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REVIEW

Plant-derived anticancer agents: a promising treatment for bone metastasis

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Bone metastasis is a very frequent complication of advanced cancer, and it remains an incurable disease. Current therapies that have been approved for the treatment of bone metastases delay the occurrence of skeletal-related events and can extend the patient's lifespan by a few years. However, they will not cure or cause the regression of established bone metastases, and new side effects are emerging after prolonged treatment. Thus, new therapies are severely needed. There are compelling evidences from *in vitro* and *in vivo* preclinical studies that support the use of compounds derived from plants to treat several forms of cancers including bone metastasis. More than 25% of the drugs used during the past 20 years were directly derived from plants, whereas another 25% are chemically altered natural products. Still, only 5–15% of the ~250 000 higher plants have ever been investigated for bioactive compounds. There is a growing interest for the study of anticancer drugs with relatively low side effects that target specific key signaling pathways that control the establishment and progression of the cancer metastasis. Therefore, further studies are needed to identify new natural compounds with high efficiency in cancer prevention and treatment. Extensive reviews about plant-derived agents and their use in cancer have been published, but none when it comes to the treatment of bone metastases. Only a few of these compounds have been evaluated for the treatment of bone metastasis; here we describe some of the most prominent ones that are having the potential to reach the clinic soon.

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Plants as a Source of Cancer Therapeutic Drugs

Medicinal plants have been used as traditional treatments for numerous human diseases for ages in several parts of the world. They have also had an important role as a source of effective anticancer agents. Over 60% of the currently used anticancer agents are derived from natural sources such as plants, marine organisms and microorganisms.[1]

Anticancer drugs derived from plants that are currently in clinical use can be categorized into four main classes of compounds: vinca (or Catharanthus) alkaloids, epipodophyllotoxins, taxanes and camptothecins.[2] The search for anticancer agents derived from plants started in the 1950s with the discovery and development of the vinca alkaloids and the isolation of the cytotoxic podophyllotoxins. The vinca alkaloids vinblastine and vincristine were isolated from the Madagascar periwinkle (*Catharanthus roseus* or *Vinca rosea*). These alkaloids and their semisynthetic derivatives block mitosis at the metaphase stage causing cell apoptosis, and they have been used in the treatment of leukemias, lymphomas, Kaposi's sarcoma (KS) and testicular, breast and lung cancers for more than 40 years.[2,3] The podophyllotoxins were isolated from the resin of *Podophylum peltatum*, and they also block mitosis during the metaphase by inhibiting tubulin polymerization-causing apoptosis. However, their use in clinic failed because of their severe side effects. Further studies lead to the development of semisynthetic derivatives of podophyllotoxins such as etoposide and teniposide that inhibit the catalytic activity of DNA topoisomerase II inducing irreversible breaks in the DNA and apoptosis. Etoposide and teniposide are used in the treatment of lymphomas, as well as acute leukemia and small-cell lung, testicular, ovarian and bladder cancers.[4] In 1960, the United States National Cancer Institute (NCI) initiated an extensive plant collection program leading to the discovery of many novel chemotypes including taxanes and camptothecins. The taxanes were isolated from *Taxus brevifolia* and other conifers from the genus *Taxus*. They stabilize the microtubules suppressing their depolymerization to tubulin leading to cell death. Taxanes have been used for the treatment of ovarian

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cancer, metastatic breast and lung cancers and KS.[2,5] The camptothecins are alkaloids isolated from *Campthotheca acuminate*, *Ophiorrhiza pumila* or *Mapia foethida*. Camptothecin inhibits DNA topoisomerase I. The semisynthetic analogs of camptothecin, topotecan and irinotecan, are in clinical use. Topotecan is used for the treatment of ovarian and small-cell lung cancers, whereas irinotecan is used for the treatment of colorectal cancer.[2,5]

Currently, the Natural Products Branch, part of the Developmental Therapeutic Program at the NCI, collaborates with agencies throughout the world to collect thousands of plants and marine organisms for the extraction and screening of antitumor properties of extracts. The NCI is also committed to the conservation of biological diversity. To date, only 1% of the ~500 000 plant species worldwide has been phytochemically investigated.[6] Therefore, it is likely that many new bioactive compounds are waiting to be discovered and developed into treatment for pathologies such as bone metastasis.

Bone Metastasis

Bone metastases are a frequent complication of cancer and can occur in up to 80% of patients with advanced breast or prostate cancer.[7] The consequences of bone metastases are often devastating. Once tumors metastasize to bone, they are incurable and cause severe pain, fractures, spinal nerve compression and paralysis.[8] Tumor cells in bone secrete multiple osteolytic factors that stimulate bone resorption, releasing growth factors from the bone matrix that, in turn, stimulate tumor growth and further bone destruction. This leads to the establishment of a feedforward cycle, which is responsible for tumor growth and invasiveness in bone.[9] The diversity of the pro-osteolytic factors secreted by cancer cells makes it unlikely that therapies targeting a single factor will be effective to treat bone metastases. Currently, antiresorptive drugs such as bisphosphonates are the standard of care for the treatment of patients suffering from skeletal complications and bone metastatic cancer.[10] Bisphosphonates bind to the bone and inhibit osteoclast-mediated bone resorption, thus preventing the release of all the different growth factors contained in the bone matrix. Besides, bisphosphonates inhibit tumor cell adhesion, migration, invasion and proliferation and induce cell death in a wide range of cell lines *in vitro*.[11] Consequently, bisphosphonates reduce skeletal tumor burden in multiple *in vivo* models of breast, prostate and lung cancer bone metastasis.[12] However, bisphosphonate efficiency is more limited in patients, and they are associated with serious side effects such as osteonecrosis of the jaw, as well as nephrotoxicity.[13,14] It is therefore imperative to identify new therapeutic agents that will be (i) more effective in treating bone metastasis, (ii) that can prevent bone metastases and (iii) that have a low toxicity and limited side effects.

Surprisingly, among the large amount of plant-derived anticancer agents reported, only four compounds (plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone), honokiol (3′,5-di-(2-propenyl)-1,1′-biphenyl-2,2′-diol), curcuminoid and halofuginone (7-bromo-6-chloro-3-[3-[(2*r*,3*s*)-3-hydroxy-2-piperidyl]-2-oxopropyl]-4(3*H*)-quinazolinone)) have been tested in bone metastasis, and only two of them (curcuminoids and halofuginone) are currently tested in clinical trials for diverse cancers, but not for bone metastasis. Here we review the properties of these compounds and their effects on bone metastasis. We also include the description of resveratrol (3,4′,5-trihydroxy-*trans*-stilbene), a promising natural agent with high potential for the treatment of cancer and metastasis.

Plumbagin

Plumbagin is derived from the root of *Plumbago zeylanica* also known as Chitrak, an Ayurvedic plant (Hindu traditional medicine) (**Table 1**). The Plumbaginaceae family is found in semiarid regions of the Mediterranean basin and of central Asia. In the United States, it is found on the Pacific coast and it grows in Florida, and from Texas to Arizona. Plumbagin has been shown to exert a broad range of pharmacological properties, including antioxidant, anti-inflammatory, anticancer, antibacterial and anti-fungal activities. The properties are exerted through a variety of mechanisms, including signal transducer and activator of transcription 3 (STAT3) activation,[15] induction of p53 and c-Jun N-terminal kinase expression in human non-small-cell lung cancer cells,[16] inhibition of the nuclear factor-κB (NF-κB)/Bcl-2 pathway[12] and by downregulating the expression of the chemokine receptor CXCR4.[17]

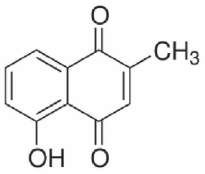
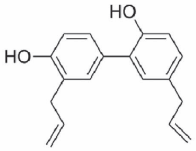
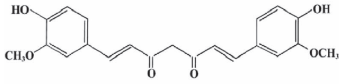
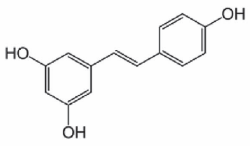
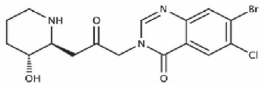
The antitumor effects of plumbagin have been shown in several cancers including squamous cell carcinoma,[18] leukemia[19] and myeloma,[15] as well as breast,[17,20] ovarian,[21] pancreatic,[22] lung,[23] liver[24] and cervical cancers.[25]

In bone, breast cancer cells increase bone resorption by producing factors such as the parathyroid hormone-related protein (PTHrP) and interleukins that stimulate the osteoblasts and increase the production of the receptor activator of NF-κB (RANK) ligand (RANKL). RANKL interacting with its receptor RANK in osteoclast precursors activates the NF-κB signaling pathway inducing osteoclastogenesis.[26] Various tumor cells can also produce RANKL directly causing osteoclastogenesis.[27] Therefore, selective modulation of the RANK/RANKL axis is used for the treatment of cancer-induced bone loss and bone-related diseases such as osteoporosis.

In bone metastasis, Sung *et al.*[28] demonstrated that plumbagin modulates RANK/RANKL signaling, osteoclastogenesis and breast cancer-induced osteolysis. In mouse monocytes, used as osteoclast precursors, plumbagin suppressed NF-κB activation induced by RANKL through sequential inhibition of activation of IκBα kinase, IκBα phosphorylation and IκBα degradation. Plumbagin also suppressed the differentiation of RAW264.7 macrophage cells into osteoclasts induced by RANKL or by human breast cancer cells (MDA-MB-231 and MCF-7) or human multiple myeloma cells (U266).[28] In mice bearing bone metastasis, caused by the intracardiac inoculation of MDA-MB-231 breast cancer cells, a treatment with plumbagin ($2\,mg\,kg^{-1}$) by intraperitoneal injection five times a week during 28 days decreased the size and number of osteolytic lesions.[28] Micro-computed tomography analysis of proximal tibia showed that plumbagin treatment preserves the bone volume and significantly increases the trabecular bone volume .[28] These results suggest that plumbagin can efficiently abrogate RANKL signaling and reduce the development of bone metastases.

Despite the beneficial effects of plumbagin against several cancers and bone metastasis, this molecule has not yet been tested in clinical trials, which may be partly because of some of its side effects. Plumbagin toxicity has been evaluated in

Table1 Plant-derived anticancer agents in preclinical and clinical studies for the treatment of cancer and bone metastases

Molecule	Structure	Preclinical Cancer Studies	Cancer Clinical Trials
Plumbagin 		Leukemia, Myeloma, Breast, Prostate, Ovarian, Pancreatic, Liver, Cervical and skin cancer Breast cancer bone metastasis	No
Honokiol 		Lung, Colon, Liver, Breast and Prostate cancer Prostate cancer bone metastasis	No
Curcumin 		Head squamous cell carcinoma, Myeloma, Pancreatic, Bladder, Breast, Colon and Ovary cancer Breast cancer bone metastasis	Colon cancer Breast Cancer NCT00113841-Multiple Myeloma
Resveratrol 		Neuroblastoma, Myeloma, Breast, Prostate, Colon, Pancreatic and Lung cancer metastasis	Colon Cancer
Halofuginone 		Glioma, Wilms tumor, Hepatocellular carcinoma, Bladder, Prostate, and Pancreatic cancer, Melanoma bone metastasis	Progressive advanced solid tumors HIV—Kaposi's Sarcoma

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rodents. Side effects were dose-dependent and include diarrhea, skin rash and hepatic toxicity.[29] In addition, female rats treated with plumbagin failed to conceive and pregnant rats were more likely to abort when receiving plumbagin.[30] Further studies to characterize the extent of plumbagin's toxic effects are needed.

Honokiol

Honokiol is a bioactive natural product extracted from *Magnolia* spp. The *Magnolia* genus is distributed throughout the world principally in East and Southeast Asia. Extracts from the seed of the Magnolia tree have been used in traditional medicine in China and Japan for the treatment of digestive, anxiolytic and allergic diseases.[31]

Honokiol is a relatively small polyphenol that interacts with proteins from the cell membrane via hydrogen bonds and hydrophobic interactions (**Table 1**).[32] Honokiol targets multiple pathways involved in numerous physiological processes including development, differentiation, immunity, metabolism and cancer.[33] Some of the gene targets are transcription factors such as NF-κB and STAT3 that upregulate the expression of proteins involved in apoptosis.[33] Both NF-κB and STAT3 are also involved in the regulation of hypoxia-inducible factor alpha 1α and vascular endothelial growth factor (VEGF) involved in invasion and angiogenesis.[34] Honokiol promotes the apoptosis of many human cancer cell lines, including lung squamous cancer cells (CH-27 and HL-60), colon cancer cells (COLO 205), liver cancer cells (HepG2) and breast and prostate cancer cells.[35] Honokiol also has anti-inflammatory effects as it decreases the expression of inflammatory mediators, inflammatory genes and the proliferation of lymphocytes induced by lipopolysaccharide.[36]

In bone metastasis, Shigemura *et al.*[36] tested the effects of honokiol alone and in combination with docetaxel, a second-generation taxane that is currently used in therapy for metastatic prostate cancer on human prostate tumor xenografts in the tibia of mice.[36] Honokiol induced the apoptosis of human androgen-dependent and -independent prostate cancer cells

as well as of bone marrow, bone marrow-derived endothelial and prostate stromal cells, *via* the activation of caspase-3, -8 and -9 in a dose- and time-dependent manner. The antitumor effects of honokiol alone or in combination with docetaxel were evaluated *in vivo* in mice harboring tumors from C4-2 prostate cancer cells and treated with honokiol (100 mg kg^{-1} per day) alone or in combination with docetaxel (5 mg kg^{-1} per week) for 6 weeks. Tumor growth and responsiveness to therapy were determined by serum prostate-specific antigen (PSA), X-ray and histomorphometric analysis. Serum PSA was significantly decreased in all treated groups compared with control mice. The combined treatment group had lower serum PSA when compared with docetaxel alone, although the difference was not statistically significant. Honokiol treatment inhibited the growth of C4-2 cells injected in the bones of mice, and the combination with docetaxel enhanced tumor reduction and increased cortical bone when compared with the control group. Immunohistochemical analysis of the tumor in mouse tibia showed that honokiol plus docetaxel treatment markedly decreased cell proliferation and vascular density, and increased apoptosis compared with vehicle-treated mice.[36] These results suggest that honokiol is a promising molecule that could be used as an adjuvant treatment in combination with low-dose docetaxel for the treatment of hormone-refractory prostate cancer and its bone metastases. Honokiol appears to be well tolerated with low toxicity. However, side effects of honokiol include antithrombotic effects that could cause hemorrhage, especially in patients with hemophilia.[32]

Despite the potential benefits of honokiol alone or combined with other anticancer treatments, there are not yet any clinical trials in progress.

Curcuminoids

Turmeric (*Curcuma longa*) is a rhizomatous herbaceous plant of the ginger family from the southeast of India and Asia. Turmeric has been used in India for cooking and in Ayurvedic medicine to treat various common diseases including stomach upset, dysentery, ulcers, jaundice, arthritis, sprains, wounds, acnes and skin and eye infections.[37] The active component of turmeric is curcumin, which possesses diverse pharmacologic effects including anti-inflammatory, antioxidant, antiproliferative and antiangiogenic activities. Curcumin ((1*E*, 6*E*)-1,7-bis-(4-hydroxy-3-methoxy-phenyl)-1,6-heptadien-3,5-dione) is a bis-α,β-unsaturated β-diketone. It is soluble in dichloromethane, chloroform, methanol, ethyl acetate, dimethyl sulfoxide and acetone but insoluble in water (**Table 1**).[38] Several studies have described the anticarcinogenic effects of curcumin used alone or in combination with chemotherapy for the treatment of several cancers including head squamous cell carcinoma and breast, colon, ovarian, pancreatic and bladder cancers.[39] These anticancer properties are attributed to its effects on a variety of biological pathways. Curcumin inhibits the cell cycle and the expression of the tumor suppressor gene *p53* and of various transcription factors (such as Nrf2 and NF-κB) by modulating inflammatory signaling cascades, and it induces apoptosis. Curcuminoids can also regulate gene expression through epigenetic mechanisms. Histone modifications are among the most important epigenetic changes and by altering gene expression they can increase cancer risk. Curcumin is an inhibitor of histone deacetylases, and it induces the apoptosis of numerous cancer cell lines by inhibiting histone and p53 acetylation through specific inhibition of p300/CBP.[40] In addition, curcumin suppress osteoclastogenesis induced by RANKL through the inhibition of NF-κB activation.[41] Curcumin also blocks PTHrP secretion from tumor-like synoviocytes isolated from the joints of rheumatoid arthritis patients that can cause bone loss.[42]

Wright *et al.*[42] demonstrated the effects of curcumin on the secretion of PTHrP and in a mouse model of breast cancer bone metastasis. Curcumin inhibited PTHrP secretion induced by tumor growth factor--β (TGF-β) in MDA-MB-231 human breast cancer cells and reduced the levels of Smad2/3 phosphorylation and Ets-1, a proto-oncogene required for the activation of PTHrP promoter by Smads.[42] *In vivo*, treatment of mice bearing bone metastasis caused by MDA-MB-231 cells with curcumin (25 or 50 mg kg^{-1} every other day) for 21 days decreased the number of osteoclasts at the tumor–bone interface and the size of osteolytic lesions. However, curcumin treatment did not decrease the growth of xenografts in the mammary fat pad, suggesting that curcumin effects are specific of the bone microenvironment.[42]

To date, curcumin has completed several phase I clinical trials to evaluate its effects in different pathologies including multiple myeloma and colorectal, pancreatic, breast and prostate cancers. In advanced colorectal cancer, 15 patients with advanced colorectal cancer refractory to standard chemotherapies consumed capsules with curcumin (0.45–3.6 g per day for up to 4 months). Levels of curcumin and its metabolites in the plasma, urine and feces were analyzed by high-pressure liquid chromatography and mass spectrometry. Activity of glutamine *S*-tranferase and levels of M_1G, a marker of DNA formation, were measured in the blood of patients. The dose of 3.6 g of curcumin daily generated detectable levels of compound and conjugates in plasma and urine, and also caused the inhibition of prostaglandin E_2 (PGE_2) production in blood leukocytes measured *ex vivo*.[43] The administration of 0.5–3.6 g daily for up to 4 months was associated with mild diarrhea as its only apparent toxicity. A phase IIa clinical trial of curcumin for the prevention of colorectal neoplasia was performed in 44 smokers with eight or more aberrant crypto foci (ACF) on screening endoscopy. The effects of oral curcumin (2–4 g per day) on the procarcinogenic eicosanoids PGE_2 and 5-hydroxyeicosatetraenoic acid (5-HETE) were assessed for 30 days by liquid chromatography. Secondary end points included total ACF number and estimate of proliferation in normal mucosa using the proliferation marker Ki-67. Neither dose of curcumin reduced PGE_2, 5-HETE or Ki-67, but there was a significant 40% reduction of the number of ACF in patients treated with a 4 g dose. Curcumin was well tolerated at both doses.[44]

In advanced pancreatic cancer, curcumin was found to be safe and well tolerated in a phase II clinical trial in which 25 patients were given 8 g of curcumin per day orally until disease progression. No toxicities associated with curcumin administration were reported in the patients. A downregulation in the expression of NF-κB, cyclooxygenase-2 (COX-2) and pSTAT3 in peripheral blood mononuclear cells of patients was observed after curcumin intake.[45] Besides, the combination of curcumin with gemcitabine (a chemotherapeutic drug) against advanced pancreatic cancer was evaluated in a phase II trial.[46] Seventeen patients enrolled in the study received 8 g of curcumin orally per day for 4 weeks; gemcitabine was given at a dose of

1000 mg m^{-2}) three times a week intravenously. Nine percent of the patients who were evaluated showed a partial response. Curcumin at a dose of 8 g per day in combination with gemcitabine was well tolerated, but the efficacy of the combinations seemed modest.[46]

In breast cancer disease, docetaxel—a microtubule inhibitor—has been commonly used as a single agent to treat metastatic disease. The tolerability of the combination of docetaxel and curcumin in patients with advanced and metastatic breast cancer was evaluated in a phase I trial.[47] Fourteen patients were enrolled in this study and treated with 100 mg m^{-2} curcumin administered as a 1-h intravenous infusion every 3 weeks for six cycles. Curcumin was given orally starting with 0.5 g per day for 7 consecutive days in a cycle and escalated until a dose-limiting toxicity occurred. The recommended dose of curcumin was 6 g per day for 7 consecutive days every 3 weeks in combination with a standard dose of docetaxel.[47] Other clinical trials are still in progress to test the effects of curcumin on Alzheimer's disease and radiation-induced breast cancer.

Despite the pharmacological safety and efficacy, curcumin has not yet been approved as a therapeutic agent. This may be because of one of its limits: poor bioavailability. First, curcumin is poorly absorbed; it is also rapidly metabolized and quickly eliminated.[42] Therefore, new methods to improve curcumin delivery have been developed and are now being tested in patients, including incorporation into liposomes or into micelles.

Resveratrol

Resveratrol is a polyphenol antioxidant that is produced in various plants in response to environmental stress and pathogenic attack (**Table 1**). Resveratrol has been found in more than 70 species of plants including grapes, berries, plums and peanuts.[48] It has been shown to suppress proliferation and to induce the apoptosis of a wide variety of cancer cells, including melanoma and breast, prostate, pancreatic and lung cancers.[49] Resveratrol has been associated with beneficial effects in preclinical animal studies in various models of cancer and other diseases such as diabetes, arthritis, neurodegenerative and pulmonary diseases, as well as bone loss.[50] *In vivo* studies of mice with DMBA (dimethylbenz[*a*]anthracene)-induced mammary tumors showed that the addition of resveratrol in the diet reduces the incidence and number of tumors.[51] Treatment with resveratrol decreased in tumors the expression of *COX-2*, matrix metalloprotease (*MMP-9*) and *NF-κB* genes associated with tumor growth and invasion. The combination of resveratrol with the isoflavone from soy, genistein, was more efficient than either treatment alone in reducing the tumor multiplicity.[52] In a xenograft model in *nude* mice, resveratrol inhibited the growth of MDA-MB-231 tumor, increasing apoptosis and reducing angiogenesis.[53] In a prostate cancer model using the transgenic TRAMP mice, a treatment with resveratrol showed antitumor activities by increasing the expression of estrogen receptor-β and by decreasing the levels of insulin-like growth factor-I.[54] Sheth *et al.*[55] showed that resveratrol reduces the viability and invasiveness of PC-3 M-MM2 cells *in vitro* and their growth when inoculated subcutaneously in mice. A microRNA array profile of PC-3 M-MM2 cells treated with resveratrol showed that resveratrol decreases the expression of miR-21,[55] a microRNA found to be upregulated in a number of cancers including prostate cancer.[56] Consistently, oral administration of resveratrol in a xenograft model of prostate cancer reduced PC-3 M-MM2 tumor growth and miR-21 levels in the tumors. Resveratrol also reduced the ability of these cells to metastasize to the lungs.[55]

Resveratrol inhibited the growth of myeloma cells (RPMI 8226 and OPM-2) *in vitro* by inducing their apoptosis. In addition, resveratrol decreased the expression of the receptor RANK and the activation of NF-κB in human primary monocytes *ex vivo*. Consequently, resveratrol prevented the differentiation into osteoclasts of human primary monocytes, as well as their resorption, as shown by a decrease of resorption pits on dentin slices *ex vivo*.[57] Besides, resveratrol also had an effect on osteoblasts. When hMSC-TERT cells, derived from human bone marrow mesenchymal stem cells, were treated with resveratrol, there was an increased expression of the osteoblastic markers osteocalcin and osteopontin. Furthermore, resveratrol potentiated the effect of $1,25(OH)_2D_3$ and induced a synergistic increase of osteocalcin and osteopontin. Additional studies showed that modifications to the resveratrol formula could increase its potency. Although these new analogs did not inhibit the proliferation of myeloma cells *in vitro*, they were up to 5000 times more efficient at inhibiting osteoclast differentiation and promoting osteoblast maturation. As there are currently no efficient treatments to increase bone formation, these analogs of resveratrol could be particularly useful to treat patients suffering from bone loss or low bone mass.[58]

In addition, Castillo-pichardo *et al.*[59] showed that polyphenols extracted from red wine inhibit the growth of tumors in the mammary fat pad of mice, as well as the development of metastases to bone and the liver. These grape polyphenols include molecules such as resveratrol, quercetin and catechin that represent about 70% of the polyphenols in red wine. They have been shown to be the most effective anticancer compounds in red wine.[60] Combined grape polyphenols induced cancer cell apoptosis and were more effective than each compound alone to inhibit cell proliferation, cell cycle progression and cell migration of MDA-MB-435 cells *in vitro*.[59] In addition, the combined grape polyphenols inhibited the spontaneous development of metastases to bones, liver and lungs from a fluorescent bone metastatic variant of MDA-MB-435 when inoculated in the mammary fat pad.[59] Although this study was well performed, these results need to be nuanced, as it remains unsure as to which form of cancer MDA-MD-435 cells were derived from.

Several phase I and phase II clinical trials are currently underway for resveratrol at the National Institute of Health mainly in cardiovascular diseases and diabetes. In cancer, a phase I clinical trial to study the pharmacology of resveratrol and its metabolites in patients with colorectal cancer was completed recently.[61] Twenty patients were treated with resveratrol at a dose of 0.5 or 1.0 g before surgical resection. Resveratrol was well tolerated, and daily doses of 0.5 or 1.0 g were recommended to obtain anticarcinogenic effects.[61] Despite these interesting results, there are currently no clinical trials testing the effect of resveratrol on bone metastases.

Halofuginone

Halofuginone is a molecule derived from febrifugine, a natural alkaloid used in the traditional Chinese medicine for the

treatment of malaria (**Table 1**). Halofuginone specifically decreases type I collagen synthesis and has shown antifibrotic, antiangiogenic and antiproliferative effects.[62] Findings from extensive studies in animal models have shown that halofuginone could be effective for the treatment of cancer and fibrotic diseases. Halofuginone decreases the growth of cells from glioma,[63] bladder[62] and hepatocellular carcinoma,[64] Wilms tumor,[65] brain,[66] pancreatic[67] and prostate cancer[68] and bone metastasis from melanoma.[69]

Halofuginone has completed phase I clinical trial in patients with advanced solid tumors.[70] In this study, 24 patients with advanced solid tumors were treated with escalating doses of halofuginone ranging from 0.5 to 3.5 mg per day once or two times daily, orally. The maximum-tolerated dose was 3.5 mg per day with nausea, vomiting and fatigue. The recommended dose for chronic administration was 0.5 mg per day administered once a day orally.[70] In addition, a phase II clinical trial for the use of topically administered halofuginone in AIDS-related KS was performed in 26 patients during 12 weeks, two times daily.[71] KS response was measured locally in lesions treated with halofuginone, as well as the expression of collagen type-I mRNA. Expression of MMP-2, VEGF and KSHV-LANA (Kaposi's sarcoma-associated herpesvirus-latency-associated nuclear antigen) was evaluated by immunohistochemistry in paraffin-embedded biopsies. Halofuginone treatment inhibited collagen type I in KS lesions but it had no effect on MMP-2. Some patients showed delayed antitumor effects; however, the study lacked statistical power to make definitive statements about the efficacy of halofuginone.[71]

The precise mechanism of action of halofuginone still remains unclear and halofuginone could target multiple signaling pathways. Halofuginone inhibits TGF-β-induced phosphorylation of Smad2/3 proteins and increases Smad7 expression.[72] In addition, halofuginone modulates the PI3K/Akt and MAPK/ERK pathways causing the inhibition of Smad3 phosphorylation.[73] In mouse models of pancreatic fibrosis and acute promyelocytic leukemia, halofuginone treatment increased N-terminal phosphorylation of c-Jun kinase, inhibiting TGF-β signaling and cell proliferation.[74] Halofuginone activates the amino-acid starvation response pathway *in vivo*, and it prevents the differentiation of T-helper type 17 cells.[75]

In the past years, our group has been characterizing the effects of halofuginone in preclinical models of bone metastasis. Recently, we published the effects of halofuginone on bone metastasis from melanoma.[69] Halofuginone inhibited TGF-β signaling in melanoma cells, as demonstrated by (i) the dose-dependent reduction of the TGF-β-responsive $(CAGA)_9$ promoter; (ii) the inhibition of Smad2/3 phosphorylation induced by TGF-β; (iii) the induction of Smad7 expression by halofuginone; and (iv) the inhibition of TGF-β-regulated genes *PTHrP*, *CTGF*, *CXCR4* and *IL-11* that encode for prometastatic and osteolytic factors required for the establishment and development of bone metastasis.[76] Halofuginone inhibited melanoma cell proliferation and induced apoptosis *in vitro* in a dose- and time-dependent manner. In a mouse model of bone metastasis, systemic treatment with halofuginone reduced the establishment and the progression of melanoma bone metastases. Histomorphometry showed that halofuginone reduced the number of osteoclasts at the tumor–bone interface, which could be because of decreased expression of pro-osteolytic genes by cancer cells or because of a direct effect of halofuginone on osteoclasts and their precursors.

Halofuginone also reduced melanoma metastasis to the brain, showing that its effects are not limited to bone metastases, unlike the effects of other inhibitors of TGF-β signaling. Therefore, halofuginone is a novel promising drug that could rapidly be brought to the clinic for the treatment of patients with malignant melanoma and associated metastasis to bone and other organs.

Conclusion

Current therapies for the treatment of bone metastases improve the skeletal morbidity but fail to cure or induce the regression of established bone metastases. Therefore, more effective therapies are needed. Medicinal plants have been used for centuries for the treatment of various diseases and a wide number of modern drugs have been developed from them. Of the current anticancer drugs that are commercially available in the United States of America, 60% are of natural origin. However, the study and the development of plant-derived molecules is a long and slow process, and only a small portion of the rich diversity of plants existing on this planet have been studied for their pharmacological properties. One of the advantages of using natural compounds for cancer treatment is that they are multitarget agents. They can block more than one signaling pathway. Besides, plant-derived molecules can be used in combination with other therapeutic agents to increase the efficacy of the treatment and to reduce the probability of developing resistance to treatment. Thus, the discovery of new natural compounds that are effective against resistant tumors is an important strategy to improve cancer treatment that necessitates more attention.

Conflict of Interest

The author declares no conflict of interest.

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