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REVIEW ARTICLE

Compounds From Celastraceae Targeting Cancer Pathways and Their Potential Application in Head and Neck Squamous Cell Carcinoma: A Review

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Abstract: Squamous cell carcinoma of the head and neck is one of the most common cancer types worldwide. It initiates on the epithelial lining of the upper aerodigestive tract, at most instances as a consequence of tobacco and alcohol consumption. Treatment options based on conventional therapies or targeted therapies under development have limited efficacy due to multiple genetic alterations typically found in this cancer type. Natural products derived from plants often possess biological activities that may be valuable in the development of new therapeutic agents for cancer treatment. Several genera from the family Celastraceae have been studied in this context. This review reports studies on chemical constituents isolated from species from the Celastraceae family targeting cancer mechanisms studied to date. These results are then correlated with molecular characteristics of head and neck squamous cell carcinoma in an attempt to identify constituents with potential application in the treatment of this complex disease at the molecular level.

Keywords: Celastraceae, Triterpenoids, Quinone-methides, Cancer, Head and neck squamous cell carcinoma, Targeted therapy.

1. INTRODUCTION

Cancer presently accounts for approximately 8 million deaths per year worldwide, a number that should escalate in the next two decades according to current projections [1]. Considered a global health problem, great scientific efforts are being made in order to understand the burden of cancer and prevent an even worse scenario.

In the past 10 years, the PubMed searchable databases alone have registered over a million articles addressing cancer [2]. Results show that despite improvements in early diagnosis and treatment, most cancer patients are still lacking treatment options and that success rates in drug development are low [3, 4].

Head and neck squamous cell carcinoma (HNSCC) is responsible for about 90% of the cancers arising in the epithelial lining of the mucosal surfaces of the head and neck [5]. It is considered the sixth most prevalent cancer type worldwide, with approximately 540,000 new cases annually and 271,000 deaths, mostly due to lack of early diagnostic markers and efficient therapies [6]. Major risk factors include heavy drinking and tobacco consumption [7] and the human papilloma virus for certain HNSCC subsites [8]. Most patients are diagnosed at advanced cancer stages. At this point treatment requires complex surgeries followed by radiotherapy and/or chemotherapy, with severe consequences in speech, breathing and eating abilities [9, 10]. Noteworthy is the fact that over 50% of patients will present recurrence in less than 2 years after initial treatment with overall survival between 6 and 12 months [11, 12]. The molecular complexity of this cancer type is certainly the major drawback for the development of more efficient therapies. The use of biologically active molecules acting upon distinct cellular processes could be a desirable alternative for therapy.

Compounds isolated from plants have traditionally been considered for their medicinal properties [13]. Several commercially available drugs for cancer were developed using bioactive molecules originally isolated from plant extracts, including Velban® (also known as vinblastine, originally isolated from *Catharanthus roseus* G. Don), Oncovin® (generically known as vincristine, it was originally obtained from *Catharanthus roseus* G. Don), Taxol® (paclitaxel, originally obtained from *Taxus brevifolia*), Eldisine® (also known as vindesine, originally obtained from *Catharanthus roseus* G. Don), Navelbine® (known as vinorelbine, obtained from *Catharanthus roseus* G. Don),

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Taxotere® (generically known as Docetaxel, a semisynthetic compound derived from bacatin III, which was originally isolated from *Taxus baccata* needles), Vepesid® (also known as etoposide, it is a semisynthetic analogue of podophyllotoxin obtained from the root of *Podophyllum peltatum*), Vumon® (known as teniposide, it is a semisynthetic analogue of podophyllotoxin obtained from the root of *Podophyllum peltatum*), Camptosar® (known as irinotecan, a semisynthetic analogue of the natural alkaloid camptothecin originally isolated from the bark and stem of *Camptotheca acuminate*) and Hycamtin® (also known as topotecan, a synthetic analog of the natural chemical compound camptothecin originally obtained from *Camptotheca acuminata*), as reviewed else where [14, 15].

The Celastraceae family is among those greatly investigated for antineoplastic effects. It comprises around 100 genera and 1300 species, most of them distributed in the tropical and sub tropical regions of South America as well as in eastern Asia [16, 17]. This review presents a comprehensible collection of articles addressing the antineoplastic effect of Celastraceae plant extracts and/or chemical constituents. Genes and proteins reportedly targeted by these molecules and associated with deregulated signaling pathways in cancer are reported and special emphasis was given to HNSCC molecular features. The final aim was to tackle if plant extracts or constituents isolated from species of the Celastraceae family could be considered potential new sources for therapeutics development for this kind of cancer.

2. OVERVIEW OF PUBLICATIONS ON CELASTRACEAE AND CANCER

In order to identify research articles possibly associating Celastraceae and cancer the PubMed searchable database (accessing mostly journals indexed in the MEDLINE database, Medical Literature Analysis and Retrieval System Online), the repository PubMed Central (PMC) and the Scientific Electronic Library Online (SciELO) were used. The general terms Celastraceae (all fields) AND Cancer (Title/Abstract) were used in all instances. Publications automatically selected following the criteria described above were manually curated and only those presenting *in vitro* reports on molecular mechanisms of action of extracts and/or compounds isolated from species of the Celastraceae family were further discussed in this review. Publications reporting only cytotoxicity and cell proliferation results, reviews, retracted articles, articles published in any language other than English, and studies reporting only results other than antineoplastic-related effects were excluded. Reports found using more than one database were included only once in the total number of publications.

A total of 101 publications associating Celastraceae and cancer were identified using the search engines and electronic databases selected for this review. Most literature (61 reports, 83% of all results) was retrieved through PubMed, followed by PubMed Central (14,8%). Of these potentially useful publications, 27 were kept for further discussion in this review since they matched the final selection criteria. Only 2 publications were identified in SciELO, but they did not qualify for further analysis.

In summary, of 101 publications found using the terms "cancer" and "Celastraceae", 73% were excluded. Twenty-six of them were reports on the potential use of Celastraceae-related natural compounds for other diseases or the evaluation of specific properties of compounds isolated from species of the Celastraceae family not directly associated with cancer. Several publications focused on extraction and purification protocols of bioactive molecules, and therefore were dismissed. A total of 27 publications were selected for full-text reading. The selection results are represented in (Fig. **1**).

The 27 selected reports were published between 2005 and 2014, but most of them were published in 2008, 2012 and 2013. Prior to 2005 most publications did not report specific mechanisms of action for the studied biomolecules and were not, therefore, included in this review. China is responsible for 48% of the publications, possibly due to the widespread use of traditional medicine, often based on plant ex-

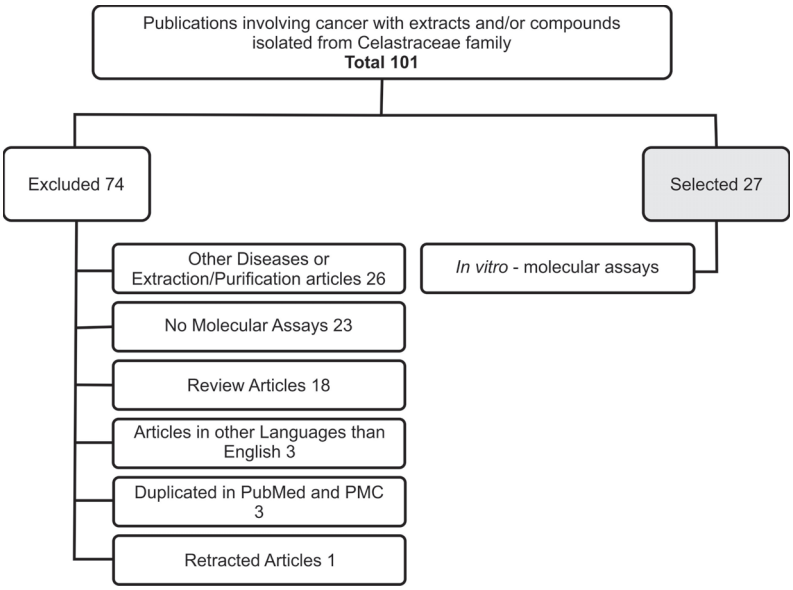

Fig. (1). Summary of screening results for publications associating Celastraceae and cancer.

tracts, in that country. The USA appears as the second country with highest number of publications (24%). The remaining of the publications came from Brazil, Korea, Taiwan and Vietnam.

3. THE ANTI-CANCER POTENTIAL OF COMPOUNDS ISOLATED FROM SPECIES OF THE CELASTRACEAE FAMILY

Accumulating evidence presented in this review indicates that several species from the Celastraceae family are potential sources of molecules that may interfere in the progression of cancer. The most studied cancer types were prostate and colon cancer, with 17% of the studies, followed by breast cancer, hepatocellular carcinoma and pancreatic cancer, with 13% of the studies each (Fig. **2**). One of the approaches for studying the anti-cancer activities of plant extracts and bioactive molecules is to directly address signaling pathways commonly deregulated in cancer. A comprehensive summary of current results using this approach is shown in (Table **1**). As shown, ten Celastraceae species were studied in the literature reviewed in this work: *Celastrus paniculatus*, *Celastrus hypoleucus, Salacia cochinchinensis*, *Maytenus ilicifolia*, *Tripterygium wilfordii*, *Tripterygium regelii, Tripterygium hypoglaucum, Euonymus alatus*, *Microtropis fokienensis* and *Perrottetia arisanensis*. The genus *Tripterygium* was the most frequently studied, followed by *Celastrus* and *Maytenus.* These species can be considered source of at least three classes of molecules: terpenoids, alkaloids and polyphenols. Most studies (92%) focused on the anti-tumoral activities of terpenoids. Terpenoids constitute a large and diverse group of naturally occurring products. They are essentially lipids, built up of isopropene units, but differ in their carbon skeleton and functional groups, characteristics responsible for their specific effects in biological systems. Three terpenoids were most cited in the selected studies: sesquiterpenoids, diterpenoids, and triterpenoids, with 4%, 39% and 57% of the citations respectively.

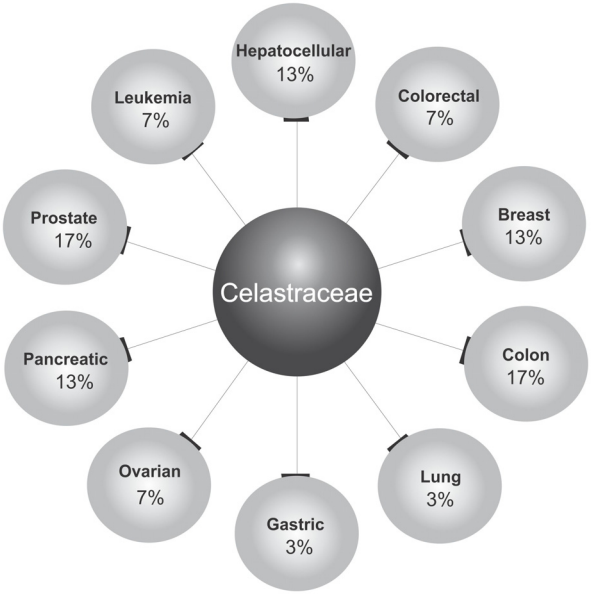

Fig. (2). Cancer types potentially treatable by compounds isolated from species of the Celastraceae family and the percentage of reports addressing each cancer type according to the reports retrieved.

Among diterpenoids, triptolide, the principal bioactive ingredient of *Tripterygium wilfordii*, has a unique structure leading to multiple biological activities [18]. According to reports, triptolide directly induces tumor cell apoptosis, but can also enhance apoptosis induced by cytotoxic agents (e.g. TNF-α and TRAIL) and chemotherapeutic agents by inhibiting NFκB activation [19]. The design and synthesis of triptolide derivatives have been motivated owed to its high potential but limited clinical use due to severe toxicity and water-insolubility. As a matter of fact, PG490-88, a derivative of triptolide, is part of a phase I clinical trial for treatment of prostate cancer in the USA [19].

The most studied triterpenoids are quinone-methides (85% of the studies on triterpenoids). Quinone-methides are common constituents of biological systems and some possess important biological activities, including DNA alkylation and DNA cross-linking [20]. In fact, oxidation to a reactive quinone-methide is the mechanistic basis of many phenolic anti-cancer drugs [21, 22]. Quinone-methides obtained from natural sources are among the most promising chemical classes for the development of new drugs against cancer [23, 24]. Naturally occurring quinone-methide triterpenoids can only be found as secondary metabolites in plants of the Celastraceae family [25]. Despite their broad pharmacological potential, including anti-cancer effects, these compounds cannot be obtained by chemical synthesis yet. Extraction from plants remains the only feasible strategy and biotechnological techniques, such as *in vitro* culture of cells, may become an alternative source in the future [25].

Celastrol and its methyl ester, pristimerin, are the most studied triterpenoid quinone-methides in cancer (Table **2**). Originally extracted from the root bark of *T. wilfordii*, an ivy, vine-like plant native to China, Japan and Korea, these compounds are currently obtained from several other species. The chemical structure of the three most studied compounds obtained from species of the Celastraceae family, namely triptolide, celastrol and pristimerin, are depicted in (Fig. **3**).

4. PRISTIMERIN, CELASTROL AND TRIPTOLIDE: MOLECULAR FUNCTIONS AND MECHANISMS IN CANCER

The triterpenes quinone-methides celastrol and pristimerin and the diterpenoid triptolide are the most studied molecules isolated from species of the Celastraceae family in regard to molecular mechanisms associated with anti-cancer effects.

Triterpene quinone-methides have been found to actively inhibit choline kinase-α, a critical enzyme in the synthesis of phosphatidylcholine, a major structural component of eukaryotic cell membranes, as reviewed in Estévez-Braun and co-authors [89]. The compounds tested in this study were further found to exhibit anti-proliferative activity against human colorectal adenocarcinoma HT29 cells *in vitro* and they also showed *in vivo* anti-tumoral activity in xenographs of HT29 cells injected into mice [89].

The anti-cancer effect of the quinone-methide pristimerin has been studied in a variety of cells *in vitro* and cancer models *in vivo*. Several molecular mechanisms underlying

Table 1. Studies addressing the anti-cancer potential of Celastraceae through their effect on commonly deregulated genes and/or proteins in cancer.

Source	Compound/extract	Chemical Class	Tumor Type	Addressed Target	References
Euonymus alatus	chlorogenic acid	polyphenol	hepatocellular carcinoma	MMP-9	[26]
*	celastrol	quinone-methide triterpenoid	prostate	Proteasome, IκB-α, Bax, p27, caspase-3	[27]
Microtropis fokienensis and *Perrottetia arisanensis*	(28-hydroxy-3-oxo-lup-20(29)-en-30-al)	triterpenoid	leukemia	PARP, Bax	[28]
Maytenus ilicifolia	pristimerin	quinone-methide triterpenoid	leukemia	Topoisomerase I	[29]
Tripterygium wilfordii	triptolide	diterpenoid triepoxide	pancreatic	caspase-3, Bax	[30]
Tripterygium wilfordii	triptolide	diterpenoid triepoxide	ovarian	caspase-3	[31]
*	pristimerin	quinone-methide triterpenoid	prostate	Bax, p27, IκBα, proteasome	[32]
Tripterygium wilfordii	triptolide	diterpenoid triepoxide	breast	Erα	[33]
*	triptolide	diterpenoid triepoxide	colon	IL-6, JAK1, STAT3, Rac1, cyclin D1, Cdk4	[34]
*	celastrol	quinone-methide triterpenoid	hepatocellular carcinoma	HIF-1α, VEGF, AKT, Met	[35]
Tripterygium wilfordii	triptolide	diterpenoid triepoxide	colon	c-Myc, VEGF, COX-2, CXCR4,TGF-β,	[36]
Tripterygium regelii	celastrol	quinone-methide triterpenoid	breast	Bax, Bcl-2, cytocrome *c*	[37]
*	triptolide	diterpenoid triepoxide	pancreatic	triptolide combined with HCPT : topoisomerase, caspase-9/caspase-3, NF-κB	[38]
Celastrus hypoleucus	oleanen	triterpene	colon	caspase 9, Bim	[39]
Tripterygium wilfordii Hook F	triptolide	diterpenoid triepoxide	gastric	In association with cis-platine: pro-caspase3 and 9, NF-κB/p65, Bax, cytochrome *c*	[40]
*	triptolide	diterpenoid triepoxide	colorectal	14-3-3 epsilon	[41]
Celastrus orbiculatus	leaf extract	-	hepatocellular carcinoma	VEGF	[42]
*	pristimerin	quinone-methide triterpenoid	pancreatic	cyclin D1, cyclin E, Cdk2, Cdk4, Cdk6, p21, p27, pro-caspase-3, Bax, Bcl-2, Bcl-xl, NF-κB/p65	[43]
Celastrus paniculatus	(1a,2a,8b,9b)-1,8-bis(acetyloxy)-2,9-bis(benzoyloxy)-14-hydroxy-bdihydroagarofuran	sesquiterpenoid	breast	AKT, ERK, p38, NF-κB, Bcl-2	[44]

(Table 1) contd….

Source	Compound/extract	Chemical Class	Tumor Type	Addressed Target	References
Salacia cochinchinensis	pristimerin	quinone-methide triterpenoid	breast	HER-2, FASN, AKT/ERK1/2, p38, JNK, mTOR/p70s6K/4E-BP1	[45]
Maytenus ilicifolia	aqueous extract from aerial parts,	-	hepatocellular carcinoma	caspase-3, Bcl-2	[46]
Tripterygium wilfordii	triptolide	diterpenoid triepoxide	colon	caspase-3,-8,-9; PARP, Bax, Bcl-2, Bcl-xl,	[47]
*	pristimerin	quinone-methide triterpenoid	prostate	PARP-1, procaspase-3 and -9, cytochrome c , Bcl-2	[48]
*	pristimerin	quinone-methide triterpenoid	pancreatic	PARP-1, procaspase -3, -8 and -9, cytochrome *c*, Akt, NF-κB, Foxo-3α, cyclin D1, Cox-2, VEGF, pS6K1, 4E-BP1, Bcl-2, survivin	[49]
Tripterygium hypoglaucum	alkaloid rich root extract	alkaloids	colon	caspase-3, Bcl-2,Bcl-xl, XIAP	[50]
*	pristimerin	quinone-methide triterpenoid	prostate	Cyclin D, Cyclin E, Cdk2, Cdk4, Cdk6, p21, p27, PARP, Bcl-2, Bcl-xL, Bax, Bak, Bad, Survivin, XIAP, cIAP	[51]
**	celastrol	quinone-methide triterpenoid	ovarian, lung	Caspase- 3, 8, 9, IκB, pIκB, E-cadherin	[52]

(*) Commercially obtained (**) obtained from other researchers (donation or collaboration).

Table 2. Celastrol and pristimerin in cancer.

Compound	Tumor	References
celastrol/pristimerin	lung	[35, 53-59]
celastrol/pristimerin	prostate	[27, 32, 48, 51, 55, 59-66]
celastrol/pristimerin	breast	[37, 45, 55, 59, 67-71]
celastrol	liver	[35, 55, 69, 72]
celastrol/pristimerin	cervical	[41, 73-75]
celastrol	oral squamous cell	[55, 76, 77]
celastrol	thyroid	[78]
celastrol	gastric	[55, 79-81]
celastrol/pristimerin	pancreatic	[43, 49, 82, 83, 84]
celastrol/pristimerin	colorectal	[85]
celastrol	bladder	[86]
celastrol	melanoma	[58, 87]
celastrol/pristimerin	glioma	[55, 59, 88]
celastrol	myeloma	[55]
celastrol	kidney	[55, 74, 89]
celastrol/pristimerin	leukemia	[29, 66, 69]
celastrol/pristimerin	ovarian	[59, 90]

the pristimerin effects were proposed; some of them have been confirmed through studies in more than one tumor type. Pristimerin was found to induce apoptosis in hormone-sensitive (LNCaP) and hormone-refractory (PC-3) prostate cancer cell lines [91]. Pristimerin increased annexin V-binding and cleavage of PARP-1, procaspases-3 and -9-induced mitochondrial depolarization, cytochrome c release from mitochondria, generation of reactive oxygen species (ROS), and downregulation of BCL-2 and survivin expression via proteasome-dependent degradation [51, 91]. However, in the study, overexpression of BCL-2 rendered prostate cancer cells resistant to pristimerin. Pristimerin inhibits protein expression of CD133 and CD44, reduces VEGF expression and the expression of pro-inflammatory cytokines such as interleukin [IL]-1, -6, -8, TNF-α and interferon-γ in human prostate cancer PC-3 cells, also preventing the growth of xenografted PC-3 tumors into the bone of nude mice [91]. Pristimerin inhibited VEGF-induced vasculogenesis of bone marrow derived-endothelial progenitor cells by suppressing proliferation, adhesion and migration, possibly due to decreased phosphorylation of VEGF receptor-2, AKT and eNOS [91]. Pristimerin also inhibited the proliferation of (HER2)-positive SKBR3 human breast cancer cells, possibly due to changes in FASN and AKT expression. The changes in HER2, FASN and AKT expression induced by pristimerin altered the phosphorylation levels of various mitogen-activated protein kinases (MAPK), including ERK1/2, p38 MAPK, and JNK, and lowered levels of phosphorylated mTOR and its downstream targets, such as p70S6K and 4E binding protein-1 (EIF4EBP1) [45]. Pristimerin inhibited migration and invasion of cells, and co-treatment with the mTOR inhibitor, rapamycin, additionally suppressed these cellular functions [45].

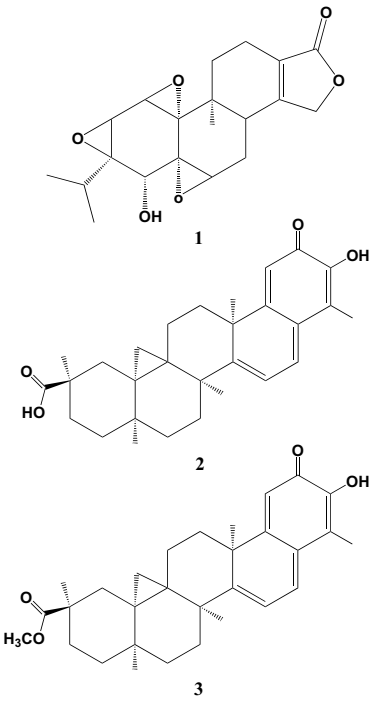

Fig. (3). Chemical structure of triptolide (1), celastrol (2) and pristimerin (3), originally isolated from species of the Celastraceae family. ChemDraw 12.0 software (currently available through PerkinElmer) was used to draw these structures.

Pristimerin decreased cell proliferation of human pancreatic cancer cells (BxPC-3, PANC-1 and AsPC-1) in a dose- and time-dependent manner [43]. The work reported that treatment of pancreatic cancer cells with pristimerin resulted in G1-phase arrest associated with a marked decrease in the level of cyclins CCND1 and CCNE, and cyclin-dependent kinases (CDK-2, -4 and -6) with concomitant induction of CDK inhibitors, CDKN1A (p21WAF1) and CDKN1B (p27KIP1). Pristimerin treatment also resulted in apoptotic cell death through cleavage of caspase-3, modulation in the expression of BCL-2 family proteins and inhibition of the translocation and DNA-binding activity of NF-κB [43].

In human pancreatic ductal adenocarcinoma cells (Mia-PaCa-2 and Panc-1) pristimerin inhibited the proliferation and induced apoptosis [49]. These effects were characterized by increased Annexin V-binding and cleavage of poly (ADP-ribose) polymerase (PARP)-1 and activation of procaspases -3, -8 and -9 [49]. The induction of apoptosis was associated with the inhibition of the pro-survival AKT, NF-κB and mTOR proteins and downstream targets, such as FOXO3A, CCND1, COX-2, VEGF, p-70S6K1, p-4E-BP1, and protein kinase C-ε (PKCε), as well as of anti-apoptotic BCL-2 and survivin (also known as BIRC5) but not BCL-xL. Additionally, pristimerin induced mitochondrial depolarization and the release of cytochrome C from the mitochondria [49].

Pristimerin was found to possess potent cytotoxic effects, inducing apoptosis and inhibiting proliferation in U87 human glioma cells [86]. Pristimerin activated caspase-9, -3, and PARP cleavage. Pristimerin also increased the generation of ROS, and induced the subsequent release of cytochrome c from the mitochondria into the cytosol [86]. In ovarian cancer cells, pristimerin also induced apoptosis through cleavage of PARP-1, procaspases-3, -8 and -9 activity and enhanced mitochondrial depolarization [88]. The study also showed downregulated levels of p-AKT, p65 subunit of NF-κB and p-mTOR, and downregulated expression of NF-κB-regulated genes encoding anti-apoptotic proteins, such as BCL-2, BCLxL, c-IAP1 and survivin (BIRC5), therefore promoting apoptosis [88].

In a somewhat different approach, pristimerin was shown to inhibit human telomerase reverse transcriptase (hTERT) expression and activity in human pancreatic cancer cells [90]. The compound inhibited hTERT expression by suppressing the transcription factors Sp1, c-Myc and NF-κB, which are known to control hTERT gene expression, and inhibited protein kinase AKT, which phosphorylates and facilitates hTERT nuclear import and its telomerase activity [90].

Celastrol and triptolide extracted from the Chinese herb *Tripterygium wilfordii* Hook F (also known as Lei Gong Teng or Thunder of God Vine) were also found to exhibit marked anti-tumoral effects [62]. Triptolide efficiently inhibited cell growth and induced cell death in human prostate cancer LNCaP and PC-3 cell lines *in vitro* as well as inhibited the xenografted PC-3 tumor growth in nude mice *in vivo* [62]. Tumor cell apoptosis was induced through the activation of caspases and PARP cleavage and reduced SUMO-specific protease 1 (SENP1, a potential biomarker and therapeutic target for prostate cancer) expression in dose- and time-dependent manner resulting in an enhanced cellular

SUMOylation in prostate cancer cells [62]. Meanwhile, triptolide decreased c-Jun expression, and suppressed c-JUN transcription activity. On the one hand, silencing of SENP1 or c-JUN in PC-3 prostate cancer cells decreased cellular viability, suggesting that the cytotoxicity of triptolide could result from triptolide-induced downregulation of SENP1, or c-JUN. On the other hand, ectopic expression of SENP1, or c-JUN significantly increased the viability of prostate cancer cells upon triptolide exposure, indicating that rescuing these triptolide downregulated proteins could inhibit cell toxicity induced by triptolide [62].

Several other studies have addressed the effects of tripolide on cancer cells. For instance, exposure of BE(2)-C human neuroblastoma cells to triptolide resulted in reduction in cell growth and proliferation [92]. Along with cell cycle arrest in the S phase and inhibition of the colony-forming ability of BE (2)-C neuroblastoma cells observed *in vitro*, reduction of tumor development and growth of tumor grafts was seen *in vivo* [92]. Triptolide significantly decreased the proportion of regulatory T cells and lowered the levels of FOXP3 transcription factor (also known as scurfin) in the spleen and axillary lymph nodes of tumor-bearing mice [93]. Production of IL-10 and TGF-β in peripheral blood and spleen were also decreased and the production of VEGF in tumor-bearing mice was inhibited [93]. Triptolide attenuated colon cancer growth *in vitro* and *in vivo* [94]. Using a proteomic approach, the authors found 14-3-3ε, a cell cycle- and apoptosis-related protein, cleavage and perinuclear translocation, to be induced by triptolide in human colon cancer cells [94].

Triptolide was shown to enhance cisplatin-induced cytotoxicity in human gastric cancer SC-M1 cells [40]. After low-dose combined treatments with triptolide and cisplatin, a decrease in viability with a concomitant increase in apoptosis was observed in SC-M1 cells but not in normal cells [40]. Apoptosis induced by the combined treatments was accompanied by a loss of mitochondrial membrane potential and release of cytochrome c and triptolide also increased the cisplatin-induced activation of caspase-3 and -9 and the downstream cleavage of PARP in SC-M1 cells *in vitro* [40]. The combined treatment completely suppressed *in vivo* tumor growth of gastric tumor grafts in mouse xenograft model [40]. In liver cancer, the combination of triptolide plus chemotherapeutics (cisplatin, 5-fluorouracil) reduced liver cancer cell viability and enhanced apoptosis compared with single treatment *in vitro* [95]. Furthermore, cells treated with triptolide plus chemotherapeutics exhibited marked production of intracellular ROS and caspase-3 activity, induced BAX expression, and inhibited BCL-2 expression [95].

Celastrol, a known natural 26S proteasome inhibitor, promotes cell apoptosis and inhibits tumor growth [27, 55, 62, 63]. Celastrol inhibited the proliferation of various human tumor cells, including multiple myeloma, hepatocellular carcinoma, gastric cancer, prostate cancer, renal cell carcinoma, head and neck carcinoma, non-small cell lung carcinoma, melanoma, glioma, and breast cancer (with concentrations as low as 1 μM). Celastrol decreased protein levels of CCND1 and CCNE, but increased the CDKN1A and 1B protein levels, activated caspase-8, -9, and -3, as well as induced cleavage of BH3 interacting-domain death agonist (BID) and PARP. The apoptotic effects of celastrol were preceded by activation of JNK and repression of AKT signaling [55].

Celastrol was found targeting multiple molecular components, including activating transcription factor 2 (ATF2), mitochondrial respiratory chain complex I, heat shock protein 90, beta1 integrin, Kv11.1, the alpha subunit of a potassium ion channel, ERBB2, estrogen receptor α, as well as affecting the activities of p38 MAPK and AKT/mTOR pathways in various tumor cells [56-58, 60, 64].

Celastrol induces apoptosis in human cervical cancer cells by targeting a proteasome catalytic subunit β1, endoplasmic reticulum (ER) protein 29 (ERP29) and mitochondrial import receptor Tom22 (TOM22) [73]. Celastrol was found to induce ER stress and induced translocation of BAX into the mitochondria, further upregulating BIM and TOM22, possibly involving glycogen synthase kinase-3β in these events [73]. Celastrol could also induce paraptosis-like cytoplasmic vacuolization in cancer cell lines including HeLa cells, A549 cells and PC-3 cells derived from cervix, lung and prostate, respectively [41]. Celastrol directly affects the biochemical properties of tubulin heterodimer *in vitro* and reduces its protein level *in vivo* [74]. At the cellular level, celastrol induces synergistic apoptosis when combined with conventional microtubule-targeting drugs and manifests an efficacy toward taxol-resistant cancer cells. Celastrol inhibited the cell migration and increased G1 arrest, and induced autophagy and apoptosis in human gastric cancer cells [79].

Celastrol was also found to increase the level of autophagy in the human pancreatic cancer MiaPaCa-2 xenograft tumor model. However, autophagy inhibitor 3-MA could improve the therapeutic effect of celastrol *in vitro* and *in vivo* [96]. Celastrol could inhibit proliferation of human osteosarcoma cells accompanied by G2/M phase arrest, activation of caspase-3, -8, and-9, as well as triggering autophagic pathway, as evidenced by formation of autophagosome and accumulation of LC3B-II protein [97]. Intriguingly, inhibition of apoptosis enhanced autophagy while suppression of autophagy diminished apoptosis in osteosarcoma cells upon celastrol exposure. Celastrol also induced JNK activation and ROS generation, while the JNK inhibitor significantly attenuated celastrol-triggered apoptosis and autophagy while ROS scavenger could completely reverse them [97]. Celastrol induced autophagy in human androgen receptor (AR)-positive prostate cancer cells, while the AR knockdown resulted in enhanced autophagy induced by celastrol, and autophagy inhibition by miR-101 mimic was found to enhance the cytotoxic effect of celastrol in prostate cancer cells [98].

Celastrol decreased gastric cancer cells viability via reduced IκB phosphorylation, nuclear p65 subunit protein levels and NF-κB activity [81]. Furthermore, celastrol could increase miR-146a expression and upregulation of miR-146a expression could suppress NF-κB activity. However, downregulation of miR-146a expression can reverse the effect of celastrol on NF-κB activity and apoptosis in gastric cancer cells. Combination of TRAIL and celastrol induced apoptosis in human pancreatic cancer cells through upregulation and dephosphorylation of EIF4E-BP1 protein [82]. Celastrol

was also found to exhibit anticancer activity in KU7 and 253JB-V bladder cells by inducing apoptosis, inhibition of growth, colony formation and migration *in vitro* and *in vivo* [84]. Celastrol was shown to decrease expression of specificity protein transcription factors Sp1, Sp3 and Sp4 and several Sp-regulated genes/proteins including VEGF, survivin and CCND1 and fibroblast growth factor receptor (FGFR)-3 [84].

Suberoylanilide hydroxamic acid (SAHA) is a promising histone deacetylase inhibitor approved by the US Food and Drug Administration but its clinical application for solid tumors is partially limited by decreased susceptibility of cancer cells due to NF-κB activation [52]. As an NF-κB inhibitor, celastrol exhibits potent anti-cancer effects but has failed to enter clinical trials due to its toxicity [52]. The combination of celastrol and SAHA exerted substantial synergistic efficacy against human cancer cells *in vitro* and *in vivo*, accompanied by enhanced caspase-mediated apoptosis [52]. This combination inhibited the activation of NF-κB caused by SAHA monotherapy and consequently led to increased apoptosis in cancer cells [52]. Interestingly, E-cadherin was dramatically downregulated in celastrol-resistant cancer cells and E-cadherin expression was closely related to decreased sensitivity to celastrol. However, the combination treatment significantly augmented the expression of E-cadherin, suggesting that mutual mechanisms contributed to the synergistic anti-cancer activity [52]. Furthermore, the enhanced anticancer efficacy of celastrol combined with SAHA was validated in human lung cancer 95-D xenografts in mice *in vivo* without increased toxicity [52]. These synergistic anti-cancer effects of celastrol and SAHA could be underlined by their reciprocal sensitization, which was simultaneously regulated by NF-κB and E-cadherin [52].

The mechanistic effects of plant extracts have also been addressed to certain extent. A spray-dried extract of *Maytenus ilicifolia* was shown to induce apoptosis in human hepatocellular HepG2 cells and human colorectal carcinoma HT-29 cells via down-regulation of BCL-2 and activation caspase-3 [46]. *Celastrus orbiculatus* extract significantly inhibited cell viability and induced apoptosis of human hepatocellular carcinoma LM6 cells in a dose-dependent manner [96]. In this study, apoptosis was accompanied by an increased BAX expression and decreased BCL-2 expression, induced release of cytochrome C, activation of caspase-3, and cleavage of PARP [99]. Furthermore, activation of ERK, p38 MAPK, and JNK phosphorylation, and downregulation of AKT phosphorylation was observed [96]. Compound oleanen from *Celastrus hypoleucus* also exhibits antitumor activity toward human cervical cancer cells by increasing in activity of caspase -3, -7, and -6, as well as a proapoptotic protein BIM [39].

Emerging evidence shows that quinone-methide triterpenes exert multiple molecular mechanisms leading to decrease of tumor cell viability or even cell death, and therefore are indeed promising compounds in the context of cancer treatment. However, existing data also highlight the need for more comprehensive, far-reaching approaches and technologies that would lead to a better understanding of direct and indirect effects of these compounds on molecular processes in tumor cells *in vitro* and *in vivo*.

5. CELASTRACEAE AND POTENTIAL MOLECULAR TARGETS IN HEAD AND NECK SQUAMOUS CELLS CARCINOMA

HNSCC arises from premalignant progenitor cells that progress to invasive malignancy due to cumulative genetic alterations [100]. Conventional treatment modalities - surgery, radiation and chemotherapy - are nonselective therapies that not only cause damage to normal tissue but which are associated with systemic toxicities that reduce compliance and, consequently, the success of therapy [9, 10]. The past decade has witnessed significant improvements in the knowledge on the complex molecular abnormalities underlying the clinicopathological characteristics of HNSCC, a promising scenario for the development of novel diagnostic markers and therapeutic procedures for the clinical management of patients [8, 101]. In theory, once major molecular mechanisms involved in the pathogenesis of HNSCC are known, a cancer therapy working at the molecular level, targeting deregulated pathways may be created. In practice, a very limited number of therapeutic agents for the targeted treatment of HNSCC are currently undergoing clinical trials [102], and the only established therapeutic target is the epidermal growth factor receptor (EGFR). EGFR is a cell-surface protein that regulates cell growth and differentiation and it can be targeted by the monoclonal antibody cetuximab (™Erbitux), resulting in the elimination of signal transduction. EGFR is overexpressed in HNSCC when compared with cancer-free mucosa with predictive and prognostic value [10, 103-105]. However, despite the abundant expression in HNSCC, only a subset of patients responds to EGFR inhibitors since alternative downstream signaling pathways may remain activated [10, 11, 106-108]. These results indicate the need for combined therapy approaches and for the continuous search for new active compounds that may target molecular processes in HNSCC.

Upon comparing literature on genetic and molecular characteristics of HNSCC and data on the effects of Celastraceae-derived compounds on gene expression and protein levels, a lot of overlapping information can be found. We summarize the molecular alterations of HNSCC that have been addressed in studies on anti-cancer effects of Celastraceae-derived compounds and extracts (Table **3**).

Loss of heterozygosity at the chromosomal region 9p21 is found in 70–80% of HNSCC cases, representing the most common genetic alteration in this type of cancer and in early pre-invasive lesions [109]. The *CDKN2A* gene locus found within this chromosome encodes transcript p16 involved in G1/S cell cycle regulation through the inhibition of cyclin dependent kinases such as CDK4 and CDK6 [110]. These kinases phosphorylate retinoblastoma protein (pRB) leading to the progression from G1 phase to S phase. For instance, pristimerin was previously shown to modulate the activity of CDK-4 and -6 resulting in G1-phase arrest of various human cancer cells [43, 51]. Emerging evidence shows that triterpenes affect the expression levels of other genes related to cell cycle progression, including including cyclin D1, cyclin E, p21, p27 and c-Myc, which have also been studied in the context of HNSCC [27, 32, 34, 36, 49, 111-113]. Therefore, a therapeutic interference able to regulate these CDKs, such as one seen upon treatment of tumor cells with pristimerin, could be important potential asset helping in cell proliferation control of HNSCC, as well.

Table 3. Possible molecular targets of Celastraceae-derived compounds involved in HNSCC progression.

Molecules Involved in HNSCC Carcinogenesis	References Addressing the Celastraceae-derived Compounds and Cancer	References Addressing HNSCC
HER-2	[45]	[114]
Cdk2	[43, 51]	[111]
Cdk4	[34, 43,51]	[112]
Cdk6	[43, 51]	[111]
Cyclin D1	[34, 43, 49, 51]	[112]
Cyclin E	[43,51]	[111]
p21	[43,51]	[113]
p27	[27, 32, 43, 51]	[113]
c-Myc	[36]	[115]
Bax	[27, 28, 30, 32, 37, 40, 47, 51]	[116]
Bcl-xl	[43, 47, 50, 51]	[117]
Bcl-2	[37, 44, 46-49, 51]	[115]
NF-κB	[37, 40, 43, 44, 49]	[118]
AKT	[35, 44, 45, 49]	[119]
ERK	[44, 45]	[119]
mTOR	[45]	[119]
P70-S6	[45,49]	[120, 121]
MET	[35]	[122]
XIAP	[50,51]	[123]
PARP	[28, 47, 49,51,94]	[124]
Cytochrome *c*	[37, 40, 48, 49]	[123]
Caspase-3	[27, 30, 31, 37, 46, 47, 50, 52]	[115]
Caspase-9	[37, 39, 47, 52]	[115]
p38	[44, 45]	[119]
FASN	[45]	[125]
COX-2	[36,39]	[119]
CXCR4	[36]	[126]
Proteasome	[27,32]	[127]
IL-6	[34]	[128]
MMP-9	[26]	[129]
STAT3	[34]	[114]
Rac1	[34]	[130]
VEGF	[35, 36, 42, 49]	[131]
HIF-1α	[35]	[113]
JNK	[45]	[119]
ERα	[33]	[132]
TGF-β	[36]	[132]
Survivin	[49,51]	[134]
4EB-P1	[45,49]	[120]

Major molecular alterations in HNSCCs include the activation of the phosphatidylinositol-3-kinase/protein kinase B (PI3-K/Akt) signal transduction pathway, shown to be activated in 50–80% of HNSCCs and involved in the regulation of various cellular processes, including apoptosis, proliferation and cell cycle progression [135, 136]. (Table **3**) lists numerous genes either directly or indirectly involved with this pathway, including AKT and ERK, but also Bcl-2, Bcl-x and NF-kB [112]. PI3-K itself is considered as a novel treatment target for HNSCC [137]. The mammalian target of rapamycin (mTOR) is a cell-growth regulator also associated with the PI3-K/Akt pathway and considered a therapeutic target for HNSCC [114]. Akt activated mTOR, which in turn phosphorylates p70-S6 kinase, leads to the activation of the ribosomal S6 protein. As a matter of fact, the accumulation of the phosphorylated active form of S6 is a common event in HNSCC tissue specimens [138]. Lee and co-authors have demonstrated that celastrol inhibits the activation of PI3K/AKT/mTOR signaling cascade at various levels in melanoma cells [139]. This important result could be addressed in the context of HNSCC since the PI3K-AKT pathway is downstream of EGFR, and phosphatidylinositol-4, 5-bisphosphate 3-kinase (PIK3CA) is among the most frequently mutated oncogenes for this cancer type (approximately 20%), possibly playing a role for both HPV-negative and HPV-positive tumors [140, 141].

Compounds isolated from Celastraceae also target a hepatocyte growth factor receptor (HGFR, also known as MET, encoded by the c-MET gene), the key player in PI3K/AKT/mTOR signaling regulation. MET was found to be overexpressed in up to 84% of HNSCC cases and is being tackled as a therapeutic target for HNSCC [142]. Additionally, if the antioxidant systems that allow stem-like cancer cells to avoid oxidative stress and resist EGFR inhibition are targeted, this may sensitize the remaining surviving cells, which will become sensitive to treatment [143]. Thus, the effects of triterpenoids on the redox state of cells may also be explored in this context [144].

As an example of how Celastraceae compounds could affect HNSCC, the treatment of human tongue cancer cells with triptolide, ionizing radiation, or triptolide plus ionizing radiation was reported to oral cell colony numbers [145]. In the study, triptolide was shown to increase apoptosis and decrease the expression of anti-apoptotic proteins in oral cancer cells *in vitro*. In addition, *in vivo* a combination treatment (triptolide with radiation) synergistically reduced tumor weight and volume *in vivo* possibly via the induction of apoptosis and reduction in anti-apoptotic protein expression suggesting that this may be a promising combined modality therapy for advanced oral cancer [145].

Celastraceae triterpenoids (dihydrocelastrol and celastrol) were identified as potent inducers of unfolded protein response (UPR) signaling and cell death in a panel of oral squamous cell carcinoma (OSCC) cells [76]. The pharmacological exacerbation of the UPR was suggested to be an effective approach to eliminate OSCC cells [76]. The UPR is executed via distinct signaling cascades, whereby an initial attempt to restore folding homeostasis in the endoplasmic reticulum during stress is complemented by an apoptotic response if the defect cannot be resolved. Moreover, biochemical and genetic assays using OSCC cells demonstrated that intact protein kinase RNA-like endoplasmic reticulum kinase (PERK)-eukaryotic initiation factor 2 (eIF2)-activating transcription factor 4 (ATF4)-DNA damage-inducible transcript 3 (DDIT3, also known as C/EBP homologous protein, CHOP) signaling is required for pro-apoptotic function of UPR, and subsequent death of OSCC cells upon celastrol treatment [76].

Celastrol was found to decrease TGF-β1-induced phosphorylation of mitogen-activated protein kinase kinase kinase 7 (also known as TAK1) and RELA, and suppressed NF-κB reporter gene activity in HNSCC cells [141]. Celastrol also inhibited cell proliferation, while increasing sub-G0 DNA fragmentation and Annexin V markers of apoptosis. Furthermore, TGF-β and RELA activation promoted SMAD7 expression. In turn, SMAD7 preferentially suppressed TGF-β-induced SMAD and NF-κB reporters when compared with constitutive or TNF-β-induced NF-κB reporter gene activation. Thus, crosstalk by TGF-β via TAK1 and NF-κB promotes the malignant phenotype of HNSCC [133].

Altogether, current data on the anti-cancer effects of compounds isolated from Celastraceae (Table **3**) points to a prolific and promising field of research in which such molecules should be able to either inhibitor up-regulate key pathways involved in HNSCC phenotype. New studies in this area should contribute to bringing about additional molecules of interest to this still scarce treatment scenario.

CONCLUSION

At the clinical and molecular level, HNSCCs are characterized by extensive heterogeneity, a picture that defies their classification as a single disease. HNSCC treatment should undergo substantial changes in the near future due to the present-day exploration of its mutational landscape. However, the development of effective therapy modalities involves not only the increased understanding of the mechanisms involved in HNSCC carcinogenesis but also the identification of new molecules capable of acting upon several molecular mechanisms. Ideally compounds should distinguish themselves from conventional cytotoxic agents and from drugs that target a single step in signal transduction pathways. This review shows that a variety of compounds isolated from species from the Celastraceae family and, at times, plant extracts, have been addressed as multifunctional drugs, interfering in multiple steps in key pathways involved in the development and progression of HNSCC. Few studies have investigated the potential of Celastraceae molecules to target HNSCC features, a scenario that will hopefully change in the next few years.

LIST OF ABBREVIATIONS

Bcl-xl	=	B-Cell Lymphoma-eXtra Large
Bcl-2	=	B-Cell Lymphoma 2
XIAP	=	X-linked Inhibitor of Apoptosis Protein
PARP	=	Poly (ADP-Ribose) Polymerase
c-MET	=	Receptor tyrosine kinase acting as a proto-oncogene, also known as hepatocyte growth factor receptor (HGFR)

CDK = Cyclin-Dependent Kinase

NF-κB = Nuclear Factor-κB

FASN = Fatty Acid SyNthase

HER-2 = Human epidermal growth factor receptor

MAPK = Mitogen-Activated Protein Kinase

c-MYC = MYeloCytomatosis viral oncogene

VEGF = Vascular Endothelial Growth Factor

ERK = Extracellular Regulated MAP Kinase

JNK-c = Jun N-terminal Kinase

TGF-β = Transforming Growth Factor beta

mTOR = Mammalian Target Of Rapamycin

4EB-P1 = Translation initiation factor 4E-binding protein 1

HNSCC = Head and Neck Squamous Cell Carcinoma

CDKN = Cyclin-Dependent Kinase iNhibitor

RB = RetinoBlastoma protein

EGFR = Epidermal Growth Factor Receptor

PI3K = Phosphatidyl Inositol-3-Kinase

HPV = Human Papilloma Virus

SAHA = Suberoyl Anilide Hydroxamic Acid

PARP = Poly (ADP-Ribose) Polymerase

CXCR4 = C-X-C chemokine Receptor type 4 (CXCR-4) also known as fusin, or CD184 (cluster of differentiation 184)

hTERT = Human TElomerase Reverse Transcriptase

UPR = Unfolded Protein Response

OSCC = Oral Squamous Cell Carcinoma

HCPT = 10-Hydroxycamptothecin, anti-cancer drug

ERα = Estrogene Receptor-α

HIF-1α = Hypoxia-Inducible Factor-α

TNF-α = Tumor Necrosis Factor-α

TRAIL = TNF-Related Apoptosis-Inducing Ligand

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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