ARTÍCULO DE REVISIÓN / REVIEW ARTICLE

Anti-proliferative effect of terpenes on human prostate cancer cells: Natural sources and their potential role as chemopreventive agents**Efecto anti-proliferativo de los terpenos en células de cáncer de próstata humana: Fuentes naturales y su rol potencial como agentes quimiopreventivos**

ABSTRACT

Prostate cancer is one of the neoplastic diseases with the highest morbidity and mortality in the world. The diversity of available treatments and side effects related to therapeutic treatments are severe and affect a patient's quality of life. Thus, to creating new therapeutic alternatives to reduce morbidity and creating safe and effective therapies is a constant challenge. Recently, the use of traditional medicine and chemoprevention has gained importance. Several clinical and epidemiological studies suggest that a high-terpenoid compound-based diet is associated with a reduced risk of prostate cancer. This review is focused on the anti-proliferative effects of different terpenoids isolated from natural sources on human prostate cancer cells, with the aim of setting the basis to use these compounds as phytotherapeutic, nutraceutical and functional ingredients.

Keywords: Prostate cancer; Plant secondary metabolites; Terpenoids; antiproliferation; Chemoprevention.

RESUMEN

El cáncer de próstata es una de las neoplasias que produce gran morbilidad y mortalidad en el mundo. Los tratamientos y los efectos secundarios de la terapia son severos y afectan la calidad de vida del paciente. Por esto, es necesario crear nuevas alternativas terapéuticas para reducir la morbilidad y generar terapias seguras y efectivas es un desafío permanente. En los últimos años, el uso de la medicina tradicional y agentes quimiopreventivos han adquirido importancia. Estudios clínicos y epidemiológicos sugieren que una dieta basada en altas dosis de terpenoides se asocia a una reducción en el riesgo de presentar cáncer de próstata. Esta revisión se centra en los efectos anti-proliferativos sobre las células de cáncer de próstata en humanos de diferentes terpenoides aislados de fuentes naturales, con el objetivo de establecer las bases para utilizar estos compuestos como ingredientes fitoterapéuticos, nutraceuticos y funcionales.

Palabras claves: Cáncer de próstata; metabolitos secundarios de plantas; Terpenoides; antiproliferación; Quimiopreención.

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INTRODUCTION

Neoplastic diseases are one of the leading causes of death worldwide, especially in developing countries¹. It is expected that the number of cancer deaths will continue to increase worldwide and exceed 13.1 million in 2030. Moreover, it is expected that the number of new cancer cases will increase from 11.3 million in 2007 to 15.5 million in 2030².

Prostate cancer is considered one of the most important medical problems affecting the male population. During the last decade, it has been one of the three leading causes of

death worldwide³. In developing countries, it is the most frequently diagnosed cancer and the third deadliest type⁴. Prostate cancer is more common in elderly men, with six out of ten cases diagnosed in men older than 65 years. It rarely presents before the age of 40⁵.

Prostate cancer treatment is increasingly complex mainly because there are different available therapeutic options for each clinical stage, such as prostatectomy, radiotherapy, and hormone treatment. These options are effective, but treatment side effects are complicated (incontinence and erectile dysfunction, mainly). Thus, strategies aimed at modifying several factors that theoretically favor the development of prostate cancer have been attempted. The role of dietary supplements in the development and progression of prostate cancer has been a frequent topic of discussion and there is increasing interest in lifestyle changes that may help prevent this common disease^{6,7}.

The World Health Organization (WHO) recognizes the importance of medicinal plants in the treatment and prevention of several diseases, as well as their economic relevance since they are a potential source for new drugs that may have a much lower cost. Considering that several compounds present in plants are highly potent and selective⁸, it is interesting to focus efforts to obtain new active ingredients that provide alternative approaches for the development of drugs to treat different diseases. To date, a wide range of dietary factors have been associated with preventing the development of prostate cancer, including genistein from soybeans⁹, flavonolignans¹⁰, vitamin C and E^{11,12}, selenium¹³ and zinc¹⁴.

Terpenes or terpenoids are one of the most widespread groups of secondary metabolites in the plant and animal kingdoms. Several clinical and epidemiological studies suggest that a high terpenoid compound-based diet is associated with a reduced risk of prostate cancer^{15,16}. Their ubiquitous presence in numerous plants used for medicinal purposes and the their high quantities in food provides a high probability to get new selective and less toxic bioactive compounds for the treatment of malignant tumors¹⁷.

This review is focused on the antiproliferative effects on human prostate cancer cells of different terpenoids isolated from natural sources, with the aim of setting the basis to use these compounds as phytotherapeutic, nutraceutical and functional ingredients.

MATERIAL AND METHODS

Literature search

PubMed, Web of Science Literature Databases, Medline and Scielo were searched for articles first published between 1998 and September 2016. For this review, research related to *in vitro* (human prostate cancer cells) and the related action of terpenes were studied. The search for articles was limited to humans, English, and Spanish language. The mesh search terms were: "terpenoid prostate cancer", "terpenes prostate cancer cells", "carotenoid prostate" and the keywords "terpenes", "lycopene", "peryllil alcohol"

and others were paired with: "prostate", "cancer", "cell cycle", "proliferation". Moreover, some official websites about cancer and health, such as National Cancer Institute (NCI), WHO and National Institutes of Health (NIH) were searched. The articles containing information regarding terpene compounds isolated from natural sources and with a specific description of the mechanism of action were selected for this study. As the main goal of this review was to give insights about the specific modes of action of these metabolites, articles describing the effects of extracts from natural sources were excluded.

Current treatment strategies for prostate cancer and their complexity

Prognosis and treatment modality depends on the tumor extension stage (I to IV depending on the degree of spread and metastasis). Radical prostatectomy is generally effective in treating prostate cancer that has not spread and significantly improves overall survival. Patients benefit from radiation therapy techniques, but there is no conclusive evidence of the superiority of one modality of treatment over the other. With prostatectomy, about 5%-10% of patients have incontinence or lack of bladder control after 5 years of treatment, and 0 to 100% impotence (erectile dysfunction) depending on age and surgical technique. In more advanced tumors, radiotherapy and hormone therapy (anti-androgen, orchiectomy) are the main treatment options¹⁸. The most promising chemopreventive agents for prostate cancer are the 5- α -reductase inhibitors (5-ARIs) like finasteride or dutasteride, frequently used to treat bothersome lower urinary tract symptoms associated with benign prostatic hyperplasia and male androgenic alopecia. It has been shown that 5-ARIs reduce prostate cancer risk but may increase the risk of a more serious form of prostate cancer (high-grade or lethal prostate cancer)¹⁹⁻²¹.

Natural products: Opportunities for cancer treatment

As mentioned, there are several treatment options for localized prostate cancer including surgery, radiotherapy and hormone therapy, but the clinical management of advanced prostate cancer is a challenge²². Androgen ablation is one of the most frequently recommended treatment options for prostate cancer, but this treatment is palliative and has a limited scope for hormone-refractory cancer²³. Most cancerous tumors in the prostate grow slowly, thus many prostate cancer patients have developed the metastatic stage at the moment of diagnosis, which is inevitably followed by the hormone-refractory stage after ablation hormone therapy. Further, chemotherapy and radiotherapy are largely ineffective against advanced prostate cancer²²⁻²⁴.

The continuous increase in incidence and failure of conventional therapies against advanced prostate cancer warrant the development of novel agents for the treatment and prevention of prostate cancer. In the last decades, chemoprevention involving natural compounds has emerged as a promising and cost-effective approach to reduce

incidence and morbidity by inhibiting the precancerous events even before the occurrence of clinical disease²⁵. Research suggests that several natural compounds can help in the prevention of prostate cancer or slow down its development, for example, fruit and vegetables, such as tomatoes, pomegranates, cruciferous vegetables, among others²⁶.

Terpenes: anti-proliferative effects

Terpenes, terpenoids, and isoprenoids are the largest group of secondary metabolites (over 40,000 different molecules). These metabolites are insoluble in water and their biosynthetic origin is the Acetyl-CoA or another glycolysis intermediary, being formed by two units of isoprene (a hydrocarbon with 5-carbon atoms)²⁷. Thus, they are classified according to the theoretical units of isoprene (C₅): two units of C₅ are the monoterpenes (C₁₀); three units are sesquiterpenes¹⁵; four units are diterpenes (C₂₀); five units are sesterterpenes (C₂₅); six units are triterpenes (C₃₀); eight units are the tetraterpenes (C₄₀), over eight units of isoprene the molecules are denominated polyterpenes (C_{>40}). Terpenes are present in most green vegetables, soy and crop plants. They are also present in some marine organisms (particularly diterpenes). In nature, they have a role as messengers (alexins), insect anti-alimentary or defense agents²⁶. This function as a chemical messenger is especially important because they can influence the expression of genes in a plant or even influence the gene expression of neighboring plants²⁸.

Terpenes constitute a very interesting group of natural molecules for studies in chemistry, biochemistry, and biology, not only because of their ubiquity and availability (extraction), but also due to their potential health benefits in several pathological conditions. Thus, antihypertensive, antioxidant, anti-inflammatory, gastro-protective, antitumor and cytotoxic activities, to name a few, have been described for this type of compound²⁶. However, most of the studies about terpenes are related to the evaluation of the complete extract from different plants, and only a little research has been done with the terpene isolated from its natural source. We describe the most relevant research associated with the isolated forms of terpenes and their role in prostate cancer. Table 1 summarizes the anti-proliferative effect on prostate cancer cells exerted by each compound described in this review, individual compounds, natural or dietary sources of commonly occurring terpenes and their proposed chemopreventive mechanisms. Likewise, a list of the major human prostate cell lines described here and their characterization are summarized in Table 2.

Lycopene

Lycopene (Figure 1a) is a member of a group of natural pigments known as carotenoids. Carotenoids are synthesized by both plants and micro-organisms and are widely found in the environment, for example, they give color to many flowers, fruits, and vegetables such as *Citrullus lanatus*

(watermelon); *Solanum lycopersicum* (tomato); *Carica papaya* (papaya) and *Prunus pérsica* (peach), among others^{6,29}. The possible molecular and cellular mechanism of action of lycopene as a cancer inhibitor could be related to its antioxidant activity, regulation of antioxidant response elements (AREs), growth factors and signaling pathway modulation (IGF, PDGF, VEGF signaling), and its effects in the cell cycle. A recent study showed that lycopene induces the detention of the cell cycle in the G₁ phase, which underlies its chemopreventive properties³³. β -carotene significantly inhibited the proliferation of esophageal cancer cells in a dose- and time-dependent manner. It is noteworthy that β -carotene in anti-proliferative concentrations was nontoxic to normal esophageal epithelium Het-1A cells, but cell apoptosis analyses showed that it was able to significantly induce apoptosis in esophageal cancer cells cell lines³⁴.

Incubation of the RAS-activated prostatic carcinoma LNCaP cells (see Table 2) with a 24 hour lycopene treatment (2,5-10 μ M) reduced dose-dependently total cholesterol by decreasing 3-hydroxy-3-methyl-glutaryl CoA (HMG-CoA), thus inactivating RAS. This in turn may modulate the activation of nuclear factor (NF)- κ B and consequently, cell cycle arrest and apoptosis induction, as proven by decreased cyclin D1, pAKT levels and changes in the Bax: Bcl-2 ratio. Prostate cancer cells have cholesterol biosynthetic pathways that are resistant to decreased regulation by cholesterol³⁵. Therefore, the inhibition of HMG-CoA reductase by lycopene suggests that lycopene and/or tomato products could be potent anticancer compounds in chemopreventive and therapeutic applications³⁶.

Clinical evidence of a meta-analysis (2015), which summarized the data from 34 eligible studies (10 cohorts, 11 nested case-control, and 13 case-control studies), involved a total of 15,891 cases and 592,479 participants. This meta-analysis shows that dietary α -carotene intake is associated with reduced risk of prostate cancer (RR for dietary α -carotene intake: 0.87, 95% CI: 0.76–0.99)³⁷. As suggested, α and β carotene have been used *in vivo* as potential agents related to the reduction of prostate cancer, with good associations between blood levels of lycopene and is significantly associated with a reduction of prostate cancer risk.

Crocin

Crocin (Figure 1b) is a natural carotenoid that is found in the medicinal herb *Crocus sativus* (safron). Safron extract and crocin exhibit anticancer activity by promoting cell cycle arrest in aggressive prostate cancer cells³⁸ through a significant reduction of N-cadherin, beta-catenin expression and increased expression of E-cadherin. Additionally, crocin inhibits prostate cancer cell invasion and migration through the down-modulation of metalloproteinase and urokinase, showing potential antitumor effects in biologically aggressive prostate cancer cells³⁸. Crocin has an *in vitro* antiproliferative/cytostatic effects in prostate cancer cells with low pro-apoptotic activity³⁹.

Table 1
Effect exerted by each type-terpene compound on Prostate cancer cells and their natural sources.

Publication	Agent	Mechanism of action	Natural source	Cell line
(Overmüller-Jevic et al., 2003), (Palozza et al., 2010) (Tejos-Solis et al., 2013), (Teodoro et al., 2012), (Wei and Giovannucci, 2012);	Lycopene	<ul style="list-style-type: none"> - Cell cycle arrest in G₁ stage. - Anti-carcinogenesis. - Changes on mevalonate pathway and on RAS activation. - Regulation of AREs. - Growth factors and signaling pathways modulation (IGF, PDGF, VEGF signaling). - Inhibition of HMG-CoA reductase with inhibitory effects over the growth of human cancer cell. 	<i>Carica papaya</i> (papaya), <i>Citrus x paradise</i> (pink grapefruit), <i>Solanum lycopersicum</i> (tomato), <i>Citrullus lanatus</i> (watermelon).	LNCaP
(D'Alessandro et al., 2013)	Crocin	<ul style="list-style-type: none"> - Proliferative block in G₀/G₁ phase. - Increases the expression levels of CDK inhibitors. - Decreases the levels of cyclin D1 and cyclin B1. - Increases the levels of Bax and reduce the level of Bcl-2, in a concentration-dependent manner. - Activation of mitochondrial-associated apoptosis due to activation of caspases. 	Medical herb saffron (<i>Crocus sativus</i> L.).	LAPC-4, CWR22, LNCaP, C42B, DU145, PC-3
(Yang et al., 2006)	Celastrol	<ul style="list-style-type: none"> - Inhibits the proteasomal chymotrypsin activity in a concentration-dependent manner. - Increases levels of IκB-α, Bax, and p27 protein. 	Root bark of the Chinese medicine <i>Tripterygium wilfordii</i> Hook F.	PC-3, VCaP
(Sundin et al., 2012)	Perillyl alcohol	<ul style="list-style-type: none"> - Inhibits telomerase activity. 	Oils from <i>Prunus avium</i> (cherries), <i>Officinalis</i> (lavender), <i>Mentha Piperita</i> (peppermint) <i>Apium graveolens</i> (celery seeds).	DU145, PC-3
(Shen et al., 2007)	A cycloartane-type triterpenoid (24-en-1α,2α,3β-triol) and an aliphatic alcohol glycoside (octadecane-1,2S,3S,4R-tetrol 1-O-α-L-rhamnopyranoside)	<ul style="list-style-type: none"> - Inhibits expression of AR. - Decreases the PSA level. 	Resinous exudates of <i>Commiphora opobalsamum</i> .	PC-3, LNCaP, DU145
(Yang et al., 2013)	Spongian terpenoid: Furanoditerpenoid spongia-13(16),-14-dien-19-oic acid.	<ul style="list-style-type: none"> - Inhibits AR transcriptional activity. 	Marine sponges and Shell-less mollusks (nudibranchs) that feed on the sponges.	LNCaP, PC-3
(Kotake-Nara et al., 2005)	Neoxanthin and Fucoxanthin	<ul style="list-style-type: none"> - Decreases the expression of Bax and Bcl-2 proteins, in a time-dependent manner. - Induces caspase-3-dependent apoptosis. 	Neoxanthin: <i>Spinacia oleracea</i> (spinach). Fucoxanthin: <i>Undaria pinnatifida</i> (wakame), <i>Hijikia fusiformis</i> (hijiki).	PC-3, LNCaP
(Li et al., 2012), (Ikezoe et al., 2003)	Oridonin	<ul style="list-style-type: none"> - Decreases the expression of Bcl-2 protein. - Increases the expression level of Bax and Caspase-3. - Autophagy-inducing activity in a concentration-dependent manner. - Increases the expression of LC3 protein. - Induces cell cycle arrest at the G2/M phase. - Increases the expression of P21 protein. 	<i>Rabdosis rubescens</i> .	PC-3, LNCaP
(Zhang et al., 2010), (Kassi et al., 2007), (Zhang et al., 2009)	Ursolic acid	<ul style="list-style-type: none"> - Induction of apoptosis and Bcl-2. - Inhibition of cell invasion by down-regulating matrix metalloproteinase-9 via inhibition of Akt. - Induction of JNK activation resulting in Bcl-2 phosphorylation. 	<i>Rosemarinus officinalis</i> (rosemary), <i>Eriobotrya japonica</i> (medlar), <i>Calluna vulgaris</i> (heather), <i>Ocimum sanctum</i> (basil), <i>Malus domestica</i> (apple tree).	PC-3, LNCaP, DU145

RAS: rat's sarcoma; AREs: antioxidant response element; IGF: insulin-like growth factor; PDGF platelet-derived growth factor; VEGF: vascular endothelial growth factor; HMG-CoA: 3-hydroxy-3-methyl-glutaryl Coenzyme A; CDK: cyclin-dependent kinase; IκB-α: nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha; AR: androgen receptor; PSA: prostate-specific antigen; Bcl-2: B-cell lymphoma 2, LC3: Microtubule-associated protein 1A/1B-light chain 3; Akt: Protein kinase B; JNK: c-Jun N-terminal kinase.

Table 2					
Characteristics of human Prostate cancer and immortalized cell lines associated with terpenes assay described in this review.					
Cell line	Androgen receptor	Androgen sensitivity	5 α -Reductase	PSA	Source
LNCaP	+	AS	-	+	Lymph node metastasis in Caucasian male ⁸⁶
LAPC-4	+	AS	NR	+	Derived from xenograph established from a lymph node metastasis ⁸⁶
CWR22	+	AS	NR	+	Xenograph human primary Prostate cancer cells ⁸⁷
C42B	+	AI	+	++	Metastatic subline of LNCaP, derived after coinoculation of LNCaP and fibroblasts ⁸⁶
DU145	-	AI	-	-	Central nervous system metastasis ⁸⁶
PC-3	-	AI	-	-	Lumbar Metastasis ⁸⁶
VCaP	+	AS	-	+	Vertebral metastatic-cancer of the prostate ⁸⁸
AS: androgen sensitive; AI: androgen independent; NR: non-reported.					

The antiproliferative effects of saffron extract and crocin were evaluated in five different human prostate cancer cell lines (see Table 2) representing different phases in the progression of prostate cancer, specifically: LAPC-4, CWR22, and LNCaP (positive for androgen receptor (AR) and sensible to androgen), C42B (AR-positive and androgen independent), DU145 and PC-3 (AR-negative and androgen independent). After 48 hours of exposure to 0.1-8 mg/mL crocin (trans-crocin-4 and trans-crocin-3 purified from saffron), crocin showed antiproliferative effects (doses-dependent) in LAPC-4, CWR22 and LNCaP cells (measured by cell growth inhibitor assay, flow cytometry analysis of cell cycle distributional and caspase activity), and at a 2mM, the proliferation significantly reduced to 18%, 34.2%, 12%, and 30% in LAPC-4, LNCaP, DU145 and PC-3, respectively³⁹.

Prostate cancer is regulated by AR, which plays a role in the development and progression of the tumor. One relevant aspect in prostate cancer is the conversion from an androgen-dependent state to an androgen-independent (castration resistant) state, which currently has no effective treatment⁴⁰. Prostate cancer presents a phenotype and genotype related with an abundance of proteins including cdk 2, cdk 4, cyclin A, cyclin D1, mTORp-ser2481, and a decrease of p21cip1, p27kip1 and p53 among others⁴¹. Prostate

cancer can sabotage the apoptosis and take control of the proliferation versus programmed cell death, contributing to the progression of cancer. Crocin could block proliferation in the G0/G1 phase, in LAPC-4 and PC-3 cell lines, increasing the levels of p21 and p27, CDK, depleting the levels of cyclin D1 and cyclin B1, and Bcl-2³⁹. This data suggests a strong role of crocin as an anti-proliferative agent, but at present there are no rigorous studies that support these effects in *in vivo* assays in prostate cancer models.

Celastrol

Celastrol (Figure 1c) is a pentacyclic triterpenoid and belongs to the family of quinone methides. It is an active compound isolated from the bark of the root of *Tripterygium wilfordii* (Thunder god vine). It has been used for years in the treatment of autoimmune diseases, chronic inflammation and neurodegenerative disorders^{42,43}. Celastrol has been shown to inhibit the growth of several types of cancer cells and suppress tumor initiation, progression and metastasis in various animal models *in vivo*, including glioma, melanoma, breast, lung, and pancreatic cancer, as well as prostate cancer⁴⁴. When PC-3 cell line was treated with ascendant doses of celastrol (0.5 to 5 μ mol/L) a proteasome inhibition was observed⁴⁵, and 2.5 μ M of celastrol inhibited 55% of total activity (associated with apoptosis induction)⁴⁶. Recently, it

was observed that celastrol treatment down-regulated the expression of IKK α , and NF- κ B pathway in PC-3 cells⁴⁷. In addition, celastrol can significantly inhibit the growth of TMPRSS2/ERG fusion (T/E fusion gene is present in the majority of all prostate cancers) expressing in prostate cancer cells both *in vitro* (VCaP cell line) and *in vivo* (nude mice) through targeting three critical signaling pathways: AR, ERG and NF- κ B⁴⁴, and down-regulate the expression of hERG channel, and arresting in G0/G1 DU145 cells³⁶. The targeting of the proteasome by celastrol suppresses proliferation, invasion, and angiogenesis by inducing the apoptotic machinery and attenuating constitutive NF κ B both *in vitro* and *in vivo*⁴⁴⁻⁴⁷. With the current information, celastrol could thus be developed as a new therapeutic agent for hormone-refractory prostate cancer⁴⁶.

Perillyl alcohol

Perillyl alcohol (Figure 1d) is a monoterpene isolated from the essential oils of *Lavandula officinalis* (lavender), *Mentha piperita* (peppermint), *Prunus avium* (cherries), *Apium graveolens* (celery seeds), and several other plants. In animal studies it has been shown to exhibit a chemopreventive and cytotoxic anti-tumor activity^{48,49}, with regression of pancreatic⁵⁰, mammary, and liver tumors, being a chemopreventive agent for colon, skin, and lung cancer, and as a chemotherapeutic agent for neuroblastoma, prostate and cancer colon^{51,52}.

Perillyl alcohol effects telomerase activity in prostate cancer cells. When it is incubated (400 μ M) for 16 hours with DU145 or PC-3, telomerase activity is inhibited in both cell lines, with decrease in activity of approximately 75% in PC-3 and 91% in DU145⁵³. The study suggest that this compound could be important as a preventive coadjuvant or sensitized agent over the telomerase activity in prostate cancer and be highly useful for intervention of prostate cancer.

A cycloartane-type triterpenoid and an aliphatic alcohol glycoside (octadecane-1,2S,3S,4R-tetrol 1-O- α -L-rhamnopyranoside).

The cycloartane-type triterpenoids (CTT) (Figure 1e) and aliphatic alcohol glycoside (AAG) (Figure 1f) have been isolated from a variety of sources such as the resinous exudates of *Commiphora opobalsamu* (Balsam of Meca), *Commiphora myrrha* (myrrh), *Tillandsia recurvata* (Jamaican Ball Moss), and *Chrysanthemum morifolium* (Florist's daisy). The resinous exudates of these plants have biological value for their anti-inflammatory and antimicrobial effects⁵⁴⁻⁵⁸. They exhibit moderate inhibitory activity against PC-3 cells with IC50 values from 2.26 to 5.7 for CTTs and 7.1 μ M for AAG, but are less active against LNCaP cells with IC50 values of 22.1 and 23.6 μ M, respectively. In addition, CTTs also reduces the viability of DU145 with an IC50 value of 1.67 μ M⁵⁷⁻⁵⁹. CTT induces cell cycle arrest and apoptosis by regulation of Bcl-2, p53 and caspase 3 and 9⁵⁸⁻⁶⁰. The AR protein levels decrease both compounds at 10 μ M (in LNCaP cells), but CTT shows a more potent inhibitory

effect on the expression of AR. Transient transfection was performed to measure the androgen-dependent secretion of prostate-specific antigen (PSA) in order to determine if AAG and CCT are able to block androgen action. The results show that the PSA protein level decreased with the treatment of CCT and AAG, suggesting that androgen action was inhibited. Although CCT is more efficient inhibiting AR expression, it is less effective than AAG in the transient transfection assay⁶⁰. The antiproliferative mechanism of these molecules requires further studies.

Spongian terpenoid: Furanoditerpenoid spongia-13(16),-14-dien-19-oic acid

Spongian diterpenoids or spongian structural derivatives are commonly found in marine sponges and shell-less molluscs (nudibranchs)^{61,62}. The sponges have these products as a natural defense to preserve their corporal mass, often soft and unprotected, in order to act against microorganisms which block the water and food inlet, or against predators⁶¹.

The spongian diterpenoid-type compounds spongia-13(16),-14-dien-19-oic acid (S1) (Figure 1g) and the structurally related semi-synthetic analogues S2 and S3, have been studied for their ability to inhibit AR transcriptional activity. S2 is produced by reducing the furan functionality in Spongia-13(16),-14-dien-19-oic acid and S3 is produced by reducing the 17 β carboxylic acid functionality in Spongia-13(16),-14-dien-19-oic acid. These diterpenoids have anti-androgenic properties including inhibition of both androgen-dependent proliferation and AR transcriptional activity by a mechanism that competes with androgen for AR-ligand-binding domain AR-(LBD) and blocks the essential interaction between the N-terminal domain and the C-terminus LBD of AR (N/C interaction) required for androgen-induced AR transcriptional activity. LNCaP cells treated with 10 μ M S1 or S3 both inhibit androgen-dependent proliferation with S1 being significantly more effective than S3. Results are comparable to bicalutamide, a non-steroidal anti-androgen clinically used to block AR and proliferation of prostate cancer cells, while S2 had no effect. To ascertain the specificity of S1 and S3 for attenuating AR-dependent proliferation, PC-3 cells were employed, because these cells do not express a functional AR, the results showed that none of the compounds inhibit cell proliferation, demonstrating the specificity of action⁶³. The potential activities against prostate cancer of these compounds are mainly related to their capacity to inhibit the androgen-dependent proliferation, which is an interesting line of investigation but limited in their potential action for future *in vivo* assays.

Neoxanthin and Fucoxanthin

Neoxanthin (Figure 1h) is a carotenoid and an allenic xanthophyll. It is the major xanthophyll found in green leafy vegetables such as *Spinacia oleracea* (spinach)⁶⁴. Fucoxanthin (Figure 1i) is a brown seaweed pigment that is found in edible seaweeds, such as *Undaria pinnatifida* (wakame)⁶⁵ and *Hijikia fusiformis* (hijiki)⁶⁶. Both carotenoids have the

characteristic structure of 5,6-monoepoxide and an allenic bond. It has been shown that neoxanthin and fucoxanthin can induce apoptosis in human colon carcinoma (HCT116 cell line) and PC-3⁶⁷. Apoptosis induction might be one of the important biological actions of different carotenoids^{68,69}.

Neoxanthin and fucoxanthin can induce apoptosis in PC-3⁷⁰. After incubation for 48 hours with both compounds, PC-3 cells shows morphological changes and formation of apoptotic bodies. The degree of DNA fragmentation by *in situ* TdT-mediated dUTP nick end labeling (TUNEL) staining and the apoptosis is significantly higher with neoxanthin and fucoxanthin treatment. In addition, in one experiment expression of Bax and Bcl-2 proteins were down regulated at 20 μ M, but Bcl-XL protein remained unaltered. These results suggest that neoxanthin and fucoxanthin induce apoptosis by a mechanism different from the other apoptosis-inducing reagents (such as Indole-3-carbinol (I3C), N-benzyl-N-hydroxy-5-phenylpentamide (BHPP) and Baicalein) (71,72), which modulate the ratios of Bcl-2 and Bcl-XL protein levels to the Bax protein level. Further, the levels of procaspase-3 and PARP decreased, after treatment for 24 h. Therefore, neoxanthin and fucoxanthin induced the caspase-3-dependent apoptosis. The capacity of these compounds to induce apoptosis and cell cycle arrest (associated to Bcl-2 and Bcl-XL) make these compounds an interesting strategy for further *in vivo* analyses.

Oridonin

Oridonin (Figure 1j) is a diterpenoid extracted from the Chinese traditional medicine (*Rabdosia rubescens*). This compound has various pharmacological activities, such as antiinflammatory, antibacterial and antitumor properties^{73,74}. Oridonin inhibits the proliferation of PC-3 cells in a time and concentration-dependent manner. In one study, the level of Bcl-2 was down-regulated significantly in PC-3 cells treated with different concentrations of oridonin (0-40 μ mol/L) for 48 hours but the expression level of Bax and Caspase-3 enhanced with the increased concentration of oridonin⁷⁵. In a recent study, the mechanism of oridonin-induced autophagy in PC-3 cells was studied⁷⁶. A PC-3 cell was treated with different concentrations of oridonin (5, 10, 15, 25, 50 and 100 μ M) for 24 or 48 hours, showing a large number of autophagic bodies and pre-autophagosomal structures with double membrane in cytoplasm, which indicated that oridonin treatment caused formation of acidic vesicular organelles.

In addition, the expression of common autophagy marker proteins such as LC3-I and LC3-II (LC3-I is converted in LC3-II when a pro-autophagic signal is perceived by the cell) has been evaluated. The results suggested that oridonin increases the expression of the LC3 protein and promotes the conversion of LC3-I to LC3-II. Further, the expression of beclin 1 gene has been analyzed. Oridonin promotes beclin 1 gene expression at the transcriptional level in a concentration-dependent manner. The results to date provide evidence for autophagy-inducing activity of

oridonin in PC-3 cells in a concentration-dependent manner.

In LNCaP cells, similar results have been observed⁷⁷. After treatment with different concentrations (10, 25, 40, 60 and 100 μ M) at different times (12, 24, 48 and 72 h), oridonin induced cell growth inhibition in a concentration and time-dependent manner. Oridonin treatment for 24 hours induced an increase of G2/M phase cells, increased the LC3 expression, and levels of LC3-I and LC3-II were up-regulated after 12 hours of treatment. Auto-phagosomes were also observed in the cytoplasm and the apoptotic rate was increased from 5.3% for 24 h to 31% for 48 h. In addition, the expression of P21 increased, likely related to the blocking of 3-MA (a specific autophagy inhibitor) because after 3-MA treatment (co-treatment with oridonin), oridonin-induced autophagy was inhibited and the increase of P21 was blocked. Also, in LNCaP, oridonin inhibit cellular proliferation by block cells in G0/G1 phase by up-regulating p21WAF1 protein (involved in regulation of cycle cell) in a p53-dependent manner⁷⁸. In summary, autophagy might be a possible mechanism to explain the antitumor effect of oridonin against prostate cancer, there are also potential effects of proliferation suppression and the induction of caspase-mediated apoptosis.

Ursolic acid

Ursolic acid (UA) (Figure 1k), a pentacyclic triterpenoid derived from medicinal plants, such as *Rosemarinus officinalis* (rosemary), *Eriobotrya japonica* (medlar), *Calluna vulgaris* (heather), *Ocimum sanctum* (basil), *Malus domestica* (apple tree), among others. UA has been reported to suppress the proliferation of a variety of tumor cells, to induce apoptosis, and to inhibit tumor promotion, metastasis, and angiogenesis^{79,80}. In animal studies, UA has been shown to be chemoprotective, inhibit tumor metastasis and inhibit the development of benign prostatic hyperplasia^{80,81}.

UA has an impact on induction of apoptosis and Bcl-2 in PC-3 and LNCaP cells by inhibition of cell viability at low concentrations (32.6 and 15.7 μ M, respectively)⁸². Also, UA is structurally similar to dexamethasone, but dexamethasone (an anti-androgen/anti-glucocorticoid compound) shows variable effects on the cellular proliferation of PC-3 and LNCaP cells, indicating that the UA action may be a better alternative to glucocorticoids in combined chemotherapies for the treatment of prostate cancer⁸³. Other studies of the role of UA in prostate cancer include the inhibition of cell invasion by down-regulating matrix metalloproteinase-9 via inhibition of Akt treatment of PC-3 with doses of 80 μ M of UA and the induction of JNK activation resulting in Bcl-2 phosphorylation and apoptosis of DU145 cells at 50 μ M of UA, with a apoptotic range of 47.1 ± 1.7 %. Thus, indicating that UA induces typical apoptosis in DU145 cells^{84,85}. The results observed for UA suggest that the suppression of cell growth and the induction of apoptosis would be the mechanism against prostate cancer cell proliferation, and would be an interesting strategy for further *in vivo* analyses.

Figure 1. Chemical structures of all type-terpenoids compounds described. (a) lycopene, (b) crocin, (c) celastrol, (d) perillyl alcohol, (e) cycloartane-type triterpenoid (24-en-1 α ,2 α ,3 β -triol), (f) aliphatic alcohol glycoside (octadecane-1,2S,3S,4R-tetrol-1-O- α -L-rhamnopyranoside), (g) spongian diterpenoid-type (spongia-13(16),-14-dien-19-oic acid), (h) neoxanthin, (i) fucoxanthin, (j) oridonin, (k) ursolic acid.

Other terpenes with a role in prostate cancer

Terpenes obtained from medicinal plants and studied for anti-tumorigenic potential have increased over time. Every year new studies make it possible to find molecules with a role in prostate cancer and the list will continue to grow. Some examples of recently explored molecules are: i) oleanane, which has been proved to inhibit mTor and Akt in PC-3 cells at extremely low concentrations (0.62 to 10 μ M)^{86,87}; ii) tirucallic acid, which inhibits Akt/mTOR signaling and induces simultaneous oxidative stress and apoptosis in PC-3⁸⁸; iii) curcubitacin, which has antiproliferative activity and is a potent inhibitor of cell growth *in vitro* (PC-3 and LNCaP cells), an effect related to disruption of cytoskeletal integrity by actin aggregation and cofilin-actin rod formation leading cell cycle arrest, cytokinesis failure and mitochondrial ROS production with consequent cellular apoptosis^{89,90}; iv) capilliposide (a novel oleanane triterpenoid saponin) has been demonstrated to have a role as cytotoxic and anti-tumor effects over PC-3 cells, associated with the release of cytochrome c from mitochondria and by the activation of caspase pathways⁹¹; v) Linalool (monoterpenoid) induces sub-G1 cell cycle arrest with a significant increase of apoptotic cells. The results showed that treatment with different concentrations of linalool for 48 h led to an increase in the population of prostate cancer cells in the sub- G0/G1 phase (apoptotic population) ($p < 0.01$)⁹². Mechanistically, all the compounds described here have been implicated in a wide range of biological pathways and processes; thus, these compounds are potential candidates for anti-cancer agents.

CONCLUSIONS

Plants and their medicinal potential have been one of the major sources for the discovery of a number of current anticancer drugs used clinically. While there are studies about isolated substances from different species of plants and marine organisms, an active compound or nutrient that acts as a complementary and chemo-preventive agent by itself for prostate cancer has not been found. While the observed results from *in vitro* models show potent effects with specific compounds, in most of the cases these are due to synergic action and the interaction between a large spectrum of micro-nutrients that perform the beneficial action. In this regard, terpenoid-type compounds are especially important, since their effects on health have been demonstrated in different areas and could be of interest as coadjuvant or complementary molecules in current therapies for prostate cancer. These compounds are a very attractive group to continue being explored in *in vivo* models, mainly for their ubiquity and easy extraction.

On the basis of the results presented here, it is evident that these kinds of compounds are a viable alternative to be incorporated in prostate cancer chemopreventive protocols. Thus, the use of terpenoid-type compounds as nutraceuticals and functional foods is a potentially power

tool for chemoprevention.

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