Pharmacological Research 155 (2020) 104689

Contents lists available at ScienceDirect

Pharmacological Research

journal homepage: www.elsevier.com/locate/yphrs

Astaxanthin anticancer effects are mediated through multiple molecular mechanisms: A systematic review

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ARTICLE INFO

Keywords:
Proapoptotic
Cancer prevention
Cell cycle
Malignancy
Survivina
Staxanthin

ABSTRACT

During the latest decades, the interest on the effectiveness of natural compounds and their impact on human health constantly increased, especially on those demonstrating to be effective on cancer. Molecules coming from nature are currently used in chemotherapy like Taxol, Vincristine or Vinblastine, and several other natural substances have been showed to be active in reducing cancer cell progression and migration. Among them, astaxanthin, a xanthophyll red colored carotenoid, displayed different biological activities including, anti-nflammatory, antioxidant, proapoptotic, and anticancer effects. It can induce apoptosis through downregulation of antiapoptotic protein (Bcl-2, p-Bad, and survivin) expression and upregulation of proapoptotic ones (Bax/Bad and PARP). Thanks to these mechanisms, it can exert anticancer effects towards colorectal cancer, melanoma, or gastric carcinoma cell lines. Moreover, it possesses antiproliferative activity in many experimental models and enhances the effectiveness of conventional chemotherapic drugs on tumor cells underling its potential future use. This review provides an overview of the current knowledge on the anticancer potential of astaxanthin by modulating several molecular targets. While it has been clearly demonstrated its multitarget activity in the prevention and regression of malignant cells in *in vitro* or in preclinical investigations, further clinical studies are needed to assess its real potential as anticancer in humans.

1. Introduction

Cancer is the second-leading cause of death in the world, by generation of an uncontrollable proliferation of abnormal cells able to grow *in situ* or also able to overrun contiguous tissues and to extent to other organs in the body, through the circulatory or lymphatic systems [1]. GLOBOCAN estimated that the incidence and mortality worldwide are continuously rising year by year reaching 18.1 million new cases and

Abbreviations: Akt, protein kinase B; anti-PCNA, proliferating cell nuclear antigen; Bax, Bcl-2 associated protein; Bcl-2, B-cell lymphoma 2; BDNF, brain-derived neurotrophic factor; bw, body weight; CdC2, cell division cycle 2; CDK, cyclin-dependent kinase; COX-2, cyclooxygenase-2; DMH, dimethyl hydrazine; ECM, extracellular matrix; EMT, epithelial-mesenchymal transition; ERK, extracellular signal-regulated kinases; FAK, focal adhesion kinase; Fas-L, Fas ligand; FoxO3, Forkhead box O3; GSK-3β, Glycogen synthase kinase 3; GSTM2, glutathione S-transferase M2; HAS, human serum albumin; HDL, high-density lipoprotein; hENT1, human equilibrative nucleoside transporter; HO-1, Heme oxygenase; HPV, human papilloma virus; HSV, herpes simplex virus; IAP, inhibitor of apoptosis proteins; IKKβ, inhibitor of nuclear factor kappa-B kinase; IL-, Interleukin-; JAK1, Janus kinase 1; MAPK, mitogen-activated protein kinase; miRNAs, microRNA; MMP, matrix metallo proteinases; mTOR, Mammalian target of rapamycin; NF-kB, nuclear factor kappa-light-chain-enhancer of activated B cells; NMBA, *N*-nitro-somethylbenzylamine; NQO1, NAD(P)H quinone oxidoreductase 1; Nrf2/ARE, nuclear factor-erythroid 2-related factor 2/antioxidant response elements; p70S6K, p70 ribosomal protein S6 kinase; pBCEC, porcine brain capillary endothelial cells; pCNA, proliferating cell nuclear antigen; PI3K, phosphatidylinositide 3-kinases; PPAR-γ, peroxisome proliferator-activated receptor; PSA, Prostate Specific Antigen; ROS, oxygen reactive species; RRM1-2, ribonucleotide reductase subunit M1 and M2; RTKs, receptor tyrosine kinases; SIRT1, sirtuin 1; SOD, superoxide dismutase; STAT3, Signal transducer and activator of transcription 3; TGF-, transforming growth factor-; TLR-4, toll-like receptors 4; TNF, tumor necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand; VEGF, Vascular Endothelial Growth Factor; VEGFR2, Vascular Endothelial Growth Factor receptor; Wnt, Wingless-INT; XPC, xeroderma pigmentosum complementation group C; ZEB1, Zinc finger E-box binding homeobox 1

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https://doi.org/10.1016/j.phrs.2020.104689
Received 28 November 2019; Received in revised form 4 February 2020; Accepted 10 February 2020
Available online 11 February 2020

9.6 million deaths in 2018. Moreover, the incidence to develop cancer in men is higher than women (one in 5 men and one in 6 women worldwide) as well as the mortality (one in 8 men and one in 11 women) [2]. Worldwide, it is estimated to be 43.8 million the number of people alive within 5 years of a cancer diagnosis (the 5-year prevalence). For decades, surgery, chemotherapy, radiotherapy and molecular target therapy represented the only antitumoral strategies available. Nowadays, significant efforts have revolutionized these traditional cancer treatments with the advent of promising strategies as cancer immunotherapy and precision medicine. Nevertheless, chemotherapy is still widely used and the importance of chemical compounds, in particular coming from natural sources, is widely recognized, like Taxol, Vincristine and Vinblastine. Moreover, the interest on the comprehension of the mechanistic aspects that lead to the beginning and the growth of cancer is increasing [3–5].

Natural products are gaining success in our society and almost half of the best-selling pharmaceuticals were derived or inspired from nature. Several testing procedures as preclinical, clinical phases I to III, and preregistration, included 547 FDA-approved natural products and derivatives. They derived mainly form plants (47 %), bacteria (30 %), and fungi (23 %) [6].

Among natural substances, several have been demonstrated to act specifically on biological targets involved in prevention or treatment of malignant cell as astaxanthin (3,3′-dihydroxy-ß,ß'-carotene-4,4′-dione) (Fig. 1). It was discovered for the first time in lobsters, in 1938, and it was employed in aquaculture for the pigmentation. In 1991, it gained approval as a supplement for food, thanks its antioxidant features, biological and physiological activities [7]. The polar-nonpolar-polar layout allows to astaxanthin to precisely fit into the cell membrane [8,9]. Moreover, the hydroxyl (OH) and the carbonyl (C = O) present in each ionic ring of astaxanthin make the molecule more polar, esterifiable, and highly antioxidant [10].

In particular, astaxanthin is one of the most important terpene unsaturated compound [11] derived from *β*-carotene by 3-hydroxylation and 4-ketolation, catalysed by *β*-carotene hydroxylase and *β*-carotene, in both ionone end groups, changing the colour from yellow to red [12]. This reaction occurs naturally only in living organisms, such as few bacteria, fungi, and some unicellular green algae, because the ketolation is limited to these species [12,13]. There are many different natural molecular isomers of astaxanthin with unique three-dimensional shape (Fig.1). In detail, the presence of two stereogenic carbon atoms in C3 and C3′ position give three stereoisomers for astaxanthin: a pair of enantiomers (3*S*,3′*S*- and 3*R*,3′*R*-astaxanthin) and an optically inactive *meso* form (3*R*,3′*S* astaxanthin) [14,15]. Given the higher stability, the predominant natural astaxanthin form usually used as a dietary supplement and in clinical trials is the all-*trans* 3*S*,3′*S* astaxanthin [8,15]. Nowadays, a large proportion of astaxanthin is synthetically produced starting from the ketoisophorone obtained from petroleum [10]. Only a small part of commercial astaxanthin is extracted from *Heamatococcus pluvialis* and *Xanthophyllomyces dendrorhous*, providing the bioactive 3*S*,3′*S* stereoisomer [12]. In astaxanthin-producing organisms, the free form of the molecule is uncommon because it is particularly unstable and susceptible to oxidation; in fact, the most common form of astaxanthin is present as acyl monoesters or diesters (esterified with one or two fatty acids such as palmitic, oleic, stearic or linoleic acid) or conjugated with proteins or lipoproteins. In *H. pluvialis*, the 99 % of astaxanthin is as acyl monoesters and diesters, which make up astaxanthin currently available in commercial field [8].

Indeed, the microalgae *H. pluvialis* represents the primary natural source of astaxanthin containing up to 4% of the total cellular dry weight of 3*S*,3′*S* isomer. It is commonly used for human applications such as food and beverages, dietary supplements, and cosmetics; in fact, in humans, astaxanthin intake derives almost exclusively from seafood [8]. Other natural sources are represented by *X. dendrorhous* (*Phaffia rhodozyma*) and *Zantophyllumiss dendorhousis* yeasts, *Chlorococcum* sp., *Chlorella zofingiensis* algae, marine *Agrobacterium aurantiacum* bacterium and other marine organisms such as salmon, lobster, crab and shrimp [7]. On the other hand, the synthetic form of molecule obtained from yeast (mutated *X. dendrorhous*) and bacteria sources (aerobic bacteria *Paracoccus carotinifaciens*) are predominantly used in the aquaculture sector [16]. Astaxanthin displayed several interesting biological activities including, proapoptotic, anticancer, antioxidant, and antinflammatory effects, alleviating endothelial dysfunction, but its role in the prevention, effectiveness and regression on malignant cells is

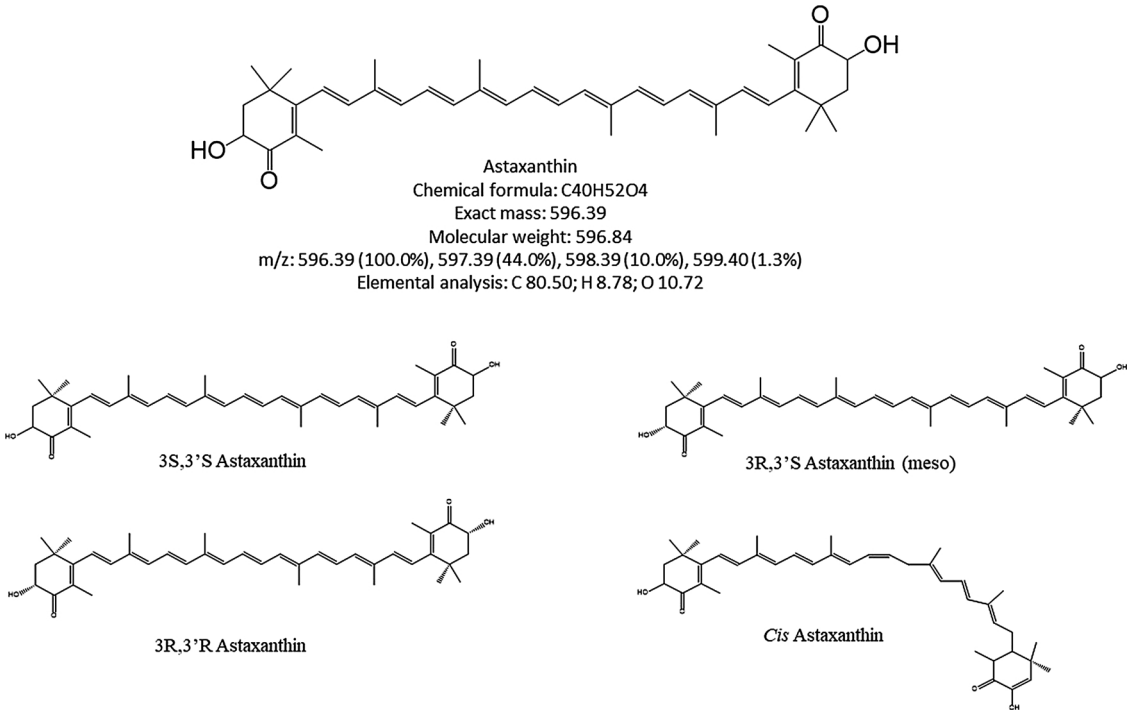

Fig. 1. Astaxanthin and isomers.

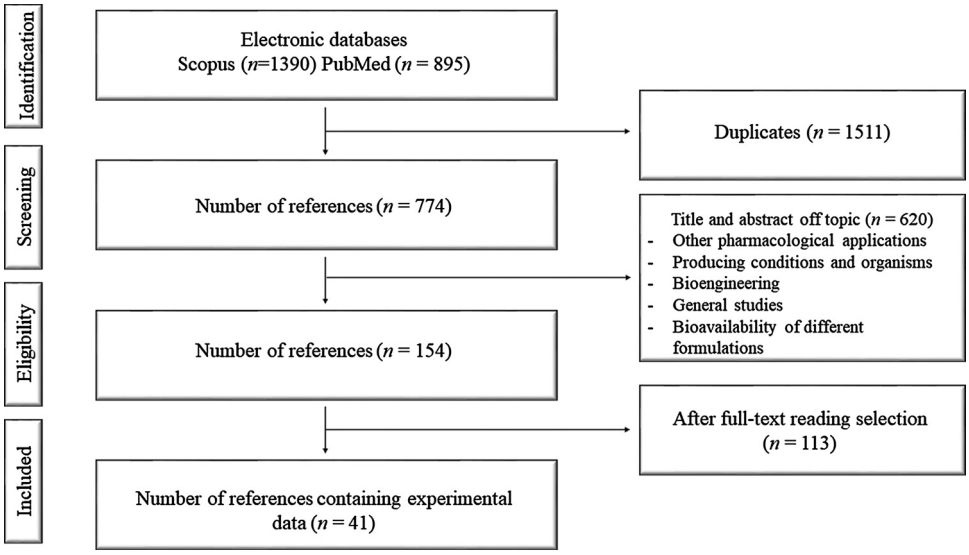

Fig. 2. Flow diagram of the systematic review literature search results based on PRISMA statement.

still under investigation [9].

In several cancers there is a deregulation of different pathways, these are responsible of cancer cell hyperproliferation, diffusion and consequently on their subsistence. In cancer, several aberrant signaling pathways contribute to its pathology, and a multi-targeted treatment could be able to modulate different pathways and up or downregulate different genes [17]. Biologically active natural products are able to target several signaling pathways simultaneously, showing advantageous in cancer therapy.

This review provides a detailed and comprehensive knowledge of the recent achievements in the field of astaxanthin mechanistic effects on the different phases of cancer prevention, malignant cell transformation and therapy analyzing the available research papers available up to now (Fig. 1).

2. Study analysis

The diagram describes the selection process of the bibliographic sources (Fig. 2). The initial search provided 2285 studies (1390 from Scopus and 895 from PubMed), out of which 1511 were duplicates among all keywords used. Out of those 774 studies, 619 were off topic basing on the exclusion criteria. Out of those 154, 113 do not contain experimental data about effective concentration. The final reference list of 41 articles comprises 6 major countries [China 24.5 %, Japan 20.4 %, India 18.4 %, Usa 12.2 %, South Korea 10.2 %, others (Iran, Italy, Malaysia, Taiwan, Thailand) 14.3 %] as shown in Fig. 3. Statistical analysis showed that the considered references have been published

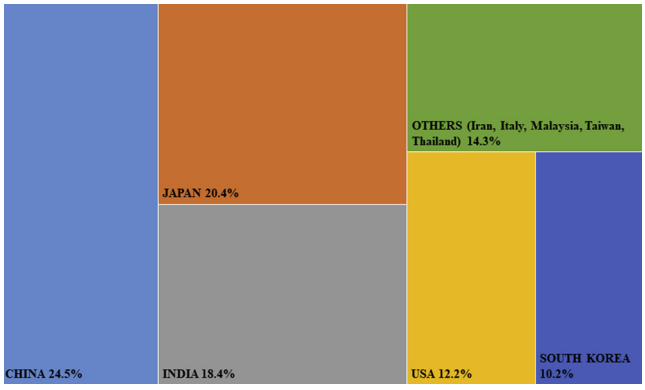

Fig. 3. Representation of distribution of author origin country.

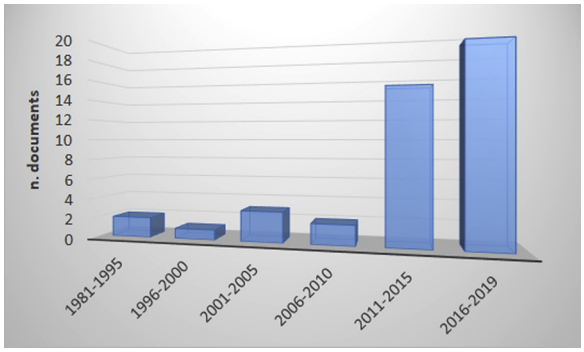

Fig. 4. Number of works published on astaxanthin and cancer.

mostly in the last few years (Fig. 4). Astaxanthin anticancer effects have been evaluated *in vitro* on several cell lines. Among them, eight cell lines belong to colon carcinoma, and HCT116 were the most studied (n = 3). Six different hepatocarcinoma cell lines have been studied, while no *in vitro* studies have been reported on tongue and urinary cancer. Regarding these tumors, 2 studies on rats and 1 study on mice have been analyzed. Moreover, *in vivo* studies regard breast carcinoma (n = 2), colon carcinoma (n = 2), and hepatocarcinoma (n = 3). Our systematic review included 27 *in vitro* studies and 14 *in vivo* reports. *In vitro* studies included biochemical and molecular analysis, comprising colorimetric and enzymatic assays, western blot, flow cytometry analysis, and immunofluorescence techniques. *In vivo* experiments were carried out on animal model as mice, rats and hamster in order to determine tumor weight, metastasis formation, and apoptosis index. Regarding the methodological quality assessment, the *in vivo* studies were selected *via* standard checklist for preclinical trials [18]. As reported in Fig. 5, all studies describing the objectives, outcomes and main findings have been considered in this review. In addition, doses, routes of administration and frequency were cited, but none of the studies showed sample size calculations and no information on blinding was reported. Basing on this assessment, 5 (35.7 %) studies established that the allocation was randomized, without describing how they performed it. Studies on randomization of animals, blinding and reported outcome measurements were generally considered of higher methodological quality. Limitations of these studies are due to the lack of these important information and the unclear results; the experimental design of some animal studies might be improved.

To better describe and understand *in vivo* and *in vitro* results of

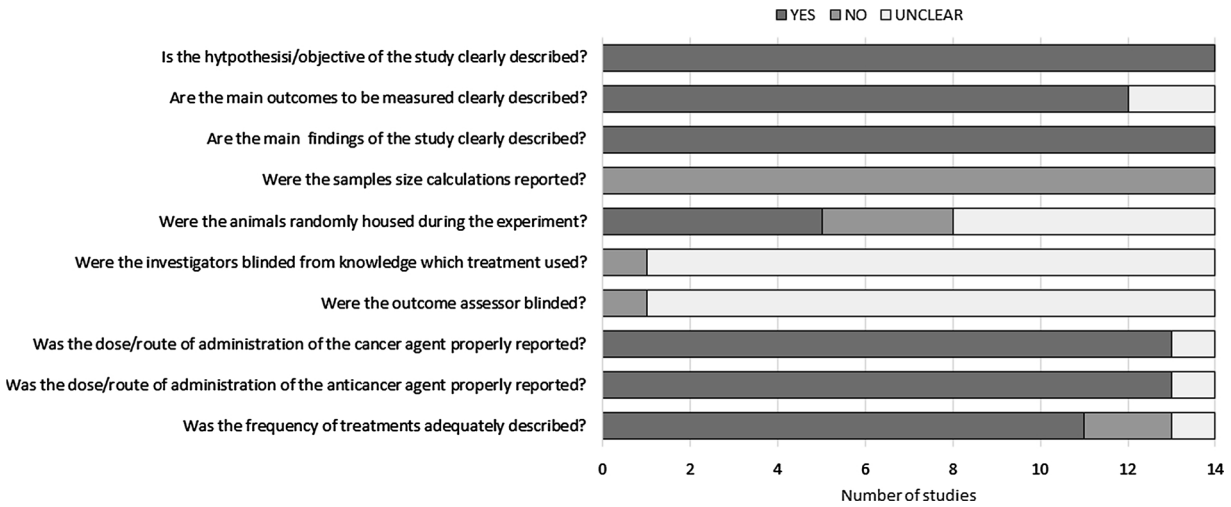

Fig. 5. Methodological quality assessment of included studies. Dark gray bars indicate the proportion of articles that met each criterion; light gray bars indicate the proportion of studies that did not and white bars indicate the proportion of studies with unclear answers.

anticancer properties of astaxanthin, we summarized them as reported below.

3. Astaxanthin pharmacological properties/anti-cancer effects *in vitro*

It is broadly accepted that elevated inflammation levels, increased oxidative stress, aging, as well as mitochondrial dysfunctions are responsible for major chronic degenerative diseases [19,20]. Due to its antioxidant potential, astaxanthin has gained considerable importance as a valuable therapeutic candidate. It shows a wide field of clinical applications, sharing the same oxidative and inflammatory etiology. Among them, the most relevant are neuroprotection, gastric ulcer care, stroke injury amelioration, kidney and liver protection, which are strictly related with cancer prevention. In fact, astaxanthin treatment reduces the release of interleukins, COX-2 and nitric oxide in lipopolysaccharide-stimulated BV2 microglia [21] and pretreatment with astaxanthin (50 μM) improves stretch injury in astrocyte model by reducing inflammatory mediators [22]. The subcellular location of astaxanthin revealed its preference for the mitochondrial membrane, suggesting its role in modulating cytochrome *c* and pro-apoptotic factors release and, thus, the protective role against oxidative stress. Astaxanthin, indeed, by activating PPAR-γ and catalase, decreases mitochondrial and intracellular oxygen ROS levels and IL-8 expression in gastric epithelial cells [23]. The ability of astaxanthin in the modulation of many inflammatory-related pathways is responsible of its preventive effects on ischemia-reperfusion injury in steatotic liver cells [24], as well as against liver fibrosis in human LX-2 cells [25]. Furthermore, the activation of Nrf2/ARE signaling by astaxanthin explains the marked attenuation of renal fibrosis in glomerular mesangial cells [26], increasing the transcription of the activity of the phase II detoxifying/antioxidant enzymes activities, such as NAD(P)H, NQO1, GSTM2, HO-1 and SOD and reducing the incidence of neoplastic transformation [1]. Moreover, it has been demonstrated that astaxanthin attenuates acute kidney injury and reduces apoptosis in tubular epithelial cells, through the inhibition of the histone deacetylase SIRT1 [27] and the related FoxO3 transcription factor [28]. Astaxanthin may also improve the nephropathy-related complications, as well as peritoneal fibrosis, limiting epithelial-mesenchymal transition, thanks to the anti-inflammatory, antioxidative and mitochondrial ROS scavenging activities in peritoneal mesothelial cells [29]. The enhancement of the antioxidant defense system, as well as superoxide dismutase and catalase, has been also demonstrated [30].

Given this evidence, it is easily intuitable that astaxanthin can exert anti-cancer effects, as cancer stems from an unbalance in oxidative status, starting with a trigger inflammatory lesion. Oxidative stress, indeed, is strictly linked to carcinogenesis, due to the related cellular damage that can occur, deriving from DNA mutations and protein aberrations. Since ever, the chemopreventive properties of natural substances have been recognized. In particular, since the '90 s years, the researchers focused the attention on the relationship between natural xanthophylls, like astaxanthin, and cancer, demonstrating protective effects against well-known cancerogenic B1 aflatoxin, through the activation of detoxification mechanisms [31] and against tongue and large bowel carcinogenesis [32]. These properties have been mainly ascribed to the radical scavenging activity [33] and reduction of superoxide and nitric oxide generation [34] back then. It has been demonstrated that astaxanthin exhibits higher antioxidant properties rather than α-tocopherol and β-carotene [35] and reduces DNA damage deriving from UVA radiation exposure [36]. Evidence of the decrease of oxidative markers at doses of 10 nM, in rat kidney fibroblasts, in 1998, has led to the study of this carotenoid as candidate to contrast skin photodamage from the past [35] to date [37], also considering its non-toxicity on normal human dermal fibroblasts [38], probably due to the non-interaction with tyrosinase activity [39]. By contrast, astaxanthin demonstrated to promote the reepithelization of wounds, by increasing the number and the recruiting of keratinocytes in a dose-dependent manner [40]. Recently, researchers have been focused on the role of astaxanthin towards skin cancer, finding that it may inhibit the neoplastic transformation of mouse epidermal cell line JB6 P + cells, with a decrease of cell viability by up to 94 % after five day-treatment with 50 μM of astaxanthin [41]. These results confirmed the study of Chen, et al. [42] on two melanoma cell lines, A375 and A2058, who showed inhibition of proliferation with 168 μM astaxanthin by up 50 % and 80 %, respectively. The antiproliferative activity of astaxanthin extends to multiple cancer lines, including human hepatocarcinoma (CBRH-7919), rat breast cancer cells (SHZ-88), and mouse Lewis lung carcinoma cells, with higher sensitivity for the first cell line, with IC_{50} values around 39 μM [43]. As regards this last tumor type, astaxanthin can suppress cancer proliferation also in two human non-small cancer lung cells, bronchioloalveolar cell carcinoma (A549), and squamous cell carcinoma (H1703), reducing, with a concentration of 20 μM, the cell colony-forming ability by 50.29 % and 39.71 %, respectively [44]. The beneficial implications of astaxanthin on liver cancer development have been also investigated in other human hepatoma cell lines, reporting that astaxanthin significantly inhibits cell growth in HepG2 cell line at a concentration of 42 μM [45], while in LM3 and SMMC-7721 with IC_{50} = 100 μM [46]. However, all reported effects are dose- and time-

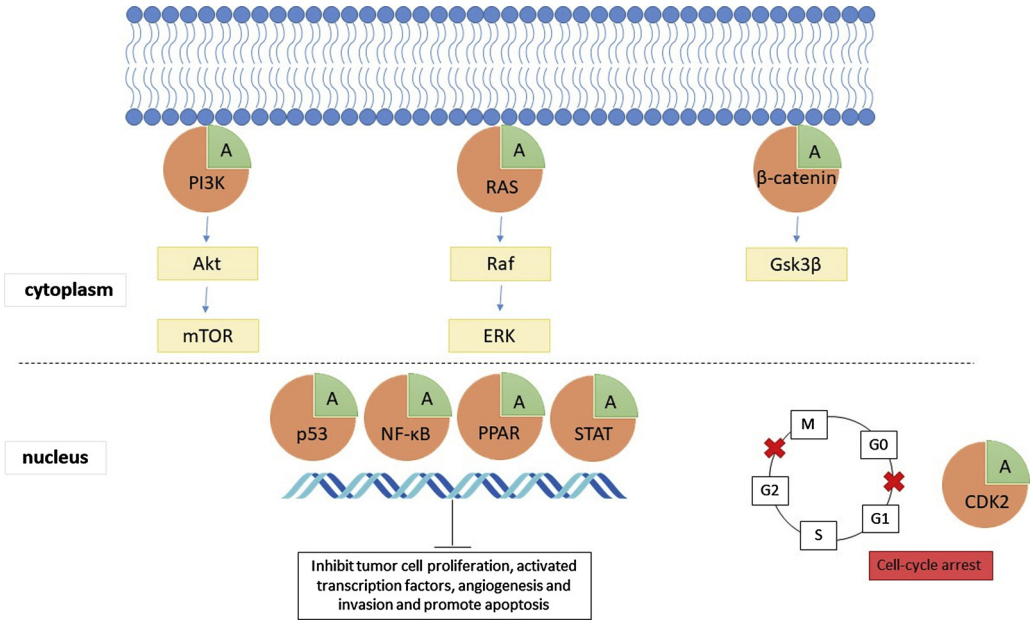

Fig. 6. Multiple mechanisms of astaxanthin for cancer therapy.

dependent, as it is clearly evident in a study conducted on hepatoma cells (H22), in which low- (20 μM) and high-dose (40 μM) of astaxanthin have been tested after 12 and 24 h, showing that the inhibitory effect is more pronounced in high-dose group and it increases with time. Interestingly, the lowest dose of astaxanthin (20 μM) inhibited cell growth with the same efficacy of cisplatin, a conventional anticancer drug, used as control [47]. By *in silico* molecular docking, Suganya and Anuradha [48] demonstrated that astaxanthin can reach higher scores compared with that obtained with a standard chemotherapic agent, sorafenib, in hepatocellular carcinoma. Previous results report time- (24 − 72 h) and dose-dependent (4 − 16 μM) antiproliferative properties of astaxanthin against HCT116 and HT-29 [49], as well as of the entire *H. pluvialis* extract (5–25 μg/mL). This extract showed 100 % inhibition on colon cancer cell viability at concentrations of 0.1 mg/mL, with a lethal concentration in 50 % of cells of 27.03 μg/mL lower than that of doxorubicin (37.6 μg/mL), used as control [50]. At the same time, these studies deny earlier results that do not ascribe a strong inhibitory potential to astaxanthin (IC_{50} > 50μM), when compared to that of other natural antioxidants, like curcumin (IC_{50} = 17.6 μM) and resveratrol (IC_{50} = 15.8 μM) [51]. The toxicity of astaxanthin on normal human oral keratinocytes (NOKs) has been investigated in parallel with the chemopreventive effects on colorectal adenocarcinoma cells (Caco-2), concluding that concentration of astaxanthin between 8 − 16 mM may inhibit Caco-2 cells proliferation, being at the same time not harmful to NOKs [52]. Furthermore, even nanoemulsions containing 25 μg/mL of astaxanthin may decrease by up 50 % the cell viability of HT-29 and stomach gastric cancer (AGS) cell lines [53]. By contrast, another study reports that the viability of AGS was not affected by astaxanthin administration, whereas other gastric carcinoma cell lines, KATO-III and SNU-1, showed a dose-dependent decrease [54]. Further studies will be necessary to establish if that depends on the bioavailability of formulation or other mechanisms are involved. In 2 human esophageal cancer cell lines (TE-1, TE-4), concentrations of astaxanthin higher than 13 μM in TE-1 cell line and between 42–84 μM in TE-4 inhibited cancer cell proliferation [55]. Moreover, in leukemia cells (K562), astaxanthin revealed the most powerful cell growth inhibitor among other tested carotenoids, with a strong activity even at low concentrations (5 and 10 μM) [56]. Even if at higher concentrations, 24 h of astaxanthin treatment may suppress the viability of oral squamous carcinoma (SCC131 and SCC4) cells with IC_{50} of 700 μM and 720 μM, respectively [57]. The multiple mechanisms at the basis of the anticancer activity of astaxanthin also include epigenetic modifications and chromatin remodeling. In prostate cancer, the control of oncosuppressor gene silencing by DNA methylation is a key strategy to protect cells from carcinogenesis [58]. In addition, the prevention and treatment of prostate carcinoma pass through the suppression of 5-*alpha*-reductase enzyme. Many natural sources showed to inhibit this enzyme activity. Among them, astaxanthin is linked to a reduction of 98 % in 5-*alpha*-reductase activity at 502 μM *in vitro,* in association with a cancer growth inhibition of 24 % at 0.2 μM [59]. Although from first observation astaxanthin seemed not significantly suppress the cell viability of breast cancer (MCF-7 and MDA-MB-468) cells [60], further investigation has demonstrated the cytotoxic activity of *H. pluvialis* extract on breast adenocarcinoma [50] suggesting a possible synergistic effect. Another study evaluated the astaxanthin nanoparticle anticancer potential against MDA-MB-231, revealing IC_{50} = 84 μM [61], while astaxanthin induces cell death in MCF-7 at concentrations of 33 mM [62]. These inconsistencies may be probably due to the synthetic origin of astaxanthin, which revealed 90 fold less potent that natural astaxanthin as demonstrated by Régnier, et al. [63] on human umbilical vein endothelial cells. However, astaxanthin has been recognized as breast cancer chemopreventive agent, [62], on the other hand, astaxanthin does not present toxicity on normal breast epithelial cells [64]. Astaxanthin also revealed an excellent adjuvant to conventional therapies, thanks to its capability to reduce irradiation-related cytogenetic effects on human peripheral blood lymphocytes at concentrations of 20.0 μg/mL [65] and for its photothermal effect. Considering its chemical structure, indeed, xanthophylls may strongly absorb the light and convert into heat in cancer cells, as demonstrated in squamous cell carcinoma (VX2) and macrophage (246.7) cell lines. This treatment led to the reduction of adverse reactions and a more site-specific action [66]. In conclusion, astaxanthin has revealed anticancer activity by multiple mechanisms, as well as apoptosis induction, cell growth inhibition, and cell cycle interferences that will be clarified in the following sections (Fig. 6, Table 1).

4. Astaxanthin induces cell cycle arrest in tumor cells

Cell cycle machinery drives and controls cell proliferation, but what happens if something goes wrong? The elucidation of the potential mechanism involved in cell viability inhibition and the changes in cell cycle progression of cancer cells are an increased investigated topic.

Table 1
Description of the main mechanisms involved in anticancer activity and effective dose in different *in vitro* and *in vivo* experimental models.

Cancer	*In vitro*		*In vivo*		Mechanisms	Ref.
	Cell line	*Cytotoxic concentration*	*Experimental model*	*Dose*		
Breast carcinoma	MCF-7	IC_{50} = 20 ± 0.2 μM			↓ cyclin D1; ↑ cell number in G0/G1 phase; ↓ migration; ↑ apoptosis; ↑ p53; ↓ Bax; ↓ Bcl-2; ↑ ROS	(64, 67, 94)
	MDA-MB-231	IC_{50} = 84 μM			↑ apoptosis; ↓ migration	(61)
			Sprague-Dawley rats	50 % (45.5 ± 0.6 mg)	↑ adiponectin	(143)
			Mice	25 mg (single dose)	↓ microelements in tumor site	(144)
Cervical adenocarcinoma	HeLa	IC_{50} ~ 50 μM			↑ cell number in G2/M phase	(80)
Colon carcinoma	HCT116	IC_{50} = 10-16 μM			↑ cell number in G2/M phase; ↑ p21; ↑ p27; ↑ p53; ↓ CDK4 and CDK6; ↑ apoptosis; ↑ caspase-3; ↑ PARP; ↑ EGFR; ↑ p-p38; ↑ p-JNK; ↑ pERK1/2; ↓ invadopodia; ↓ EMT; ↑ E-cadherin; ↓ vimentin; ↓ cortactin; ↓ MMP2; ↑ miR-29a-3p; ↓ ZEB1; ↓ MYC; ↓ cyclin D1	(49, 76, 108)
	CT-26	50 - 100 μM			↓invadopodia; ↓EMT; ↑ E-cadherin; ↓ vimentin; ↓ cortactin; ↓ MMP2; ↑ miR-29a-3p; ↓ ZEB1; ↓ MYC	(108)
	HT-29	IC_{50} = 10-16 μM			↓ cell viability	(49)
	CaCo-2	IC_{50} = 8.4 - 16.8 mM			↓ cell viability	(52)
	LS-174, WiDr, SW-480	53.0 ± 3.1 % (41.9 μM) 37.8 ± 2.1 % (41.9 μM) 40.5 ± 2.2 % (41.9 μM)			↓ cell viability	(76)
	LS-180	100 - 200 μM			↑ apoptosis; ↑ Bax; ↑ caspase 3; ↓ Bcl2	(93)
			Db/db mice	340 μM	↓ oxidative stress	(146)
			Wistar male rats	15 mg/kg	↓ ERK-2; ↓ NFkB; ↓ COX-2	(147)
Esophageal carcinoma	ECA 109 T13	IC_{50} = 295.3 μM IC_{50} = 216.1 μM			↑ cell number in G2/M phase; ↓ cyclin B1; ↓ Cdc2; ↑ apoptosis	(79)
	TE-1	IC_{50} > 13.4 μM			↓ cell proliferation	(55)
	TE-4	IC_{50} = 41.9 - 83.8 μM				
Gastric adenocarcinoma	KATO-III SNU-1	IC_{50} ≤ 100 μM			↑ cell number in G0/G1 phase; ↓ p-ERK; ↑ $p27^{Kip-1}$	(54)
Hepatocarcinoma	HepG2	IC_{50} ~ 41.9 μM			↑ cell number in G0/G1 phase; ↑ apoptosis; ↑ caspase-3; ↑ p53; ↓ c-fos-c-jun	(45)
	H22	IC_{50} = 40 μM			↑ cell number in G2 phase	(47)
	CBRH-7919	IC_{50} = 39 μM			↑ apoptosis; ↓ JAK1-STAT3; ↓ Bcl-2	(43)
	LM3 SMMC-7721	IC_{50} = 100μM			↑ cell number in G2/M phase; ↑ apoptosis; ↓ NF-κB p65; ↓ Wnt/β-catenin	(46)
	AH109A	5 μM			↓ invasion	(107)
			KM mice	2 - 4 μg/kg	↓ tumor weight	(47)
			Db/db mice	340 μM	↓ oxidative stress; ↑ adiponectin	(134)
			DBA mice	100 mg/kg (4days) 1 mg/kg (14days)	↓ lipid peroxidation; ↓ metastasis	(135)
Leukemia	K562 HL-60	IC_{50} = 25 μM			↓ metastasis; ↑ p21 stimulation; ↓ cyclin D1; ↑ PPARγ; ↑ cell number in G0/G1 phase; ↑ Nrf2; ↑ apoptosis	(56, 68, 69)
Lung carcinoma	A549 H1703				↑ cell number in G0/G1 phase; ↑ p38 MAPK; ↑ apoptosis	(44, 75)
Oral squamous cell carcinoma	SCC131 SCC4	IC_{50} = 700 μM IC_{50} = 720 μM			↑ p21 stimulation; ↓ cyclin D1; ↓ angiogenesis (↓ VEGF, VEGF2, HIF-1α); ↑ apoptosis	(57)
			Hamster	15 mg/kg bw	↓ tumor growth; ↓ PI3 K/Akt/NF-κB/STAT-3	(99, 103, 138)
Prostate cancer	LNCap-FGC	24 % (0.17 μM)			↓ cancer cell growth	(59)
			xenograft PC-3 nude mice	41.7 % (100 mg/kg bw)	↑ apoptosis; ↑ cleaved caspase-3; ↑ miR-375 and miR-487b	(142)

(*continued on next page*)

Table 1 (*continued*)

Cancer	*In vitro*		*In vivo*		Mechanisms	Ref.
Skin cancer	JB6 P +	91.4 % (50 μM)			↑ Nrf2; ↑ cell number in G1 phase; ↓ MMP1, 2 and 9; ↓ migration; ↑ apoptosis	(41, 42)
	A375	50 % (167.5 μM)				
	A2058	80 % (167.5 μM)				
Skin cancer			Mice	850 μM	↓ tyrosine nitration	(139)
Tongue neoplasm			Rats	200 μg/kg bw	↓ tyrosine activity	(140)
			male F344 rats	170 μM	↓ polyamine levels	(137)
Urinary bladder cancer			male ICR mice	85 μM	↓ cancer cell growth	(141)

Akt, protein kinase B; Bax, Bcl-2 associated protein; Bcl-2, B-cell lymphoma 2; bw, body weight; CdC2, cell division cycle 2; CDK, cyclin-dependent kinase; COX-2, cyclooxygenase-2; ERK, extracellular signal-regulated kinases; IL-, Interleukin-; JAK1, Janus kinase 1; MAPK, mitogen-activated protein kinase; miRNAs, microRNA; MMP, matrix metallo proteinases; NF-kB, nuclear factor kappa-light-chain-enhancer of activated B cells; Nrf2/ARE, nuclear factor-erythroid 2-related factor 2/ antioxidant response elements; PI3 K, phosphatidylinositide 3-kinases; PPAR-γ, peroxisome proliferator-activated receptor; ROS, oxygen reactive species; STAT3, Signal transducer and activator of transcription 3; TNF, tumor necrosis factor; VEGF, Vascular Endothelial Growth Factor; VEGFR2, Vascular Endothelial Growth Factor receptor; Wnt, Wingless-INT; ZEB1, Zinc finger E-box binding homeobox 1.

Astaxanthin induced the inhibition of cell proliferation by cell cycle arrest in various ways. Its effects might result in cell cycle arrest at G0/G1 phase as well as at G2-M phase in different cancer cells.

In HepG2 hepatoma cells, a remarkable arrest induction at G0/G1 phase was found after 24 h of astaxanthin treatment (25 and 42 μM), showing a strong reduction of cells in S and G2-M phases [45]. These findings were evidenced in other cancer types and the mechanism involved in the cell cycle arrest was investigated. Generally, the cell cycle progression is regulated by a set of CDKs. They are serine/threonine kinases that control cell cycle progression and modulate their catalytic activities by interaction with cyclins and CDK inhibitors. In malignant cells, changes in expression of CDKs, overexpression of cyclins, and under expression of CDK inhibitors have been commonly reported. The altered CDK activity represents a selective advantage for cancer cell growing. Several studies reported that astaxanthin affects the activity of CDK, repressing tumor progression. Astaxanthin as carotenoid compounds has been reported to restrain tumor cell growth stimulating p21 and inhibiting cyclin D1 [67]. As cyclin D1 controls G1 to S-phase shift, its suppression may be associated to a delay in of tumor cell line proliferation. A CDK inhibitor as p21 is responsible of G1 arrest and, consequently, cell cycle arrest [56].

In human leukemia K562 and HL-60 cells, astaxanthin activates PPARγ, resulting in p21 stimulation and cyclin D1 inhibition, leading to cell cycle arrest at G0/G1 phase [68,69]. PPARγ, belonging to the nuclear receptor superfamily, acts as a transcription factor and exerts strong anti-proliferative effects in a great range of neoplastic cells [70]. Among ligands that could target PPARγ receptors, rosiglitazone arises as an anticancer agent towards a large set of cancers including colon, breast, prostate, and lung [71]. For instance, Liu, et al. [72] reported that rosiglitazone (> 40 μM) induced apoptosis in K562 cells, depending by time and dose. Interestingly, the same effects and in the same cells were observed under astaxanthin treatment, with a significant and dose-dependently induction of cellular apoptosis and PPARγ protein expression in K562 cells. These effects might be limited by administrating antagonist molecules of PPARγ receptor [56]. Moreover, the association of astaxanthin and PPARγ was firstly described in 2005 in fibroblasts [73], when the astaxanthin-related expression of connexin 43, involved in early carcinogenesis stages, was inhibited by PPARγ antagonists [74].

In human gastric adenocarcinoma, KATO-III and SNU-1 cells, the cell cycle arrest was shown at the G0/G1 phase reducing cell proliferation at the S phase (range 0–100 μM) [54]. As ERK and Akt play central roles in cell growth and survival, their inhibition in KATO-III and SNU-1 cells by astaxanthin (100 μM) is reflected in a decrease of protein synthesis and cell proliferation, due to cyclin D1/CDK4 complex inhibition. In both cell lines, astaxanthin increased, in a concentration-dependent manner, the protein level of p27, an inhibitor of CDK, by suppression of cyclin E/CDK2 complex. These processes might induce the cell cycle arrest at the G0/G1 phase. Expression levels of p-Akt, p-Rb and cyclin D1 were not influenced by astaxanthin in KATO-III and SNU-1 cells [54]. No effects were reported on cell cycle of AGS and MKN-45 cancer cell lines when astaxanthin has been tested at different concentration (0–100 μM) [54]. The inconsistent results among cell lines might derive from their diverse features, showing different molecular characteristics, including genetic and epigenetic changes (cell cycle regulator genes, growth factors *etc.*). The antiproliferative effect on oral squamous cell carcinoma models was confirmed by the reduction of the expression of cyclin D1 along with the increase in the nuclear p21, colony formation inhibition, in both SCC131 and SCC4 oral cancer cells (0–700 μM) [57]. Astaxanthin induced G1 arrest in melanoma A2058 and A375 cells [42] and in A549 lung cancer cells measured by flow cytometric analysis [75].

Cell growth arrest might be observed after 24 h treatment with astaxanthin on colon cancer HCT116, HT-29, LS-174, WiDr, SW-480 cells [76]. In HCT116 treated with astaxanthin, the level of cyclin D1, p27 and p21 was similarly affected by the treatment with *H. pluvialis* extract [76,77]. Studies of the growth-inhibitory effects of astaxanthin (synthetic, S: meso: R = 1:2:1) and its stereoisomers (*R*- and *S*-astaxanthin) on HCT116 and HT-29 colon cancer cells were deepened. Astaxanthin stereoisomers, from *H. pluvialis* and *P. rhodozyma,* were tested without significant difference in the cell inhibition. From flow cytometry analysis, astaxanthin and its stereoisomers caused an increased number of HCT116 cells in G2/M after 48 h treatment to 1.4–2.4-times of the control cells. The cell cycle arrest is accompanied with a decrease of cells in G1/S phase and is associated to up-regulated expression of p21, and p27, as well as down-regulation of CDK4 and CDK6 [49].

In human breast cancer, astaxanthin isolated from shrimp, showed the cell cycle arrest at the G0/G1 phase associated to a down-regulation of cyclin D1 in MCF-7 cells [67], whereas Atalay, et al. [78] reported an accumulation of G2/M increase on the same cell line. However, in both reports the antiproliferative effects of astaxanthin is confirmed and differences in the astaxanthin origin, the vehicle used for the treatment, time and dose could be involved. Besides, effect of a combination of carotenoids (astaxanthin, lutein and *β*-carotene) on MCF-7 cell cycle progression has been studied suggesting that the co-administered carotenoids reduced cyclin D1 expression of G0/G1 phase more than individually. It is important to underline as no effects of astaxanthin on cell proliferation and cell cycle regulation have been reported in normal breast epithelial cells (MCF-10A) [67].

A reduction in Cyclin B1 and Cdc2 expression triggers G2/M cell cycle arrest as reported in ECA 109 esophageal squamous cell carcinoma [79] treated with astaxanthin. A significant increase of cells in the G2/M phase has been recorded as antiproliferative mechanism in other cancer cells. Unlike the HepG2, astaxanthin arrested hepatoma H22 cells in G2 phase [47], according to flow cytometry analysis. *In vitro* H22 cells showed G2 phase cells increased and G0/G1 and S phase cells decreased when low-dose and high-dose of astaxanthin were used, as also confirmed by *in vivo* studies [47]. The role of vehicle during

treatment is significant in modern drug/nutraceutical delivery, in fact, a synergism between the vehicle and active ingredients might ensure the therapeutic efficiency. Sowmya, et al. [80] investigated the role of the vehicles, THF, DMSO, FBS, for astaxanthin delivery on *in vitro* cervical adenocarcinoma HeLa cells. Results revealed that DMSO seems to be a better vehicle for astaxanthin keto-carotenoid delivery and the accumulation of G2/M phase is coupled with a cell reduction in S phase [80].

These findings showed that cell cycle arrest is a significant anti-cancer aspect induced by astaxanthin (Fig. 6). However, the anti-cancer mechanisms in cell cycle progression remain still unclear in some cancer cell lines.

5. Pro-apoptotic effects of astaxanthin

Programmed cell death is one of the possible fates of a cell. It is an essential mechanism during embryogenesis and cell differentiation, used to eliminate cells no longer needed by the body or damaged by factors such as oxidative stress, mitochondrial and/or DNA injury, growth factor deprivation, virus, radiation, chemotherapy. Apoptosis is characterized by a well-defined sequence of morphological and molecular changes, resulting in cell contraction, condensation and fragmentation itself, giving rise to the so-called *apoptotic bodies*, and finally death. Numerous intra- and/or extra-cellular molecules are involved: membrane receptors, which essentially bind factors or cytokines external to cells (*e.g.* TNF, Fas-L, TRAIL, *extrinsic via of apoptosis*), and/or proapoptotic (*e.g.* Bax, Bam, Bid) and antiapoptotic (*e.g.* IAP and Bcl2) proteins, most of which have mitochondrial origin (*intrinsic pathway of apoptosis*). To carry out the cell death program, the activity of proteolytic enzymes, called caspases, is essential [81,82]. A defective apoptotic process in any part of this complex mechanism is often involved in cancer, affecting the neoplastic transformation, the resistance to anticancer drugs and the possible metastatization [83–85]. Certainly, one of the therapeutic strategies to counteract the proliferation of tumor cells consists in aiming at the apoptotic mechanism. It is also shown that many molecules of natural origin have anti-tumor properties, aiming precisely at the intracellular mechanisms of programmed cell death, restoring, more or less consistently, a normal cellular homeostasis [86–88]. They have the undoubted advantage of high bioavailability and relatively less potent side effects. Many different phytochemical compounds of natural origin are studied and used in various types of cancer and among these many carotenoids [89–92], including astaxanthin. Its proapoptotic activity on cancer cells, has been highlighted in several studies, both *in vitro* and *in vivo* models. Specifically, an increase in astaxanthin-induced apoptosis was elucidated in colorectal carcinoma cell lines HCT-116, HT-29 [49,53,76], LS-174, WiDr, SW480 [76] and LS-180 [93], AGS gastric cells [53], breast cancer cells MCF7 [67,94], MDA-MB-231 [61,95] and MDA-MB-468 [60], human hepatocellular carcinoma cell lines LM3, SMM-7721 [46], HepG2 [96], rat HCC cells CBRH-7919 [43,97] and mouse HCC cells H22 [47], lung cancer cells A549 [75], melanoma cell lines A375 and A2058 [42], ovarian carcinoma cells SKOV3 [98], esophageal squamous cancer cells ESCC [79], rabbit squamous cancer cells VX2 [66], rat oral squamous carcinoma cells SCC131 and human oral squamous carcinoma cells SCC4 [57], chronic myelogenous leukemia (CML) cells K562 [56]. *In vivo* studies have also been carried out, which have helped to believe in an effective antitumor activity of astaxanthin through its proapoptotic effect, as reported successively.

Astaxanthin also showed its proapoptotic activity by down-regulating the expression of antiapoptotic Bcl-2, p-Bad, and survivin proteins and upregulating proapoptotic Bax/Bad and PARP proteins, together with the efflux of Smac/Diablo and cytochrome *c* into the cytosol. The inhibition of these pathways was then further confirmed in LM3 and SMMC-7721 human HCC cell lines [46], in which it was shown an activation of caspases 3 and 9, a downregulation of Bcl2 and an upregulation of Bax, markers of an apoptotic process, probably caused by NF-κB p65 and Wnt/β-catenin down-regulation *via* negative activation of PI3 K/Akt and ERK signaling pathways. Interestingly, PI3 K/Akt signaling pathway and its downstream effectors have been shown to be affected by astaxanthin in various reports. For example, addition of astaxanthin prevents cell proliferation in SCC131 and SCC4 oral cancer cells by inhibiting PI3 K/Akt and NF-κB pathways [57]. Moreover, astaxanthin exerted chemopreventive effects in the hamster buccal pouch (HBP) carcinogenesis model by inducing intrinsic apoptosis *via* the abrogation of the upstream kinase PI-3 K/Akt, and its downstream signaling pathway NF-κB [99]. Activated Akt, migrating from the cytoplasm to the cell membrane, activates its downstream pathways, such as mTOR, p70S6K, MMP-2 and NF-κB. mTOR and p70S6K enhance protein synthesis and cellular proliferation, while NF-κB reduces apoptosis-related genes transcription and upregulates the IGF-IR and MMP2 expression, associated with invasion and metastasis. Finally, in recent studies, the central role of this pathway, as astaxanthin target, has still been highlighted. Su, et al. [98] showed that astaxanthin combined treatment with human serum albumin (HAS) was able to inhibit NF-κB expression and its translocation to nucleus, promoting a cytotoxic effect in ovarian cancer cells SKOV3. Moreover, Kowshik, et al. [57] confirmed that the PI3 K/NF-κB /STAT3 axis is a specific target of astaxanthin in promoting cellular apoptotic process. The involvement of STAT3 was also examined by Song, et al. [97], resulting that astaxanthin could induce the apoptosis of rat hepatocellular carcinoma CBRH-7919 cells by inhibiting the expression of STAT3 and its upstream activator JAK1, so dysregulating the expression of genes involved in apoptosis [100].

In LS-180 colorectal cancer cells, astaxanthin increased expression of Bax and caspase 3 and decreased that of Bcl2, triggering the apoptotic process [93]. It was showed that a nanoemulsion of astaxanthin and alpha-tocopherol (a type of vitamin E) was able to induce mitochondria-mediated apoptosis in HT-29 and AGS colorectal and gastric cancer cells [53] through ROS generation and damaged mitochondrial membrane potential, opening up encouraging outlook for the use of new formulations well balanced between high efficacy, high absorption rate and low toxicity. A different extraction procedure was used to obtain astaxanthin from shrimp by-products and use it to induce apoptosis in HepG2 hepatocellular carcinoma cells through the upregulation of p53 expression, the activation of caspase 3 and a down-regulation of c-Jun and c-Fos [96]. Some carotenoids were tested, also in combination with the anthracycline drug doxorubicin, against MDA-MB-468 [60], MCF7 and MDA-MB-231 breast cancer cells [95], to induce oxidative stress-mediated apoptosis, showing that lutein and beta-carotene were more potent cell growth inhibitors and more effective in triggering the apoptotic process compared to fucoxanthin and astaxanthin. However, another study revealed a synergistic efficacy of astaxanthin plus β-carotene and lutein carotenoids on molecular events in MCF-7 cells, inducing enhanced cytotoxicity and oxidative stress, higher cellular uptake/accumulation of astaxanthin along with β-carotene and lutein to synergistically trigger apoptosis [67]. In HCT116 and HT-29 colon cancer cells, different stereoisomers of astaxanthin stimulated apoptosis, as suggested by the analysis of increased levels of caspase 3, PARP, EGFR, p-p38, p-JNK and pERK1/2 [49]. A formulation as gold nanoparticles of astaxanthin [61] showed cytotoxic effect against MDA-MB-231 breast cancer cell, inducing apoptosis and revealing itself also useful as contrast agent in photoacoustic imaging.

6. Astaxanthin attenuates cancer diffusion

Cancer diffusion includes a complex cascade of events as angiogenesis, migration and invasion of cancer cells to new tissues and promising anti-cancer molecules might be able to modulate and attenuate these multiple hallmarks.

In the last years, the cell migration targeting has become a therapeutic approach worth to be investigated for cancer treatment [101]. *In vitro* wound healing and invasion assays were performed to evaluate

the anticancer effect of astaxanthin. MCF7, MDA-MB-231 breast cancer cell lines were associated to a direct diminution in distance migrated when treated with astaxanthin (10–50 μM), than normal breast epithelial cells [64]. It has been found that 42 μM astaxanthin is associated with a reduction in cell migration to 50.2 % in A375 and 78.1 % in A2058 through the suppression of MMPs 1,2,9 expression [42].

MMPs, zinc-binding endopeptidases, play a crucial role in cancer and they can promote the cancer metastasis by breaking the ECM [102].

The effect of astaxanthin in tumor angiogenesis has been poorly investigated. Angiogenesis is the physiological mechanism for the formation of new blood vessels from preexisting ones during normal processes as embryogenesis, menstruation, and wound healing. In case of tumor cells, the release of angiogenic stimulators lead to an uncontrolled vessel growth. Several reports suggested that astaxanthin might mediate their inhibition. Indeed, angiogenesis was limited in oral squamous cell carcinoma after astaxanthin treatment [57,103]. Astaxanthin significantly decreases the expression of MMP-2, MMP-9, VEGF, VEGFR2 and HIF-1α nuclear translocation in 131 and SCC4 oral squamous carcinoma cells by JAK-2/STAT3 signaling pathway [57]. Normally, JAK/STAT pathway is involved in the growth and development processes, while in case of cancer, the dysregulation is associated to over proliferation and metastasis [104–106]. Recently, it has been reported that astaxanthin supplementation might affect this pathway and downstream events, in the HBP carcinogenesis model [103]. Accordingly, astaxanthin downregulates the expression of STAT-3 target genes involved in invasion (MMP-2 and MMP-9) in this model.

In rat ascites hepatoma cells (AH109A), 5 μM of astaxanthin inhibited the invasion, allowing to counteract the metastatic process [107].

Astaxanthin induced the EMT in CT26 and HCT116 colon cancer cells by modulation of the expression level of specific markers; in particular astaxanthin increased the expression of epithelial marker E-cadherin and reduced the mesenchymal and invadopodia markers vimentin and cortactin, respectively [108]. One fancy hypothesis about carcinogenesis confers a role to miRNAs, small noncoding RNAs responsible of post-transcriptional control of gene expression. Interestingly, astaxanthin increased miR-29a-3p and miR-200a expression in colorectal cancer cells inhibiting the ability of these cells to colonize surrounding tissues [108]. mRNA and the protein expression of MMP2 were suppressed in HCT116 and CT26 cells. These results indicated that increased miR-29a-3p by astaxanthin suppressed MMP2 expression by direct targeting 3′UTR of mRNA and affected the metastatic processes of colon cancer cells. In addition, the increased expression of miR-200a in CT26 cells, and the related ZEB1 restore, reduced invasion and migration extension. ZEB1 stimulates EMT and promotes metastasis by E-cadherin repression. In colon cancer cells, astaxanthin administration reduced ZEB1 mRNA and MYC oncogenic transcription factor protein and mRNA expression [108].

Since it has been demonstrated that viral infections may be one of the major causes of tumor occurrence, researchers have been focused on new natural approaches in counteracting virus-associated cancer [109]. It is well known that HPV is one of the main etiologic factors for cervical and testicular neoplasia, while HSV have implications on nasopharyngeal cancer. In this context, natural carotenoids with antiviral properties might represent a useful tool in virus-associated tumor treatment. Donà, et al. [110] revealed that astaxanthin is able to preserve sperm by reducing the amount of HPV16-L1 bound onto membranes, showing its antiviral potential. Given the promising results of carotenoids [111] in decreasing the persistence of HPV and the important antiviral activities of astaxanthin-rich *H. pluvialis* extract [112], further investigations should be addressed to evaluate the correlation between astaxanthin and decrement of testicular and cervix cancer development risk.

7. Astaxanthin chemosensitizing effects

Chemosensitization is a strategy to potentiate the cytotoxic effect of anticancer drugs. Astaxanthin as other natural compounds showed an important synergistic effect with antineoplastic drug in addition to their cancer chemoprevention activity. For example, co-treatment of breast cancer cells (MDA-MB, MCF-7) with astaxanthin and a minimal cytotoxic concentration of doxorubicin significantly reduced cell viability compared to control cells [95]. Particularly, astaxanthin allowed to reduce doxorubicin dose (0.12 μM in MCF-7 and 0.28 μM in MDA-MB-231 cells) and IC_{50} value of co-treatment (6.6 ± 0.1 μM in MCF-7 cells and 9.5 ± 0.2 μM in MDA-MB-231 cells) was significantly lower than astaxanthin alone (23.5 ± 0.1 μM in MCF-7 cells and 26.6 ± 0.2 μM in MDA-MB-231 cells) [95]. AlQahtani, et al. [113] reported as astaxanthin treatment increased percentage of cells in G2/M, the best phase for the activity of doxorubicin due to maximum expression of its target enzyme topoisomerase II. Moreover, the carotenoid increased doxorubicin cellular uptake in tumor cells by inhibition of P-glycoprotein pump, important for absorption, distribution and elimination of the drug [113]. Antioxidant and anti-inflammatory properties of astaxanthin are also important to reduce doxorubicin neurotoxicity due to increase of ROS production and proinflammatory cytokines expression. Astaxanthin showed neuroprotective effect in male albino rats treated with combination of astaxanthin (25 mg/kg/week) and doxorubicin (2 mg/kg/week). Particularly, it restored hippocampal function and cholinesterase activity and reduced ROS and proinflammatory cytokines production [114].

The synergistic effect of astaxanthin has also been demonstrated with other anticancer drugs. Astaxanthin enhanced cytotoxic effect of erlotinib in human lung carcinoma cells A549 and H1975 by reducing expression of XPC, an important protein in recognizing DNA damage *via* activation of p38 MAPK [115]. Different combination of astaxanthin and carbendazim, fungicide and also antitumor agent against a variety of mammalian carcinoma, decreased cell viability of MCF-7 human breast carcinoma cells increased after 24 h of treatment. Particularly, different doses of astaxanthin (8, 17, 25 μM) combined with carbendazim (15 μM) led to a higher anti-proliferative effect than carbendazim alone. Moreover, co-treatment increased G2/M phase arrest compared to the control but not to carbendazim alone [78]. Astaxanthin thanks to its antioxidant activity can protect normal tissue from damage of oxidative stress of different antineoplastic dugs. Several studies [116–118] demonstrated as treatment with astaxanthin reduced cisplatin nephrotoxicity and gonadal toxicity in male cancer patients treated with cyclophosphamide. Further, astaxanthin treatment reduced ROS generation in C57BL/6 mice exposed to 4 Gy radiation and improved hematopoietic injury [119].

Astaxanthin resensitized gemcitabine-resistant human pancreatic cancer cells to gemcitabine. Co-treatment with the carotenoid increased cell chemosensitizer by acting on key target of gemcitabine resistance like human equilibrative nucleoside transporter (hENT1) and ribonucleotide reductase subunit M1(RRM1) and M2 (RRM2) and inhibited the gemcitabine-induced epithelial-mesechymal transition phenotype by promoting expression of epithelial cell markers instead of mesechymal ones [120]. Indeed, these studies evidence as astaxanthin enhanced tumor cells sensitization to chemotherapy and limit its adverse reactions.

8. Astaxanthin in pre-clinical studies

From the first assumptions about the correlation between blood carotenoids levels and cancer prevention in the 1980s [121], many evidence have been collected to confirmation of the *in vivo* anticancer properties of astaxanthin (Table 1). The inflammatory nature of the major chronic pathologies, as well as cardiovascular and neurodegenerative diseases, diabetes and cancer makes the xanthophyll astaxanthin, with its antioxidative properties, one of the most promising

therapeutic agents in this context. Hyperglycemia, indeed, is associated with an increment of oxidative stress, with consequent tissue and cellular damages. Astaxanthin revealed *in vivo* protective properties in preserving pancreatic cell functions on diabetic mice model (db/db) [122]. Moreover, it restored diabetes-dependent liver complications in rats [123], as well as reduced renal impairment in db/db mice [124,125] and protected neurons from hyperglycemic damage, preventing depression in mice [126]. By counteracting oxidative stress, astaxanthin has a pivotal role in the context of neurodegenerative disorders and aging. Astaxanthin treatment with a dose of 10 mg/kg/day improved learning and memory impairments in mice models of cerebral ischemia-reperfusion [127] and modulated inflammatory mediators release [21]. Astaxanthin treatment (25 mg/kg) might restore brain histopathological architecture and protect against doxorubicin-induced memory impairment [114]. In the same way, the antioxidant properties of astaxanthin are responsible for cardioprotective effects on rats [128], antiatherosclerotic properties on hyperlipidemic rabbits [129], prevention of bone loss in ovariectomized mice [130], as already shown in previous reported *in vitro* experiments. Moreover, oral administration of astaxanthin (100 mg/kg) reduced skin severity symptoms in murine model of atopic dermatitis [131]. To confirm the *in vitro* implications of astaxanthin in eye care, *in vivo* treatment with oral astaxanthin (100 mg/kg) also attenuated retinal damage [132]. As a microenvironment of chronic inflammation is strictly linked to the promotion of carcinogenesis, the chemopreventive role of astaxanthin might be related to the reduction of inflammation. As reported by Chiu et al. (2016), indeed, the reduction of hepatoma development in rats is associated with the reduction of hepatotoxicity [133]. Shao et al. (2016) confirmed their own *in vitro* results also on mice, showing an inverse correlation between astaxanthin dose and tumor weight [47]. In light of the results of Ohno et al. (2016), astaxanthin might help in liver chemoprevention in obese people. In fact, they found that dietary supplementation with 340 μM for twenty weeks markedly attenuated hepatocarcinogenesis in db/db mice [134]. Astaxanthin treatment might allow stopping cancer cells at an early stage of invasiveness. Therefore, 14-days oral treatment with 1 mg/kg/day of astaxanthin in DBA mice is associated to a strong inhibition of metastases, while the administration of 100 mg/kg/day for 4-day exerted a modulatory effect on natural killer cells that have a crucial role in antiproliferative activity and the inhibition of cancer metastatic process, improving antitumor immune responses [135]. As shown in *in vitro* data on LX-2 cells, the role of PPARs in liver carcinogenesis is pivotal. *In vivo* astaxanthin treatment might modulate the expression of PPAR-α and PPAR-γ in liver and gastric tissue, respectively, with an improvement of oxidative status and profound implications in chemoprevention and carcinogenesis [136]. As already reported by *in vitro* studies, astaxanthin *in vivo* contrasted DNA damages induced by aflatoxin B1 [31], as well as suppressed tongue neoplasm development, when supplementation with 170 μM was included in rats diet [137]. With the involvement of the same pathways observed *in vitro*, the *in vivo* chemoprevention of astaxanthin has been demonstrated in the hamster buccal pouch model of oral carcinogenesis by dietary supplementation of 15 mg/kg of astaxanthin which resulted in a significant delay in tumor development [57,138]. The *in vitro* chemotherapeutic effects of astaxanthin against skin cancer were also confirmed in skin carcinogenesis mice model with a decreased number of papillomas after topical application of 850 μM of astaxanthin [139]. Once again, the modulation of tyrosinase activity might explain the reduction of skin tumor growth exerted by astaxanthin (200 μg/kg) on rats [140]. The first proofs of chemopreventive efficacy of xanthophylls on urinary bladder carcinogenesis date from the 1990s, when Tanaka, et al. [141] observed that 20-weeks administration of 85 μM in male ICR mice markedly reduced the incidence of preneoplastic lesions. The ability of astaxanthin to exert anti-tumorigenic activity was also observed in nude PC-3 prostate cancer mice. In this model astaxanthin significantly inhibited tumor growth by increasing the levels of miR-375 and miR-487b in tumor tissues at high (100 mg/kg) doses with a decrease in cancer growth by up to 41 % by apoptosis induction [142].

High doses of astaxanthin revealed efficient also in mammary cancer suppression. Supplementation with 0.4 %, but not with 0.04 %, halved the incidence of palpable mammary carcinoma and reduced tumor weight [143]. In breast cancer bearing-mice, a single intratumoral injection of an aqueous solution containing 25 mg of astaxanthin reduced microelements concentration in tumor tissues, contributing to provide anticancer effects [144]. Nakao, et al. [145] demonstrated that astaxanthin pretreatment in mice prevented mammary tumor development, but it was unable to stop cancer progression in late stages [145]. Astaxanthin treatment with 340 μM for 8 weeks reduced oxidative stress associated to precancerous lesions in colon cancer-bearing db/db mice [146]. Doses of 15 mg/kg are associated with a decrement of inflammatory mediators, involved in cancer progression, revealing astaxanthin as a helpful chemotherapic agent in rat colon carcinogenesis model [147]. Most recently, it was demonstrated that astaxanthin in rats may reduce NFκB levels, preventing progression of NMBA-induced esophageal cancer in early stage of the onset [148]. The results of *in vivo* investigations on the safety of synthetic astaxanthin were in agreement with those obtained *in vitro*, showing no genotoxicity. The increment of benign liver adenoma in female rats after astaxanthin supplementation seemed to be due to a rodent specific sensitivity, not shared with humans [149]. By contrast, it exerted a protective role against germ cell toxicity under cisplatin therapy. Indeed, 5-weeks of oral treatment with 25 mg/kg of astaxanthin is associated with sperm DNA repairing in male mice [116]. Astaxanthin reconfirmed, also *in vivo*, to act as a valuable aid to conventional chemotherapy in case of drug-resistance, thanks to the photothermal effects. As shown in VX2 tumor rabbit models, the intratumoral dose of 300 μg/mL of astaxanthin followed by laser irradiation is associated with complete and selective tumor eradication [66].

Astaxanthin is often called the “king of carotenoids”, thanks to the well-recognized benefits on human health, but despite its “regal” title, even that *in vitro* and preclinical anti-cancer activity has been widely investigated. To the best of our knowledge, there are no clinical trials or well-defined clinical studies assessing the efficacy of astaxanthin administration reported in patients suffering of any kind of cancer [150].

9. Conclusion and future perspectives

This review evidences the recent advances in the detailed description of anti-cancer eff ;ects of astaxanthin. The *in vitro* studies reported the diverse targets through which astaxanthin reduces the tumorigenesis, mainly through the cell proliferation inhibition, cell cycle arrest and apoptosis induction. *In vivo* preclinical studies, were able to assess the efficacy of astaxanthin treatment in model organisms. Furthermore, astaxanthin enhanced tumor cells sensitization to chemotherapy and limit its adverse reactions. In this context, astaxanthin could be considered as a potential candidate to apply in combining therapy in combating cancer in resistance developing patients to conventional therapy.

Further *in vivo* experiments are required to deeply investigate the anti-cancer eff ;ects of astaxanthin.

Given the lack of clinical evidence, randomized, double-blind, placebo-controlled trials in cancer patients are necessary in order to evaluate the anticancer astaxanthin effectiveness in a more reproducible and safe way. Finally, from scientific results, astaxanthin arises as a valuable alternative in anticancer therapy.

Acknowledgements

This work was supported by the Regione Basilicata; the Fondazione Enrico Mattei DGR n. 1490 del 4/12/2014, vs rep. n. 163 n8; and the Regional Project ALIMINTEGRA, GO NUTRIBAS financed on 16.1 PSR Basilicata founding ex D.G.R. n° 312/17 CUP: C31G18000210002.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.phrs.2020.104689.

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