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REVIEW

Carnosol: A Phenolic Diterpene With Cancer Chemopreventive Potential

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Cancer is an unbeaten health challenge for the humankind. After striving for decades to find a cancer cure, attention has now been shifted to reduce the morbidity and mortality from cancer by halting the course of tumor development. Numerous bioactive phytochemicals, especially those present in edible and non-edible plant species, have been reported to reduce the risk of many cancers. Multiple lines of evidence suggest that carnosol, a phenolic diterpene present in rosemary (*Rosmarinus officinalis* L.), holds the promise of preventing certain types of cancer. A remarkable progress has been made in delineating the biochemical mechanisms underlying the chemopreventive effects of carnosol. Results from in vitro cell culture studies as well as animal model experiments have revealed that carnosol inhibits experimentally induced carcinogenesis and exhibits potent anti-oxidative, anti-inflammatory, antiproliferative and apoptosis inducing properties. Moreover, carnosol enhances the sensitivity of chemoresistant cancer cells to chemotherapeutic agents. The purpose of this review is to shed light on the detailed mechanistic aspects of cancer chemoprevention with carnosol.
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Key Words: Carnosol, Antiinflammatory, Antioxidants, Antiproliferative, Antiangiogenic, Chemosensitization

INTRODUCTION

Cancer chemoprevention with natural products, especially the phytochemicals present in various edible and non-edible medicinal plants, has received significant interest over the last several years.[1,2] The discovery and development of several clinically used anticancer drugs (e.g.,vincristine and paclitaxel) have been originated from plant sources.[1] A great deal of research on identifying chemopreventive phytochemicals has explored a large number of plant secondary metabolites, such as carotenoids, alkaloids, flavonoids, chalcones, xanthones, coumarins and terpenoids, with anticancer properties.[1] A large number of laboratory-based as well as epidemiological studies have demonstrated the cancer chemopreventive effects of rosemary (*R. officinalis* L.),[3] a Mediterranean herb that is commonly consumed as diet. According to a case-control study, intake of diet containing rosemary can reduce the risk of lung cancer.[4] Preclinical studies with rosemary extract have shown the antioxidant, antimutagenic, anti-inflammatory and anticancer effects of this plant.[3] Administration of rosemary extract by gavage has been reported to inhibit 7,12-dimethylbenz(*a*)anthracene (DMBA)-induced mouse skin papilloma formation[5] and rat mammary carcinogenesis.[6] These anticancer properties of rosemary are attributable to its major polyphenolic constituents, such as carnosol, carnosic acid, rosmanol, rosmarinic acid, and ursolic acid. Carnosol (Figure), an *ortho*-diphenolic dipterpene, has been first isolated in 1942 from the plant *Salvia carnosa* (sage) and its chemical structure has been elucidated in 1964.[7] Subsequently, carnosol has been extracted from many other plant species including rosemary.[8-10] This mini review addresses the biochemical basis of cancer chemoprevention with carnosol.

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IN VIVO ANTITUMOR EFFECTS OF CARNOSOL

Several studies have demonstrated the potential of carnosol to inhibit experimentally induced carcinogenesis in animal models. Topical application of carnosol (3 or 10 μmol) prior to administration of 12-*O*tetradecanoyl phorbol-13-acetate (TPA) twice a week for 20 weeks significantly inhibited the multiplicity of papillomas in DMBA-initiated mouse skin. This skin cancer preventive effect of carnosol in mouse skin was attributable to its inhibitory effect on TPA-induced activation of ornithine decarboxylase enzyme, which is a hallmark of tumor promotion.[5] When carnosol (200 mg/kg body weight) was administered intraperitoneally for 5 days to rats challenged with DMBA, the compound inhibited DMBA-DNA adduct formation and reduced the multiplicity of mammary adenocarcinomas. However, dietary administration of carnosol (1%) failed to affect DMBA-DNA adduct formation.[6] Although the reason for this discrepancy in bioactivity of carnosol between oral and intraperitoneal routes of administration is not clear, it can be presumed that the bioavailability of carnosol upon dietary administration may not be adequate to affect mammary DMBA-DNA adduct formation. Treatment of adenomatous polyposis coli (*APC*)$^{min+}$ mice, which develops spontaneous colon tumors and resembles human familial adenomatous polyposis, with a diet containing carnosol (0.1%) attenuated the multiplicity of intestinal tumors.[11] This study also demonstrated that carnosol diminished the phosphorylation of β-catenin, which normally resides in cell membrane by interacting with an adherens junction protein E-cadherin, and increased the localization of both β-catenin and E-cadherin at the intestinal enterocyte membrane.[11] Oral administration of carnosol (30 mg/kg body weight) for 5 days in a week for 4 weeks suppressed the growth of human prostate cancer (22Rv1) cells xenograft tumors in nude mice and decreased the serum level of prostate-specific antigen in tumor-bearing mice.[12] The protective effects of carnosol against HCl/ethanol-induced mouse gastric lesions[13] and carbon tetrachloride-induced rat liver damage[14] suggest the potential of this compound to prevent gastric and hepatocellular carcinogenesis.

BIOCHEMICAL BASIS OF CANCER CHEMOPREVENTION WITH CARNOSOL

Currently available literature on the mechanistic basis of cancer chemopreventive effects of carnosol indicates that the compound can interfere with diverse intracellular signaling pathways involved in the development of tumors (Figure). Oxidative stress and inflammation, through production of reactive oxygen species (ROS) and a wide variety of inflammatory mediators, contribute to neoplastic transformation of cells by oxidative and/or covalent modifications of important cellular macromolecules, such as proteins, lipid and nucleic acids.[15] Exposure to

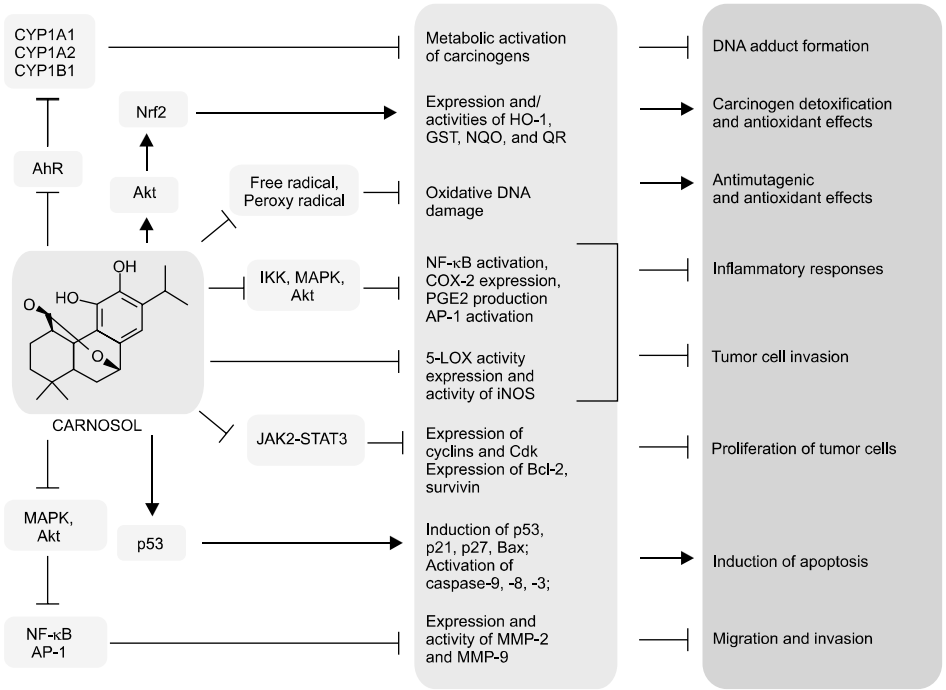

Figure. A schematic diagram showing the molecular targets of carnosol for cancer chemoprevention. CYP, cytochrome p450; AhR, arylhydrocarbon receptor; Nrf2, nuclear factor erythroid-related factor-2; HO-1, heme oxygenase-1; GST, glutathione-S-transferase; NQO, NAD(P)H: quinine oxidoreductase; QR, Akt, Akt/protein kinase B (PKB); quinine reductase; IKK, inhibitor kappa B (IκB) kinase; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor-kappa B; COX-2, cyclooxygenase-2; PGE_2, prostaglandin E2; AP-1, activator protein-1; 5-LOX, 5-lipoxygenase; iNOS, inducible nitric oxide synthase; JAK2, Janus-activated kinase 2; STAT3, signal transducer and activator of transcription 3; Cdk, cyclin-dependent kinase; Bcl-2, B-cell lymphoma-2; Bax, Bcl-2-associated X protein; MMP, matrix metalloproteinase.

genotoxic carcinogens initiates transformation of cells, which then undergo clonal expansion to develop benign tumors, and subsequently progress to complete malignancy. The entire course of tumor development involves altered biochemical events, such as loss of density-dependent inhibition of growth, independence of external growth stimuli for cell proliferation, defective cell cycle control, evasion from apoptosis, enhanced angiogenesis, host tissue invasion and metastasis, altered metabolism, and the ability to escape host immune responses. Collectively, these features constitute the hallmarks of cancer.[16] Carnosol has been shown to elicit chemopreventive effects by (1) blocking the bioactivation of carcinogens, (2) enhancing antioxidant and/or detoxification enzyme activities, (3) suppressing tumor-promoting inflammation, (4) inhibiting cell proliferation and inducing apoptosis selectively in cancer cells, and (5) blocking tumor angiogenesis and invasion. Table summarizes the biochemical

Table. Biochemical basis of anticancer effects of carnosol

Molecular mechanisms	Experimental models	References
Inhibition of carcinogen metabolism		
↓ B[α]P-DNA adduct formation, ↓ gene expression and activity of CYP1A1, ↑ expression of GST-pi and QR enzymes	Incubation of B[a]P-stimulated BEAS-2B cells with carnosol (1 μg/mL) for 6 or 24 hr	Offord et al.[18]
↓ B[α]P-DNA adduct formation, ↓ mRNA and protein expression of CYP1A1 and CYP1B1, ↓ Hsp90-ATPase activity, ↓ AhR expression	Human oral leukoplakia (Msk-leuk1) or HaCaT cells treated with carnosol (5 or 10 μM) prior to incubation with B[α]P	Mohebati et al.[19]
Induction of cytoprotective proteins		
↑ Intracellular glutathione level, ↑ gene expression of GCLC and GCLM, ↑ nuclear localization of Nrf2, ↑ Nrf2-ARE reporter gene activity	Carnosol treatment (5 or 10 μM) of HepG2 cells	Chen et al.[29]
↑ HO-1 mRNA and protein expression, ↑ HO-1 promoter activity, ↑ phosphorylation of Akt, ↑ nuclear localization of Nrf2, ↑ Nrf2 binding to the *ho-1*-ARE promoter sequence, ↓ H_2O_2-induced cell death	PC12 cells treated with 10 μM carnosol	Martin et al.[28]
Attenuation of inflammatory responses		
↓ Expression of iNOS protein and mRNA, ↓ phosphorylation of p38 MAP kinase and ERK, ↓ IKK activity, ↓ IκBα phosphorylation, ↓ nuclear localization of c-Rel and p65, ↓ NF-kB DNA binding and reporter gene activity	Treatment of LPS-stimulated murine macrophage 264.7 cells with carnosol (5, 10 or 20 μM)	Lo et al.[32]
↓ Expression of COX-2 protein and mRNA, ↓ production of PGE_2, ↓ phosphorylation of ERK, p38 MAP kinase and JNK, ↓ PKC activity, ↓ binding of AP-1 to *cox-2* promoter	Carnosol (20, 40 or 60 μM) treatment of human mammary epithelial 184B5/HER cells	Subbaramaiah et al.[34]
↓ Phorbol ester-induced mouse ear inflammation, ↓ mRNA expression of COX-2, IL-1β, and TNF-α	Topical application of carnosol (10 or 20 μg/cm^2) to mouse skin treated with TPA	Mengoni et al.[35]
↓ LPS-induced NO production	Incubation of LPS-stimulated murine Raw264.7 macrophages with carnosol (12.5 or 25 μM)	
Inhibition of tumor cell proliferation and induction of apoptosis		
Induction of G2/M phase cell cycle arrest, ↓ expression of cyclin-A, -D1, D-2, Cdk-2, -4 -6, and Bcl-2, ↑ expression of Bax, p21 and p27, ↓ phosphorylation of mTOR, p70S6 kinase and Akt, ↑ phosphorylation of AMPKα, and 4EBP1, activation of caspase-8, and caspase-9	Treatment of PC3 prostate cancer cells with carnosol (20, 40, and 60 μM)	Johnson et al.[38]
Induction of subG1 arrest, ↑ caspase-3 activity	HL-60 cells treated with carnosol (25 or 50 μM)	López-Jiménez et al.[39]
↓ Cell proliferation and the expression of AR	Treatment of LNCaP and 22Rv1 prostate cancer cells with 20 or 40 mM carnosol	Johnson et al.[12]
Interacts with ligand binding domain of ERα, ↓ Cell proliferation and the expression of ERα,	Treatment of NCF-7 breast cancer cells with 20 or 40 μM carnosol	Johnson et al.[12]
↓ Cell viability and induces apoptosis, activation caspase -9 and caspase-3, cleavage of PARP, ↑ generation of ROS, ↑ expression of p53 and Bax, ↓ phosphorylation of JAK2, Src and STAT3, ↓ STAT3 DNA binding activity and the reporter gene activity, ↓ expression of cyclin D-1, D-2 and survivin	HCT116 colon cancer cells incubated with carnosol (50 or 100 μM)	Park et al.[42]

Table. Continued

Molecular mechanisms	Experimental models	References
Suppression of angiogenesis, cell migration and invasion		
↓ Migration and capillary tube formation, and ↓ MMP-2 activity	Incubation of bronchial aortic endothelial cells and HUVECs with carnosol (25 or 50 μM)	López-Jiménez et al.[39]
↓ TNFα-induced cell migration by blocking the expression of MMP-9	TNFα-stimulated vascular smooth muscle cells treated with carnosol	Chae et al.[44]
↓ Cell migration and invasion of melanoma cells. ↓ phosphorylation of MAP kinases and Akt, ↓ activation of NF-κB and AP-1, ↓ MMP-9 expression and activity	B16/F10 melanoma cells treated with 5 or 10 μM carnosol	Huang et al.[45]

B[α]P, benzo[α]pyrene; CYP1A1, cytochrome p450 1A1; GST-pi, glutathione-*S*-transferase-pi; QR, quinine reductase; Hsp90, heat shock protein 90; AhR, arylhydrocarbon receptor; HaCaT, human keratinocyte; GCLC, glutamate cysteine ligase catalytic subunit; GCLM, glutamate cysteine ligase modifier subunit; Nrf2, nuclear factor erythroid-related factor-2; HO-1, heme oxygenase-1; Akt, Akt/protein kinase B (PKB); ARE, antioxidant response element; iNOS, inducible nitric oxide synthase; MAP, mitogen-activated protein; IKK, inhibitor kappa B (IκB) kinase; NF-κB, nuclear factor-kappa B; LPS, lipopolysaccharide; COX-2, cyclooxygenase-2; PGE_2, prostaglandin E2; ERK, extracellular signal-regulated protein kinase; JNK, c-Jun-N-terminal kinase; PKC, protein kinase C; AP-1, activator protein-1; IL-1β, interleukin 1β; TNF-α, tumor necrosis factor α; NO, nitric oxide; TPA, 12-*O*-tetradecanoyl phorbol-13-acetate; Bcl-2, B-cell lymphoma-2; Bax, Bcl-2-associated X protein; mTOR, mammalian target of rapamycin; AMPKα, 5'-AMP activated kinase-α; 4EBP1, 4E-binding protein-1; PC3, prostate cancer.

basis of anticancer effects of carnosol.

1. Modulation of carcinogen metabolism

Tumor initiation is a first and irreversible process that begins with the damage of cellular DNA upon exposure to genotoxic carcinogens. However, many carcinogens are inactive *per see*, and are activated to form ultimate carcinogens through biotransformation process. The metabolically activated carcinogens can cause DNA damage, thereby initiating tumor development through activation of oncogenes and inactivation of tumor suppressor genes. While the hepatic cytochrome p450 (CYP) enzymes catalyze the phase I biotransformation of pro-carcinogens to generate active carcinogens, a series of detoxification enzymes, such as glutathione-*S*-transferase (GST), NAD(P)H: quinine oxidoreductase (NQO), NAD(P)H: quinine reductase (QR), and glucuronyltransferase, etc. rapidly eliminates metabolically activated carcinogens through phase II biotransformation process.[17] Thus, the blockade of the activities of CYP enzymes and the induction of Phase II detoxification enzymes can prevent carcinogenesis. While the transcriptional activation of CYP enzymes is mainly regulated by arylhydrocarbon receptor (AhR), the detoxification enzymes are transcriptionally activated largely by a redox-sensitive transcription factor, nuclear factor erythroid-related factor-2 (Nrf2). Carnosol has been reported to inhibit benzo[α]pyrene (B[α]P)-induced DNA-adduct formation through inhibition of gene expression and activity of CYP1A1, and induction of mRNA levels of GST-pi and NQO in human bronchial epithelial cells.[18] Likewise, carnosol attenuated B[α]P-induced CYP1A1 and CYP1B1 activity through downregulation of AhR in human keratinocyte (HaCaT) cells.[19] This study also demonstrated that carnosol inhibited the activation of AhR, which is a client protein of a chaperone heat shock protein 90 (Hsp90), by blocking Hsp90 ATPase activity. Thus, the skin cancer chemopreventive effects of carnosol may be attributed to its modulation of B[α]P metabolism. The induction of GST and QR enzyme activities by carnosol[20] may account for its inhibitory effects of DMBA-DNA adduct formation and mammary carcinogenesis. In another study, carnosol increased the protein and mRNA expression of NQO1 and its enzyme activity in rat liver cells.[21]

Aflatoxin B1 (AFB1), a potential hepatocarcinogen, is converted to its genotoxic metabolite, AFB1-8, 9-epoxide by CYPlA2 and CYP3A4 enzymes.[22,23] The active metabolite AFB1-8, 9-epoxide may be detoxified through glutathionylation catalyzed by GST enzymes. Thus, the inhibitory effects of carnosol-rich rosemary extract on CYP1A2 and CYP3A4 activities in immortalized human liver epithelial cells,[24] and the induction of hepatic GST enzyme activity by carnosol[20] suggest that the compound may prevent hepatocarcinogenesis through metabolic inactivation of potential carcinogens.

2. Antioxidant effects of carnosol

The generation of ROS and depletion of intracellular antioxidants cause oxidative damage to cellular macromolecules. Carnosol and its structural analog carnosic acid account for the 90% of antioxidant effects of rosemary. Aruoma et al.[8] have reported that carnosol possesses peroxyl radical scavenging property and has been shown to inhibit Cu^{2+}-induced oxidation of low density lipoproteins and the generation of lipid free radicals in mouse liver microsomes. However, carnosol failed to affect superoxide anion production from xanthine/xanthine oxidase system.[25]

Another plausible mechanism of the inhibitory effects of carnosol on lipid peroxidation is that the compound can alter the membrane phospholipid order. As compared to phospholipid membrane-free assay, about 4 to 6 times more potent antioxidant activity of the compound was observed when analyzed in a phospholipid membrane-based assay. The study also revealed that the compound decreased the number and/or the mobility of water molecules located at the polar head group region of the membrane phospholipid.[26]

Carnosol has been reported to activate a variety of cellular antioxidant/detoxification enzymes, collectively known as cytoprotective proteins. Treatment with carnosol induced the expression of an antioxidant enzyme heme oxygenase-1 (HO-1) in BV2 microglial cells[27] and rat pheochromocytoma (PC12) cells,[28] and protected these cells from interferon-γ (IFN-γ)-induced inflammatory responses and hydrogen peroxide (H_2O_2)-induced oxidative stress, respectively. According to the latter study, carnosol activated Nrf2 via phosphorylation of upstream kinase Akt. Transfection of cells with dominant negative Nrf2 or treatment with pharmacological inhibitor of Akt abrogated carnosol-induced expression and the promoter activity of HO-1.[28] Genes encoding cytoprotective proteins harbor antioxidant response element (ARE), alternatively known as electrophile response element. The transcriptional activation of cytoprotective proteins is mainly regulated through Nrf-2-mediated ARE activation.[17] Carnosol increased the Nrf2 binding with *ho*-1-ARE, thereby resulting in HO-1 expression. Incubation of human HepG2 cells with carnosol resulted in increased intracellular level of glutathione (GSH) and the expression of GSH synthesizing enzyme glutamate cysteine ligase catalytic subunit (GCLC) and modifier subunit (GCLM) through nuclear accumulation of Nrf2 and the enhanced ARE activity, and protected cells from H_2O_2-induced cell death. Transfection of cells with Nrf2 siRNA abrogated carnosol-induced expression of GCLC and GCLM.[29]

3. Attenuation of inflammatory responses

Chronic inflammation is a predisposing factor for tumor development. While inflammation induces oxidative stress, excessive accumulation of ROS further amplifies inflammatory tissue damage. ROS or a variety of inflammatory mediators, such as prostaglandins (PGs), nitric oxide (NO), interleukins (IL) and chemokines promote tumorigenesis. In fact, the close association and signaling cross-talk between inflammatory immune cells and tumor cells create an inflammatory microenvironment within the growing tumor. Therefore, suppressing tumor-associated inflammation is a pragmatic approach for cancer chemoprevention.[30] Representative pro-inflammatory enzymes, such as inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) are overexpressed in many cancers.[31] While COX-2 is a rate-limiting enzyme in the synthesis of PGs, iNOS catalyzes the oxidative deamination of L-arginine to produce NO. Both PGs and NO have tumor promoting roles.[15] The aberrant expression of COX-2 and iNOS, and subsequent production of various inflammatory mediators involve inappropriate amplification of cell signaling pathways comprising various upstream kinases, such as mitogen-activated protein (MAP) kinases, phosphatidylisonositol-3-kinase (PI3K), Akt, inhibitor kappa B (IκB) kinase (IKK) and Janus-activated kinase (JAK), and their downstream transcription factors, such as nuclear factor-kappa B (NF-κB), activator protein-1 (AP-1), and signal transducer and activator of transcription (STAT).[15,31] Blockade of the activation of these inflammatory signaling molecules constitutes the biochemical basis of cancer prevention with various anti-inflammatory agents.[30]

Pretreatment of murine macrophage RAW264.7 cells with carnosol significantly attenuated lipopolysaccharide-induced mRNA and protein expression of iNOS, and the production of NO. This study also demonstrated that carnosol attenuated the IKK activity and the phosphorylation and degradation of IκBα, and subsequent NF-κB DNA binding by blocking the phosphorylation of p38 MAP kinase and extracellular signal-regulated protein kinase (ERK).[32] Incubation of mouse peritoneal cells with carnosol diminished lipopolysaccharide-induced nitrite production without affecting cell viability.[33] Carnosol inhibited phorbol ester-induced protein and mRNA expression of COX-2 and the production of PGE_2 in human mammary epithelial cells (184B5/HER), a cell line neoplastically transformed with neu oncogene. The study also reported that the compound negated the DNA binding of AP-1 through inhibition of the protein kinase C activity and phosphorylation of ERK, p38 MAP kinase, and c-Jun-N-terminal kinase. The overexpression of c-Jun abrogated the inhibitory effect of carnosol on TPA-induced COX-2 expression.[34] Topical application carnosol attenuated TPA-induced mouse ear edema formation as revealed by reduced leukocyte infiltration and diminished mRNA expression of COX-2, IL-1β, and tumor necrosis factor-α.[35] Treatment of human polymorphonuclear leukocytes with carnosol reduced the production of inflammatory leukotrienes via blockade of 5-lipoxygenase activity.[36]

4. Inhibition of cell proliferation and induction of apoptosis

Carnosol inhibited proliferation and induced apoptosis in several human cancer cells. The compound induced G2/M phase

cell cycle arrest and reduced mitotic exit of human colon cancer (Caco-2) cells via upregulation of cyclin B expression.[37] Likewise, carnosol induced G2/M phase cell cycle arrest in human prostate cancer (PC3) cells in a concentration-dependent manner.[38] The antiproliferative effects of carnosol in PC3 cells were mediated through the downregulation of various cyclins (A, D1, and D2), and cyclin-dependent kinases (Cdk)-2, -4, -6, and upregulation of Cdk inhibitors, p21 and p27. Furthermore, carnosol-induced apoptosis in PC3 cells was mediated through the activation of caspase-8 and -9, induction of Bax and inhibition of B-cell lymphoma-2 (Bcl-2) expression. The antiproliferative and apoptosis inducing effects of carnosol in these cells were associated with increased phosphorylation of 5'-AMP activated kinase-α and translation initiation factor 4E-binding protein-1, and the decreased phosphorylation of mammalian target of rapamycin, ribosomal protein p70S6 kinase and Akt.[38] The antiproliferative effects of carnosol in human leukemia (HL-60) cells were associated with subG1 arrest, activation of caspase-3 and induction of apoptosis.[39]

The activation of androgen receptor (AR) is a key molecular switch in prostate carcinogenesis. Computer modeling study and subsequent time-resolved fluorescence resonance energy transfer assay have shown that carnosol can bind with the ligand binding domain of AR and exhibits receptor antagonistic, but not agonistic, property.[12] Treatment of human prostate cancer LNCaP and 22Rv1 cells with carnosol decreased the expression of AR. This study also reported that carnosol interacted with the ligand binding domain of estrogen receptor-α (ERα), which is implicated in mammary carcinogenesis. Incubation of human mammary cancer (MCF-7) cells with carnosol decreased the expression of ERα. These findings suggest that carnosol can act as a dual inhibitor of AR and ERα, and can be effective in preventing prostate and breast carcinogenesis.[12]

The induction of cytotoxicity in pro-B and pre-B acute lymphoblastic leukemia cell lines by carnosol was associated with the depolarization of mitochondrial membrane and decrease in the expression of anti-apoptotic protein Bcl-2.[40] Treatment of adult T-cell leukemia/lymphoma (ATL) cells with carnosol induced cell death through depletion of cellular GSH level, which was reversed by pretreatment with a GSH precursor N-acetyl cysteine. These findings suggested that carnosol altered cellular redox status. Proteomic analysis also revealed a marked increase in the expression of reductases, enzymes of the glycolytic pathway and pentose phosphate pathways in carnosol-treated ATL cells.[41] In our recent study, we found that carnosol significantly reduced the viability of human colon cancer (HCT116) cells and induced apoptosis via generation of ROS, activation of p53, Bax, caspase-9 and caspase-3, the cleavage of poly-(ADP-ribose) polymerase (PARP), and inhibition of Bcl-2 and Bcl-xl expression. Moreover, carnosol attenuated STAT3 activation through modulation of upstream kinases JAK2 and Src, and diminished the expression of cell proliferation markers, such as survivin, cyclin-D1, -D2, and -D3.[42]

Another plausible molecular target of carnosol is peroxisome proliferator activated receptor-γ (PPARγ), which is known to exert antitumor effects. Treatment of COS-7 cells transfected with a Gal4-driven PPARγ luciferase gene construct with carnosol induced the PPARγ activity.[43]

5. Suppression of angiogenesis, cell migration, and invasion

The increased tumor angiogenesis and the ability of tumor cells to migrate and invade through host stromal tissue are the critical biochemical events in tumor promotion and progression. The chemopreventive effects of carnosol can partly be ascribed to its antiangiogenic property. Carnosol inhibited migration of human bronchial aortic endothelial cells (BAEC) and human umbilical vein endothelial cells (HUVEC) and blunted the tube formation by these cells in Matrigel plug assay, which was associated with decreased activity of matrix metalloproteinase-2 (MMP-2). Moreover, chorioallantoic membrane assay showed the in vivo antiangiogenic effect of the compound.[39] In another study, carnosol decreased the expression of MMP-9 and inhibited the migration of vascular smooth muscle cells.[44] Huang et al.[45] demonstrated that carnosol significantly attenuated the migration and invasion of mouse melanoma B16/F10 cells by reducing the expression and activity of MMP-9 through downregulation of NF-κB and AP-1, and these effects were mediated through the inhibitory effect of the compound on the phosphorylation of MAP kinases and Akt.

6. Chemo- and radio-sensitizing effects

Cancer cells often acquire resistance to chemotherapy and radiotherapy. Many chemopreventive phytochemicals have been shown to alleviate chemoresistance and radioresistance, thereby suppressing tumor growth when combined with chemotherapy or radiation therapy. Carnosol has been shown to induce apoptosis in human ovarian cancer (A2780) as well as its cisplatin-resistant (A2780CP70) daughter cell lines.[46] One of the mechanisms of chemoresistance is the upregulation of a drug efflux transporter protein, p-glycoprotein (P-gp). When multidrug-resistant human KB epidermoid carcinoma (KB-C2) cells were

incubated with carnosol in presence of daunorubicin, the compound increased cellular accumulation of daunorubicin. According to this study, carnosol stimulated the P-gp ATPase activity, suggesting that the compound may function as a substrate of P-gp ATPase by competitively occupying the drug binding site of the enzyme.[47] Carnosol also exhibited radiosensitizing effects as shown by the increased apoptosis of B16/F10 melanoma cells upon irradiation with X-radiation, while the compound prevented radiation-induced cytotoxicity in normal prostate epithelial cells.[48]

CONCLUSIONS

Our current understanding that cancer is a systemic multifactorial disease has led us to consider that targeting a single gene may not be effective in eradicating cancer. This has been evidence from our decades-long effort in developing anticancer drugs that many single gene targeting chemotherapeutics showed limited clinical success. Worldwide, the prevalence of cancer is still on rise and requires an appropriate strategy to reduce the morbidity and mortality from cancer. Over the last several decades, a great deal of research has demonstrated that cancer can be prevented by changing life-style factors, such as consumption of diet rich in fruits and vegetables, regular exercise, and cessation of smoking etc. Plants contain a wide variety of bioactive phytochemicals, which are known to prevent carcinogenesis through the modulation of diverse biochemical pathways. Systematic research on a natural diterpene, carnosol, has revealed its multi-targeting effects on various cancer hallmarks. Despite the progress in understanding the mechanisms of cancer chemoprevention with carnosol, there is dearth of knowledge about the effect of the compound on the metastasis and immune escape by tumor cells, and its pharmacokinetic properties. None-the-less, evidence from current preclinical studies suggests carnosol as a promising cancer chemopreventive agent.

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CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

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