 73.78 µM. The anti-tumor effect of scopoletin in HeLa cells is due to the induction of cell death by apoptosis, related to DNA damage, the increased expression of pro-apoptotic proteins Bax and caspase 3, 8, and 9, and the decrease in the expression of anti-apoptotic proteins Bcl-2. Scopoletin also induced a cell cycle arrest in the G2/M phase, stopping cell growth and proliferation by inhibiting phosphorylation and blocking the PI3K/Akt signaling pathway[27]. The PI3K/Akt pathway is considered one of the most important in the positive regulation of various types of cancer because it phosphorylates and modulates the expression of genes such as mTOR, Bad, FOXO, and GSK3 that regulate cell survival, proliferation, and metastasis. Blocking this pathway sends the cell to apoptosis, inhibits the cell cycle, and inhibits essential cellular processes in cancer such as proliferation, angiogenesis, invasion, migration, and metastasis[50]. Evidence suggests that scopoletin could have significant potential in cancer therapy by reducing cell growth and invasiveness, particularly against cervical cancer (Figure 3).

Osthole

Osthole (7-methoxy-8-isopentenoxycoumarin; OST), also known as osthol, belongs to simple coumarins, originates from the phenylpropanoid pathway and is present in several medicinal plants such as *Cnidium monnieri* and *Angelica pubescens*[11,51]. Many experiments have suggested that osthole exhibited multiple biological activities covering anti-inflammatory[21], neuroprotective[52], osteogenic[53], cardiovascular protective[23], antimicrobial[54], antiparasitic[55], and anti-tumor activities[25,56]. Osthole inhibits cell proliferation[56], inducing cell cycle arrest[56], promoting apoptosis[57], inhibiting cell migration and invasion[58], overcoming chemoresistance[26]. It also induces the production of reactive oxygen species[26], promoting anti-tumor immune responses[11,59], triggering DNA damage[60], and inducing autophagy and pyroptosis[61]. The antitumor effects of osthole have been observed in various kinds of tumor cells, including breast cancer[25], ovarian carcinoma (OC)[62], endometrial carcinoma (EC)[63], head and neck squamous cell carcinoma (HNSCC)[64], hepatocellular carcinoma (HCC)[65], human gastric cancer[56], esophageal squamous cell carcinoma (ESCC)[66], renal cell carcinoma (RCC)[67], nasopharyngeal cancer (NPC)[68], lung cancer[69], leukemia[70], osteo-

sarcoma (OS)[58], human melanoma[71], glioma[72], and cervical cancer cells[26].

In SiHa, CaSki, C-33A, and HeLa cervical cancer cell lines, osthole has been shown to inhibits cell viability, proliferation, migration, invasion, and induce cell death in a concentration- and time-dependent manner. It also promotes cell DNA damage induced by radiation and acts as a sensitizer of chemoresistance to cisplatin, notably reducing cell proliferation and inducing apoptosis[11,26,57,60,61]. Likewise, using female BALB/c nude mice as *in vivo* models, co-treatment of osthole with cisplatin showed a better effect in reducing tumor growth compared with the cisplatin alone group[26]. The effect of osthole in reducing cervical cancer cell migration and invasion was attributed to the modulation of gene expression in cell epithelial-mesenchymal transition (EMT) markers, including upregulated epithelial markers E-cadherin and β-catenin and downregulated mesenchymal markers N-cadherin, vimentin and MMP-9. Osthole co-treatment with radiation enhanced DNA damage in cervical cancer cells and inhibited DNA damage repair inhibiting phosphorylation of the ataxia-telangiectasia mutated gene (ATM). Osthole combined with radiation inhibits p-ATM, a protein activated when cells suffer DNA double-strand breaks; osthole prevents the phosphorylation of γH2AX and, therefore, blocks DNA damage repair[60]. Activation of the nuclear factorκB pathway occurs in irradiated tumor cells and has been shown to induce cancer cell resistance to cisplatin[73]. Osthole also inhibits NF-κB signaling, attenuating the activation of IKKα and decreasing the translocation of p50/p65 into the cell nucleus[60]. The effect of osthole as a sensitizer of chemoresistance to cisplatin has been further attributed to blocking the PI3K/Akt signaling pathway, which represses the expression of Nrf-2, an oncogenic transcriptional factor associated mainly with chemoresistance; it plays a critical role in protecting cells against oxidative stress through the regulation of antioxidant systems in tumor cells, such as NQO1, HO-1, and GCLC, which are overexpressed in various types of cancer, including cervical cancer[26,74]. The role of these antioxidant systems is to prevent oxidative damage to cells; their inhibition can alter the redox balance in cancer cells and lead to cell death[75]. The repression of Nrf-2 by osthole reduced the expression of these antioxidants in cervical cancer models and increased ROS concentrations[26]. In fact, the anti-tumor effect of osthole has been related to the triggering of apoptosis or necrosis due to high ROS concentrations[26,57,61,74,76].

In HeLa cells exposed to osthole, high concentrations of ROS were observed, inducing the rupture of the cell membrane and the release of LDH, the depolarization of the mitochondrial membrane, and DNA damage[57]. Damage to DNA increases the expression of the tumor suppressor gene p53, which activates the PUMA and NOXA proteins that code for the pro-apoptotic proteins Bax and Bak. The expression of these proteins induces the release of cytochrome C and Apaf-1 from the mitochondrial membrane. Subsequently, cytochrome C binds to Apaf-1 and procaspase-9, forming the apoptosome that activates caspase-9; this, in turn, activates caspase-3, triggering the last phases of apoptosis[77]. Secondary necrosis is also activated in HeLa cells exposed to osthole, which increases the levels of cleaved caspase-3 and the subsequent proteolytic cleavage of GSDME – a member of the gasdermin family that contains a cytotoxic N-terminal domain to form pores and induce membrane permeabilization, which causes cell death by necrosis/pyroptosis[61,76]. Secondary necrosis by osthole can also be triggered by ROS overproduction and DNA damage, leading to overactivation of PARP1[57]. It has been reported that extensive PARP1 activation leads to the depletion of NAD^+ from the cytosol and accumulation of poly (ADP-ribose) in the nucleus; thus, ADP-ribose can be transported out of the nucleus and induce the release of AIF from the mitochondria, whereby AIF translocates to the nucleus, causing DNA fragmentation and, hence, cell death[78]. Osthole also activates caspase-3 during mitophagy, a selective form of autophagy that maintains mitochondrial homeostasis by eliminating damaged mitochondria[79], increasing the expression and activity of PINK1/Parkin and LC3-II proteins[77]. PINK1 accumulates in the outer mitochondrial membrane, to respond to mitochondrial depolarization and recruits Parkin. PINK1 and Parkin interact with LC3-II to mark mitochondria that will be degraded and thus trigger mitophagy (Figure 4)[57,80].

Evidence shows that osthole could be a potential alternative against cancer or a promising adjuvant to decrease radioresistance and chemoresistance in cervical cancer. However, more studies are still needed to fully understand its mechanism of action.

Praeruptorin

Praeruptorin is the most important active component of *Peucedani radix* and the root of *Peucedanum praeruptorum Dunn*. These species are of a medicinal herb commonly used in traditional Chinese medicine[81]. Praeruptorins were found

to be responsible for various pharmacological properties such as calcium antagonist activity[82], anti-inflammatory action[83], cardiac protection[84], hepatoprotection[82], and anti-tumor[85-87].

Praeruptorin is an angular type of pyranocoumarin and is classified into types A (Pra-A), B (Pra-B), and C (Pra-C). Several studies have reported that all three types of praeruptorin possess anticancer activity[85-89]. In SGC7901 gastric cancer cells, both Pra-A and Pra-B at concentrations of 10-100 μM for 24 h showed antiproliferative activity and increased LDH release from mitochondria in a concentration-dependent manner. The antiproliferative effect was improved with the combined treatment of Pra-A and DOX, compared with DOX alone [89]. This suggests that Pra-A could sensitize tumor cells to the action of DOX, to observe the desired effects against tumor cells with lower concentrations of the chemotherapeutic.

Pra-B at concentrations of 10-30 μM for 24 h significantly reduced the migration and invasion of renal cells 786-O and ACHN in a concentration-dependent manner[90]. At the same concentrations and exposure time in NSCLC and A549 lung cancer cells, Pra-C suppressed the cell proliferation, colony formation, migration, and invasion of NSCLC cells and inhibited invasion and migration in A549 cells in a concentration-dependent manner[91]. The above findings provide valuable information on the potential use of Pra-A, -B, and -C alone or in combination with other drugs to optimize therapy against various types of cancer.

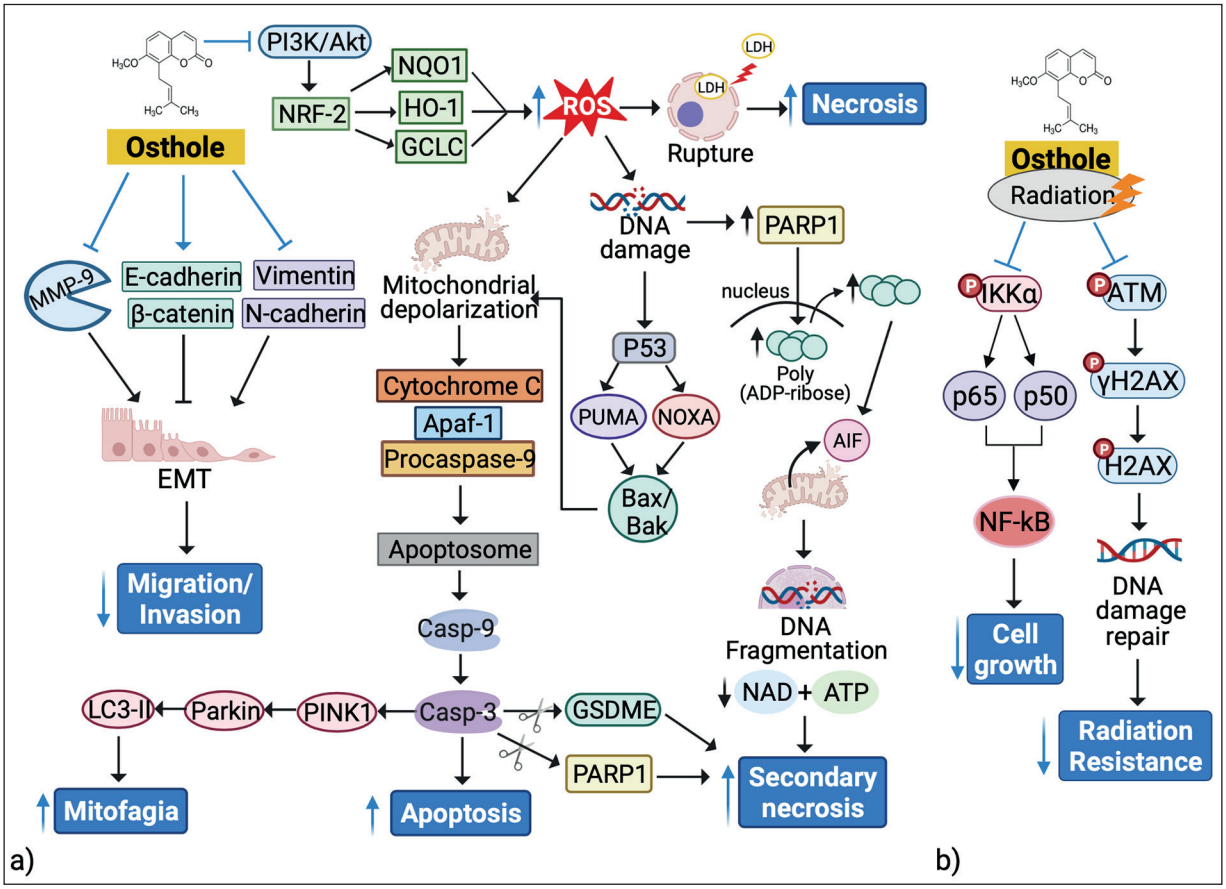


Figure 4. Mechanism of anti-tumor action of osthole against cervical cancer cells. **a)** Osthole inhibits cell migration and invasion by upregulating the EMT markers N-cadherin, vimentin, and MMP-9 and deregulating E-cadherin and ß-catenin. The cytotoxic effect of osthole is explained by the inhibition of the PI3K/Akt pathway, which deregulates the antioxidant system and produces high concentrations of ROS, altering the cytoplasmic and mitochondrial membrane and causing DNA damage; these alterations lead the cell to necrosis, apoptosis, secondary necrosis, and mitophagy. Moreover, DNA damage causes extensive PARP1 activation, nuclear accumulation of poly (ADP-ribose), and the subsequent release of AIF from the mitochondria, leading to DNA fragmentation and cell death. **b)** Co-treatment of osthole with radiation inhibits cell growth by repressing the NF-κB pathway mediated by IKKα activation. Osthole promotes radiation-induced DNA damage by repressing activating enzymes for DNA repair (ATM, γH2AX, and H2AX), which decreases resistance to radiation. Blue lines and arrows indicate the effect of osthole on molecules or cellular processes: up arrow—increase; down arrow—repression.

Unfortunately, these mechanisms have not been studied in cervical cancer cells, and although similar events may be observed, this hypothesis must be confirmed.

In SiHa and HeLa cervical cancer cell lines, Pra-A reduced colony formation and cell viability at concentrations of 10 and 20 µM per 24 h, respectively, while in HeLa cells at concentrations of 20 µM for 24 h, Pra-A caused the arrest of the cell cycle in the G0/G1 phase and inhibited cell proliferation, decreasing the expression of cell cycle regulatory proteins such as cyclin D1 and Skp2, and inducing the overexpression of negative regulators p16, p21, p27 and Rb[92]. Pra-A decreased the invasiveness of HeLa cells at concentrations of 20 µM for 24 h, inhibiting the activity of 12-O-tetradecanoylphorbol-13-acetate (TPA), a tumor promoter known to induce cell migration and invasion, dependent on the MEK/ERK1/2 signaling pathway[92,93]. Pra-A potentiated the effect of PD98059, a MEK1/2 inhibitor, which decreased the ability of cervical tumor cells to migrate, invade and metastasize by reducing the expression of MMP-2 and increasing the expression of TIMP-2, a tissue inhibitor of metalloproteinases[92]. Overexpression of MMP-2 and MMP-9 and decreased expression of TIMP-2 has been shown to promote extracellular matrix remodeling, contributing to migration and invasion during the metastasis of cervical tumor cells[94]. Considering the previous findings, Pra-A may decrease the proliferation and invasion capacity of cervical tumors. Therefore, it could be a promising therapeutic molecule for treating this neoplasia (Figure 5a).

Pra-B also showed anti-tumor activity in cervical cancer cells. At concentrations of 10 and

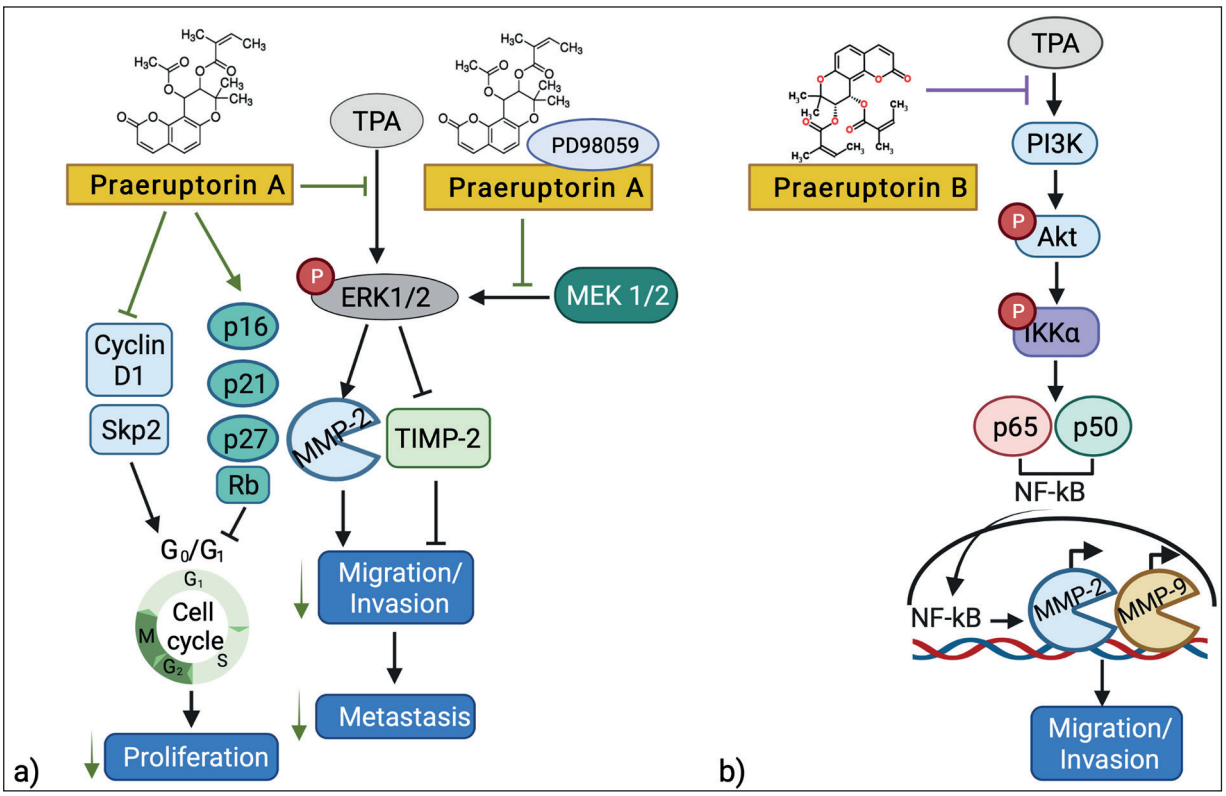


Figure 5. Mechanisms of anti-tumor action of Praeruptorin-A and -B in cervical cancer cells. a) Pra-A decreases the expression of cell cycle regulatory proteins such as cyclin D1 and Skp2, and induces the overexpression of negative regulators p16, p21, p27, and Rb, decreasing cell proliferation. On the other hand, Pra-A blocks the effect of the metastasis inducer TPA, in addition to enhancing the effect of the inhibitor PD98059 on MEK1/2; blocking the ERK1/2 signaling pathway; and therefore, reducing the capacity for migration, invasion and metastasis of the tumor cells by decreasing the expression of MMP-2 and increasing the expression of TIMP-2. Green lines and arrows indicate the effect of Pra-A on molecules or cellular processes: down arrow—repression. b) Pra-B decreases the TPA-induced migration and invasion capacity in cervical tumor cells. Pra-B blocks the activation of the PI3K/Akt-IKKα/NFκβ pathway and the expression of MMP-2 and -9 induced by TPA. By decreasing the production and activity of MMPs, the capacity for cell migration and invasion decreases. Purple line indicates the effect of Pra-B on TPA action and cellular processes; down arrow—repression.

20 μM for 24 h, Para-B significantly inhibited TPA-induced migration and invasion of SiHa and HeLa cells, targeting p-Akt/NF-κB and reducing the matrix metalloproteinase-2/-9 expression[95]. It is already known that NF-κB can regulate MMP-2/-9 at the transcriptional level[96,97]. The regulation of NF-κB by PI3K/Akt is considered an important biological activity in cancer, including cervical cancer[98]. Considering the above, Pra-B decreases the migration and invasion capacity of cervical tumor cells by blocking the PI3K/Akt /NF-κB signaling pathway, which suggests that coumarin Pra-B could be used in cervical cancer therapy to decrease the invasive potential of tumor cells (Figure 5b).

Conclusions

Several studies have shown that compounds of natural origin are pharmacologically active molecules with anti-tumor activity and a low incidence of side effects, which is why they can be the basis of new cancer therapies. These compounds include several natural coumarins recognized for their anti-tumor activity in different types of cancer without being cytotoxic nontumor cells. Auraptene, scopoletin, osthole, and praeruptorin are the most studied coumarins with activity against cervical cancer.

All coumarins studied in this review, auraptene, scopoletin, osthole, and praeruptorin, have shown a cytotoxic or antiproliferative effect on cervical tumor cells (Table I). Auraptene, scopoletin, and praeruptorin induce cell cycle arrest in cervical tumor cells, with some differences in the mechanisms reported for each coumarin. The OST-mediated blockade of the PI3K/Akt pathway induces cell death by apoptosis and necrosis, secondary necrosis, and mitophagy, which are all triggered by increased ROS. Additionally, osthole co-treatment with radiation shows antiproliferative activity by inhibiting cell growth and the IKKα/NF-κB pathway, increasing DNA damage, and blocking the phosphorylation of ATM and H2AX proteins.

Scopoletin induces an increase in the expression of pro-apoptotic proteins Bax and caspase-3, -8, and -9 and a decrease in the expression of anti-apoptotic proteins Bcl-2. Praeruptorin, mainly Pra-A, decreases the expression of cell cycle regulatory proteins such as cyclin D1 and Skp2, inducing the overexpression of negative regulators p16, p21, p27, and Rb. Although studies are needed to determine the mechanism of action of auraptene to induce cell death in cervical cancer cells, in other tumor lines, auraptene induces the intrinsic pathway of apoptosis, similar to scopoletin and OST.

On the other hand, coumarins scopoletin, osthole, auraptene, and praeruptorin inhibit the capacity for migration, invasion, and metastasis of cervical tumor cells. Pra-A and -B inhibit the expression of MMP-2 and MMP-9 by blocking the ERK1/2 and the PI3K/Akt/IKKα/NF-kß pathway, respectively, which decreases the invasive potential of the cells. Additionally, Pra-A, with the blockade of the ERK1/2 pathway, increases the expression of TIMP-2. Likewise, osthole suppresses levels of EMT proteins, including upregulated epithelial markers E-cadherin and β-catenin and downregulated mesenchymal markers N-cadherin and vimentin and MMP-9. Auraptene inhibited the expression and activity of MMP-2 and -9, although the route of signals regulating this effect has not been determined.

Furthermore, scopoletin decreases the migration capacity of HeLa cervical tumor cells but the mechanisms by which this event occurs are unknown. Additional studies are required to elucidate the mechanisms of auraptene and scopoletin to decrease the invasiveness of cervical cancer cells.

In conclusion, osthole and praeruptorin are the best-characterized coumarins in terms of their effect against the proliferation and invasiveness of cervical cancer cells, followed by auraptene and scopoletin. The effects and mechanisms of action against cervical cancer of the coumarins included in this review are summarized in Figure 6 and Table I. An important aspect to highlight is that, according to the reports, none of the coumarins included in this review showed cytotoxic activity in nontumor cells. A limitation of the possible use of coumarins as therapy against cancer is that, until now, no *in vivo* models or clinical trials have been reported that demonstrate the effectiveness of coumarins in reducing the growth and invasiveness of cervical tumor cells. Osthole enhanced the effect of cisplatin to reduce tumor growth in female BALB/c nude mice. Auraptene, osthole, and praeruptorin have also been shown to help sensitize tumor cells to radiotherapy or molecules used in cancer chemotherapy, such as FOLFOX, cisplatin, or DOX. However, the evidence in cervical cancer and the mechanisms of action of coumarins in this sense are still poorly understood; thus, more studies *in vitro* and *in vivo* are required to fully understand the mechanism of action of coumarins as adjuvants to the molecules already used in cancer chemotherapy. Considering the observations mentioned in this

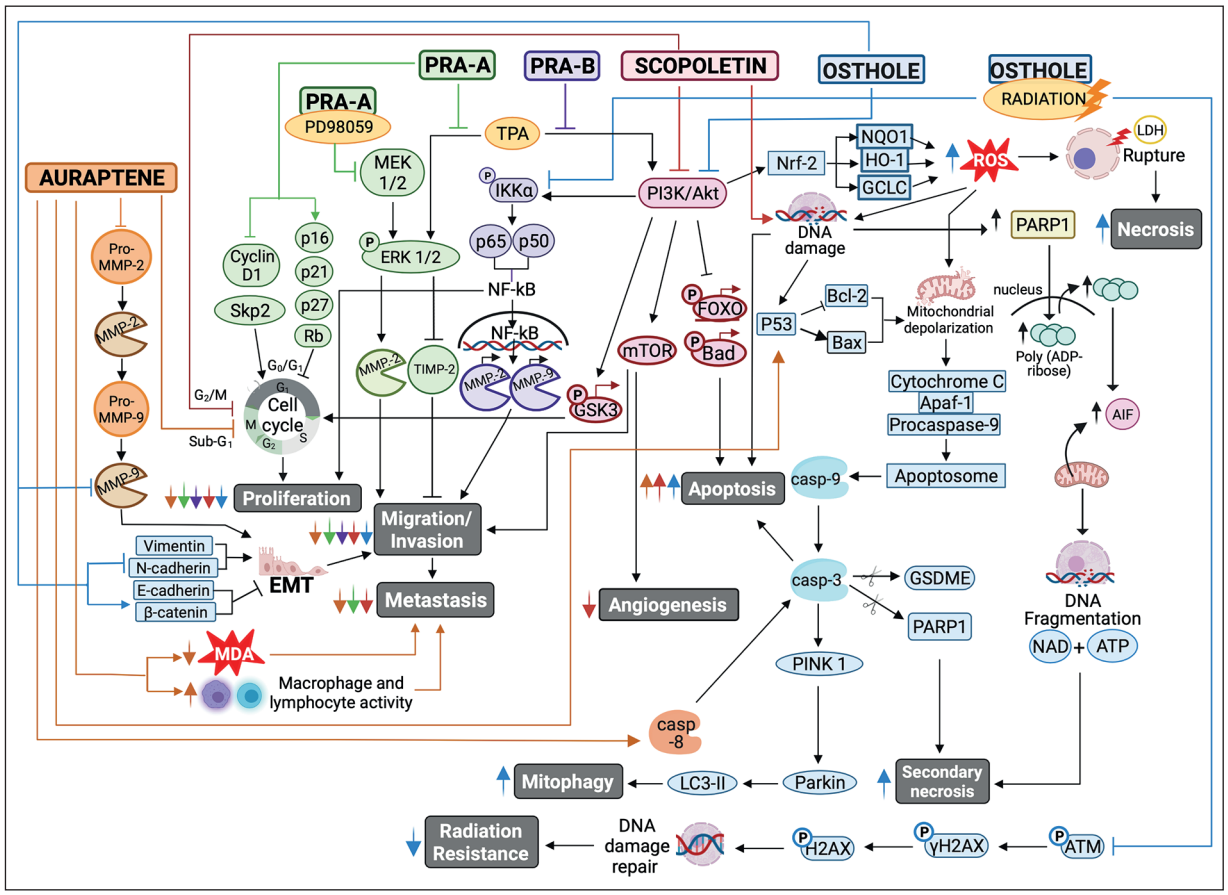


Figure 6. Global summary of the reported mechanisms of action of the most studied coumarins in cervical cancer. The coumarins auraptene, scopoletin, and osthole induce apoptosis, blocking the PI3K/Akt signaling pathway with some differences in the mechanisms of action reported. Moreover, auraptene, Pra-A, scopoletin, and osthole show antiproliferative activity by repressing the cell cycle; scopoletin activity is also due to the inhibition of the PI3K/Akt pathway, while osthole, combined with radiation, inhibits the IKKα/NF-κB pathway. In addition, osthole sensitizes tumor cells to radiation therapy, inhibiting the ATM and H2AX enzymes, that are responsible for repairing radiation-induced DNA damage. The colored lines and arrows indicate the effect of the different coumarins on the molecules or cellular processes: up arrow—increase, down arrow—repression.

review, coumarins auraptene, scopoletin, osthole, and Pra-A and -B offer excellent possibilities for developing new drugs as complementary medicine with an integrative approach against cervical cancer. We emphasize the need for *in vivo* studies and clinical trials that demonstrate the effectiveness of these coumarins in cancer therapy, particularly cervical cancer.

Conflicts of Interest

The authors declare no conflicts of interest.

Figures Design

The figures included in the manuscript were created with BioRender.

Authors' Contributions

M.A.M-C., N.N-T., and C.O.P. had the idea for the article; B.C-C., A.G-E., H.Y.L-M., D.N.M-C., A.E.Z-G., F.I.T-R., R.D-M., H.J-W., C.S-L. and M.A.M-C., performed the literature search and data analysis. The first draft of the manuscript was written by B.C-C., A.G-E., H.Y.L-M., and M.A.M-C. All authors critically revised the work and read and approved the final manuscript.

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