Review

An updated review on molecular mechanisms underlying the anticancer effects of capsaicin

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Abstract The quest for developing anticancer principles from natural sources has a long historical track record and remarkable success stories. The pungent principle of hot chili pepper, capsaicin, has been a subject of research for anticancer drug discovery for more than three decades. However, the majority of research has revealed that capsaicin interferes with various hallmarks of cancer, such as increased cell proliferation, evasion from apoptosis, inflammation, tumor angiogenesis and metastasis, and tumor immune escape. Moreover, the compound has been reported to inhibit carcinogen activation and chemically induced experimental tumor growth. Capsaicin has also been reported to inhibit the activation of various kinases and transcription that are involved in tumor promotion and progression. The compound activated mitochondria-dependent and death receptor-mediated tumor cell apoptosis. Considering the growing interest in capsaicin, this review provides an update on the molecular targets of capsaicin in modulating oncogenic signaling.

Keywords: capsaicin, chemoprevention, carcinogenesis, angiogenesis, detoxification, antioxidant, immunotherapy

Introduction

Capsaicin, (*trans*-8-methyl-*N*-vanillyl-6-nonenamide), is a pungent constituent of hot chili peppers (*Capsicum frutescens* L.) or hot red peppers (*Capsicum annum* L.) (1,2). Besides its use as a condiment, hot chili peppers are used in traditional medicine to ease neuropathic pain and reduce cholesterol level. Although capsaicin is mainly used as a therapy for peripheral neuralgia, there is a great deal of interest with the anticancer potential of the compound. The anticancer effects of capsaicin and its underlying molecular mechanisms have been extensively investigated over the last several decades. Whether capsaicin acts as a dietary carcinogenic mutagen or exerts anti-mutagenic and anticarcinogenic effects is debatable. The mutagenicity of capsaicin has been studied in both bacterial and mammalian cells in culture. Although early studies on capsaicin obtained from chili peppers provided conflicting reports of mutagenic and anti-mutagenic effects of the compound the use of synthetic and highly purified capsaicin in later studies revealed that the compound can protect against vinyl carbamate- or *N*-nitrosodimethylamine (NDMA)-induced mutagenesis in *Salmonella* Typhimurium TA100 partly by blocking the activity of cytochrome P450 2E1 (CYP2E1) (3). Subsequent studies have also revealed that synthesized pure *trans*-capsaicin does not cause mutagenicity in *S. Typhimurium* or *Escherichia coli* (4), but

rather can suppress the metabolic activation of various heterocyclic amines, thereby limiting their mutagenic potential (5). Despite a few initial reports of capsaicin-induced genotoxicity, such as the formation of micronuclei in polychromatic erythrocytes, and increased frequencies of sister chromatid exchanges (6,7), the compound appeared to be non-genotoxic when used in its highest purity grade (8). Furthermore, capsaicin significantly attenuated cyclophosphamide-induced chromosomal aberrations and DNA strand breakages in mice (9), suggesting the anti-genotoxic effect of the compound.

Although several groups of researchers have reported capsaicin to be a carcinogen or a co-carcinogen, others have demonstrated that the compound possesses anticancer properties (10). The pioneering study by Park *et al.* (11,12) demonstrated that capsaicin not only lacks tumor promoting potential but rather inhibits 7,12-dimethylbenz[*a*]anthracene (DMBA)-initiated and 12-*O*-tetradecanoyl phorbol-13-acetate (TPA)-promoted mouse skin papillomagenesis. These findings have been supported by several other studies. For example, prolonged dietary administration of a mixture of capsaicin (64.5%) and dihydrocapsaicin (32.5%) did not exhibit any carcinogenic effects in B6C3F1 mice (13). In another study, treatment of *v-Ha-Ras*-transgenic (Tg.Ac) mice with TPA significantly increased skin tumor formation but treatment with capsaicin had no effect on tumor burden, suggesting that the compound lacks carcinogenic potential

(14). In fact, a large pool of experimental findings with synthesized pure *trans*-capsaicin consistently revealed the anti-carcinogenic effect of the compound. Although accumulating evidence suggest that capsaicin modulates various hallmarks of cancer and inhibits experimental carcinogenesis in different animal models, several recent studies demonstrated the potential of the compound to promote tumor cell proliferation, migration and metastasis. This review addresses the current status and future prospects of developing capsaicin as an anticancer agent.

Role of capsaicin in the modulation of biochemical mechanisms implicated in cancer

Carcinogenesis, a multi-step process, involves three sequential phases of initiation, promotion, and tumor progression. Tumor initiation is a rapid and irreversible process that involves damage of cellular macromolecules, such as DNA, RNA, and proteins upon direct or indirect exposure to carcinogens. Tumor promotion is a reversible process involving clonal expansion of transformed cells to form benign tumors, which are premalignant in nature. Progression is the final stage of malignancy when the premalignant cells acquire the invasive and metastatic potential (15). Capsaicin can interfere with all stages of carcinogenesis (Fig. 1). The compound inhibits tumor initiation largely by blocking metabolic activation of carcinogens and fortifying cellular detoxification pathways, thereby protecting DNA from covalent modification and oxidative damage. The anti-tumor promoting effects of capsaicin are accredited to its ability to the inhibition of inflammation and tumor cell proliferation, and the induction of apoptosis in cancer cells. Capsaicin has been shown to block tumor progression by suppressing angiogenesis. Moreover, treatment with capsaicin enhances the sensitivity of various chemo-resistant cancer cells to existing chemotherapeutic agents. The following sections will focus on the mechanisms underlying the anticancer effects of capsaicin, with special focus on its molecular targets (Fig. 2).

Effects of capsaicin on phase I xenobiotic metabolizing enzymes

Phase I xenobiotic metabolizing enzymes cause bioactivation of various apparently inactive pro-carcinogens. The bioactivated carcinogens form covalent bonds with DNA, thereby resulting in direct DNA damage and the perturbation of intracellular signaling. Capsaicin has been shown to block the metabolic activation of various carcinogens. For instance, capsaicin attenuated arylhydrocarbon hydroxylase activity in human and murine keratinocytes, thereby blocking metabolic activation of BP and its subsequent interaction with DNA (16). Similarly, capsaicin inhibited the mutagenicity of tobacco-specific carcinogen NNK in *S. Typhimurium* TA1535 by blocking its metabolic activation (17,18). NNK undergoes metabolic activation through α -carbon hydroxylation by microsomal mixed function oxidases. *In vitro* incubation of NNK with hepatic or pulmonary microsomes isolated from capsaicin-treated Golden Syrian hamster inhibited the α -carbon hydroxylation of NNK (18).

Capsaicin protected against vinyl carbamate- or *N*-nitrosodimethy-

Fig. 1. Mechanisms of cancer chemoprevention with capsaicin

lamine (NDMA)-induced mutagenesis in *S. Typhimurium* TA100 tester strain through the inhibition of CYP2E1 activity (3). However, capsaicin caused less than 20% inhibition of the CYP2E1 activity in an *in vitro* test (19). According to the latter study, capsaicin inhibited the activities of CYP1A2, CYP2C9, and CYP2C19 with relatively low IC_{50} values (2.1–3.2 μ M), and the activities of CYP2B6, CYP2D6, and CYP3A4/5 with relatively higher IC_{50} values (12–38 μ M) in human liver microsomes (19). In contrast, daily oral administration of capsaicin to rats for seven days exhibited no significant effect on rat CYP1A2; however, it significantly inhibited the activity of CYP2C19 and induced that of CYP3A4 (20). While incubation of murine hepatoma Hepa-1c1c7 cells with capsaicin alone caused slight induction of CYP1A1 expression and activity, the compound inhibited 3-methyl cholanthrene (MC)-induced CYP1A1 levels through the activation of Ca^{2+} /calmodulin-dependent protein kinase and CCAAT/enhancer-binding protein β (C/EBP β) and inhibition of 3-MC-induced arylhydrocarbon transactivation. These findings suggest that the anticancer effect of capsaicin is mediated through the inhibition of expression and the activity of carcinogen metabolizing enzymes (21).

Modulation of cellular detoxification and antioxidant capacity

Cells have intrinsic ability to maintain an intracellular redox balance. A wide array of phase II detoxification/anti-oxidant enzymes facilitate elimination of metabolically activated carcinogens, thereby preventing reactive oxygen species (ROS)-mediated damage of cellular macromolecules. Capsaicin increased the protein expression and/or activities of various phase II detoxifying enzymes. The augmented activities of glutathione-S-transferase (GST) and quinone reductase by oral ministration of capsaicin protected F344 rats against chemically induced colon and tongue carcinogenesis (22). The induction of another phase-2 enzyme heme oxygenase-1 (HO-1) has been reported to confer anticancer effects. Joung et al. (23) reported that

Fig. 2. Molecular targets of capsaicin

capsaicin induced the expression of HO-1 in human hepatoma HepG2 cells through the generation of ROS and subsequent activation of a redox-sensitive transcription factor nuclear factor erythroid related factor-2 (Nrf2).

Several studies have demonstrated that capsaicin inhibits lipid peroxidation by increasing the activity of a battery of antioxidant enzymes (24-26). Intraperitoneal administration of capsaicin diminished lipid peroxidation and restored the BP-depleted activities of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glucose-6-phosphate dehydrogenase (G6PD), and GST in the lungs of Swiss albino mice (24,27), which contributed to the antitumor effect of capsaicin against BP-induced lung tumorigenesis. Capsaicin also ameliorated gastric mucosal damage in ethanol-fed Sprague-dawley rats by inhibiting the lipid peroxidation and myeloperoxidase activity (28). Moreover, capsaicin inhibited lipid peroxidation and prevented hepatic and pulmonary damage in rats exposed to several noxious stimuli, such as chloroform, carbon tetrachloride and dichloromethane (26).

Effects of capsaicin on inflammatory signaling One of the hallmarks of cancer is the persistent intratumoral inflammation (29). Thus, the anticancer activity of capsaicin may be attributed partly to its ability to modulate the aberrant activation of inflammatory signaling pathway. Although capsaicin induces ear edema in mice (30,31) and neurogenic inflammation in human skin (32), the compound suppressed the subsequent inflammatory responses upon repeated

application (30,33). Repeated administration of capsaicin suppressed the flare response in human skin by depleting the neuronal release of a pro-inflammatory mediator Substance P (34). Capsaicin-induced depletion of Substance P, which acts as a tumor promoter (35), may underlie the protective effects of the compound against of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidin (MNNG)-initiated gastric carcinogenesis. A major cause of gastric carcinogenesis is *Helicobacter pylori* infection. Capsaicin has been shown to inhibit proliferation of *H. pylori*-stimulated human gastric cancer (AGS) cells and reduce gastric inflammatory responses in *H. pylori*-challenged Mongolian Gerbils, as revealed by decreased infiltration of mononuclear cells into gastric antrum and corpus, and diminish expression of TNF α and phosphorylated I κ B α (36).

Capsaicin inhibited COX-2 expression in gastric mucosa of ethanol-fed rats and lipopolysaccharide (LPS)- or TPA-stimulated macrophages (RAW 264.7) cells (28,37). The later study also demonstrated the inhibitory effects of capsaicin on the production of prostaglandin E₂ (PGE₂) in macrophages incubated with LPS or TPA (37). In contrast, Kim *et al.* (38) reported that capsaicin inhibited LPS-induced PGE₂ production in murine macrophages but did not affect the expression of COX-2 protein and mRNA. Capsaicin also diminished iNOS expression and nitric oxide (NO) production in RAW 264.7 cells stimulated with LPS plus interferon- γ (IFN- γ) as well as in LPS-treated cultured murine peritoneal macrophages (37,38). As TRPV1 or vanilloid receptor-1 (VR1) is absent in macrophages, the inhibition of COX-2 and iNOS expression by capsaicin in these cells is mediated in a VR1/TRPV1-independent manner (37). In contrast, capsaicin elevated the

expression of COX-2 and the production of interleukin (IL)-8 and PGE₂ in VR1-expressing human keratinocytes (HaCaT), which was abrogated by co-treatment with a VR1 antagonist capsazepine (39). Thus, it appears that capsaicin can induce COX-2 expression when cells are orchestrated with functional VR1/TRPV1, but suppresses the expression of same enzyme in absence of this receptor.

Incubation of LPS-stimulated macrophages with capsaicin inhibited TNF α production, which was augmented in the presence of peroxisome proliferator activator receptor- γ (PPAR γ) agonists. Moreover, the compound caused transcriptional activation of PPAR γ -responsive element (PPRE) that was abrogated by co-treatment with a specific PPAR γ antagonist T0070907, suggesting that the anti-inflammatory activity of capsaicin is partly mediated through the activation of PPAR γ (40). A target gene of PPAR γ is liver-X-receptor (LXR) (41), which confers TRPV1-mediated protection against TNF- α -induced inflammation in macrophages (42). Capsaicin, being a TRPV1 agonist, activated LXRs in LPS-stimulated macrophages, thereby suppressed the production of inflammatory cytokines, such as IL-1 β , IL-6, and TNF α via blockade of NF- κ B activation (43). However, the compound inhibited the NF- κ B signaling and downregulated the mRNA expression and release of TNF α and IL6 in LPS-stimulated human salivary gland epithelial (HSG) cells and in primary cultures of salivary gland epithelial cells from TRPV1-/- mice (44), suggesting a TRPV1-independent mechanism of NF- κ B inactivation and anti-inflammatory effects of capsaicin.

The activation of several intracellular kinases and transcription factors, which are involved in aberrant expression of inflammatory triggers (e.g., COX-2, iNOS, and interleukins) has been modulated by capsaicin. Transcription factors such as NF- κ B, activator protein-1 (AP-1), and signal transducer and activator of transcription (STAT) are the molecular targets of capsaicin. Capsaicin inhibited LPS-induced NF- κ B activation in murine macrophages by blocking I κ B α degradation (38). Similarly, capsaicin inhibited the activation of NF- κ B by blocking the degradation of I κ B α and subsequent nuclear translocation of p65 in human myeloid cells (1) and human prostate cancer (PC3) cells (45) stimulated with TNF- α . Moreover, capsaicin negated TNF α - or TPA-induced activation of NF- κ B and AP-1 in human leukemia (K562 and U937) cells (46). Capsaicin also diminished TPA-induced DNA binding of NF- κ B and AP-1 in mouse skin (47) and cultured human promyelocytic leukemia (HL-60) cells (48). Methylation of the phenolic hydroxyl group of capsaicin rendered the compound ineffective in suppressing TPA-induced NF- κ B activation in HL-60 cells, suggesting that the phenolic hydroxyl group is critical for the inhibitory effect of capsaicin on NF- κ B activation (49). Treatment with capsaicin diminished the constitutive or IL-1 β -induced activation of NF- κ B and the production of IL-8 in malignant melanoma cells (50). Chen *et al.* (37) demonstrated that capsaicin attenuated the DNA binding of NF- κ B, AP-1 and STAT1, and inhibited the activation of upstream extracellular signal-regulated protein kinase (ERK), c-Jun-N terminal kinase (JNK) and I κ B kinase (IKK) in LPS-stimulated RAW 264.7 macrophage cells. Similarly, the inhibition of ERK, p38

MAP kinase and JNK phosphorylation by capsaicin in AGS cells resulted in the induction of apoptosis (51). In contrast, topical application of capsaicin to DMBA plus TPA-treated mouse skin upregulated the phosphorylation of NF- κ B, ERK, and p38 MAP kinase without affecting the activation of JNK (52). Thus, the effects of capsaicin on the activation of MAP kinases vary according to the cell or tissue type and/or in a stimuli-specific manner.

Role of capsaicin in regulating tumor cell proliferation The anticancer effects of capsaicin are partly mediated through the inhibition of cancer cell proliferation. Capsaicin inhibited the growth of human esophageal epidermoid carcinoma (CE 81T/VGH) cells by arresting the cell cycle at the G1 phase through the downregulation of cyclin E, cyclin dependent kinase (Cdk)-4 and -6, the transcription factor E2F, and the induction of Cdk inhibitor p21 (53). Similarly, the proliferation of human multiple myeloma cells was arrested at the G1 phase of the cell cycle by capsaicin treatment (54). According to this study, capsaicin inhibited the constitutive as well as IL-6-induced activation of STAT3 and the expression STAT3-regulated proliferative gene products, such as cyclin D1 and survivin by blocking Janus-activated kinase-1 and c-Src kinase (54). Moreover, capsaicin suppressed the proliferation of immortalized human endometriotic (55) and gastric cancer cells (36,51,56). The overexpression of tumor-associated NADH-oxidase (tNOX), also known as ENOX2, has been implicated in acquiring an aggressive phenotype of tumor cells. Moreover, the growth rate of mouse embryonic fibroblast (MEF) cells from tNOX-overexpressing transgenic mice is approximately two-fold greater than that of wild-type MEF cells (56). Capsaicin-induced G1 phase arrest in human gastric cancer (SCM) cells by decreasing the protein and mRNA expression of tNOX and the cleavage of poly-ADP-ribosylpolymerase (PARP) (56). Similar findings of capsaicin-induced G1 phase arrest and reduced cyclin D1 expression in human bladder cancer (TSGH 8301) cells was mediated through the inhibition of tNOX and sirtuin-1 (SIRT1) (57).

Treatment of human small cell lung cancer cells with capsaicin suppressed the growth of these cells in a time dependent manner, without affecting the proliferation of normal lung epithelial (NHBE) cells (58). Capsaicin-induced G1 arrest in these cells was associated with a decrease in the expression of E2F-regulated proliferative genes, such as cyclin E, thymidylate synthase, cell division cycle (cdc)-25A, and cdc6 (58). Capsaicin exhibited antiproliferative effects in human prostate cancer (PC3) cells by inducing endoplasmic reticulum (ER) stress protein growth arrest and DNA dependent gene-153 (GADD153) (59). In contrast, capsaicin increased the viability of androgen-responsive human prostate cancer (LnCaP) cells by inducing the expression of androgen receptor (AR), reducing the intracellular ceramide level and inhibiting the activation of Akt and ERK, which was blunted by TRPV1 antagonist capsazepine. Silencing AR by siRNA abrogated the proliferative effect of capsaicin in these cells (60). Several recent studies also demonstrated that capsaicin inhibited the proliferation of human pharyngeal cancer (FaDu) cells

by inducing G1/S phase cell cycle arrest by decreasing the expression of cyclin B1 and D1, and Cdk-1,-2 and -4 (61). Similarly, the inhibition of Cdk-2,-4 and-6, the generation of ROS, and the loss of mitochondrial membrane potential were associated with reduced proliferation of human bladder cancer cells upon capsaicin treatment (62). The G1/S phase cell cycle arrest by capsaicin in human adult T-cell leukemia cells was mediated through the upregulation of I κ B α and subsequent inhibition of NF- κ B activation (63). However, the compound arrested the progression of human epidermoid carcinoma (KB) cells at G2/M phase and that of human pancreatic cancer (PANC1), colon cancer (HCT116 and LoVo) cells, and nasopharyngeal carcinoma (NPC-TW 039) cells by inducing G0/G1 phase arrest (64-68). The anti-proliferative effects of capsaicin in pancreatic cancer cells was associated with the downregulation of c-Myc and cyclin D1 expression, which was mediated through decreased phosphorylation of STAT3 at tyrosine 705 residue, induction of β -catenin proteasomal degradation, and the blockade of phosphatidyl inositol-3-kinase (PI3K)-Akt signaling pathway (66,69).

Effects of capsaicin on cancer cell apoptosis The anticancer effects of capsaicin are mediated through the induction of apoptosis in various cancer cells in culture (70,71). Molecular mechanisms of apoptosis induction by capsaicin include the inhibition of plasma membrane NADH-oxidoreductase (72-75), generation of ROS, and reactive nitrogen species (75-77), the disruption of mitochondrial membrane permeability (78), caspase activation (53), ceramide accumulation (79), and p53 induction (45,80).

Involvement of ROS in capsaicin-induced apoptosis: Capsaicin generates intracellular ROS partly by blocking coenzyme-Q binding to the mitochondrial respiratory chain (81). The compound acts as a pro-oxidant by destabilizing the mitochondrial respiration, thereby decreasing mitochondrial membrane potential and inducing apoptosis in human skin cancer cells (78). The induction of apoptosis in various human cancer cells, such as those of prostate cancer (PC3) (77), esophageal carcinoma (CE 81T/VGH) (53), colorectal cancer (Colo320DM and LoVo) (82), bladder cancer (T24) (83), and pancreatic cancer (AsPC-1 and BxPC-3) (84) cells, by capsaicin involves intracellular ROS production and mitochondrial membrane depolarization. A study by Pramanik and Srivastava (85) reported that capsaicin-induced ROS diminished thioredoxin expression and increased phosphorylation and kinase activity of apoptosis signal-regulating kinase-1 (ASK1) in AsPC-1 and BxPC-3 cells. Pretreatment with antioxidants or over-expression of thioredoxin abrogated capsaicin-induced apoptosis in these cells. Capsaicin-induced generation of superoxides, induction of iNOS and production of peroxynitrite were associated with C6 glioma cells apoptosis. Similarly, the incubation of human melanoma A375 cells with capsaicin resulted in the generation of nitric oxide radical that leads to the activation of p53 and caspases, thereby inducing apoptosis (86). This was confirmed by pre-incubating cells with a peroxynitrite scavenger, ebselen, which abolished capsaicin-induced apoptosis (76). In another study, CE 81T/

VGH cells were protected against apoptosis when treated with capsaicin in presence of a Ca²⁺ chelator 1,2-bis(2-aminophenoxy) ethane-*N,N,N',N'*-tetraacetic acid (BAPTA), suggesting the role of increased intracellular Ca⁺ concentration in capsaicin induced apoptosis (53).

Capsaicin induced apoptosis in human gastric carcinoma (SCM) cells by inducing cleavage of PARP and inhibiting the expression of tNOX, an enzyme involved in the generation of ROS. Co-treatment with a pan-caspase inhibitor ZVAD-FMK reversed capsaicin-induced apoptosis by blocking the cleavage of PARP and downregulation of tNOX (56). These authors further demonstrated that the compound induced G1 phase arrest in human gastric cancer (SNU1) cells, without affecting that of TMC1 gastric cancer cells. Although capsaicin inhibited the protein and mRNA expression of tNOX in SNU1 cells, downregulation of tNOX by RNA interference sensitized relatively non-responsive TMC-1 cells to the anti-proliferative effects of capsaicin (87).

Activation of intrinsic pathway in capsaicin-induced apoptosis:

Capsaicin induced apoptosis selectively in human pancreatic cancer (AsPC-1 and BxPC-3) cells, without affecting the growth of normal pancreatic acinar cells, by increasing Bax and decreasing Bcl-2 and survivin expression, thereby stimulating mitochondrial release of cytochrome c and apoptosis inducing factor (AIF) into the cytosol (84). Similarly, capsaicin induced apoptosis in human gastric adenocarcinoma (88), murine B16-F10 melanoma (89), and human multiple myeloma (54) cells by downregulating Bcl-2 expression. The compound caused human liver cancer (SK-Hep-1) cells death partly by decreasing Bcl-2/Bax ration and activating caspase-3 (90). The induction of mitochondria-mediated and caspase-9 and -3-dependent apoptosis by capsaicin was documented in a wide variety of cancer cells including those of AGS (91), KB cancer cells (64), pharyngeal carcinoma (FaDu) cells (61), colon cancer (HCT116) cells (92), acute lymphoblastic leukemia cells (93) and the BP-induced mouse lung tumors *in vivo* (94). In addition to the inhibition of Bcl-2 expression and caspase activation, capsaicin downregulated the expression of several key tumor-promoting genes including K-*ras*, Akt, BRAF, MAPK7, and PTPN1 in acute lymphoblastic leukemia cells (93).

Capsaicin-induced apoptosis is mediated through extrinsic pathway:

A mechanism of apoptosis induction by capsaicin is the activation of death receptor (DR)-mediated extrinsic signaling resulting in caspase-8 activation (92,95). While capsaicin-induced apoptosis in HCT116 cells (92) and A375 cells (86) was mediated through elevated expression of DR4 and caspase 8 activation, the compound failed to activate DR4 in human hepatocellular carcinoma (HepG2 and Hep3B) cells; however, it induced the expression of DR5 in a SP-1-dependent manner and sensitized these cells to tumor necrosis factor-related apoptosis inducing ligand (TRAIL)-induced apoptosis (95).

Role of p53 in capsaicin-induced apoptosis: The induction of apoptosis in human prostate cancer (LNCaP, PC3, and DU-145) cells (45) and human esophageal epidermoid carcinoma (CE 81T/VGH) cells (53) with capsaicin is mediated through the induction of p53

and its target gene products such as, p21, and Bax. Capsaicin induced apoptosis in SNU-1 cells and human leukemia cell through the upregulation of p53 (96) and increased phosphorylation p53 at serine 15 residue (80), respectively. The latter study demonstrated that capsaicin-induced cell cycle arrest and apoptosis was abolished through the inactivation of p53 by antisense oligonucleotide (80). Similarly, capsaicin stabilized p53 protein by blocking p53 interaction with murine double minute-2 (MDM2) and subsequent inhibition of p53 proteasomal degradation, thereby inducing apoptosis in human colon cancer cells (67). Silencing p53 by small interfering RNA abrogated capsaicin-induced apoptosis in HCT116 and AGS cells (67,91). One of the mechanisms underlying p53 activation in human lung cancer (A549) cells by capsaicin was the reduced expression of tNOX and induction of silent mating type information regulation-1 (SIRT1) (97). The same study also demonstrated that capsaicin induced autophagy in human fetal lung cells by inducing SIRT1 deacetylase activity and subsequent decreased acetylation of p53 (97).

Capsaicin caused micro-RNA-mediated modulation of cell survival pathways:

Recent studies have demonstrated that capsaicin can modulate the expression of certain microRNAs (miR), thereby inducing apoptosis in pancreatic, prostate, and liver cancer cells. Incubation of human prostate cancer cells with capsaicin reduced androgen receptor (AR)-mediated signaling via induction of miR-449a (98). Capsaicin diminished the expression of microRNA miR-520a-5p, and combined treatment with miR-520a-5p inhibitor with capsaicin showed more potent anti-proliferative effects in K562 leukemic cells compared with capsaicin treatment alone (99). Moreover, this combination therapy suppressed STAT3 mRNA expression, resulting in reduced cell viability. Although the study attempted to establish a molecular link between miR-520a-5p and STAT3 signaling, the increase STAT3 mRNA expression by miR-520a-5p blockade failed to conclude that capsaicin-induced apoptosis in these cells were mediated through blockade of miR-520a-5p-mediated downregulation of STAT3 mRNA expression. However, others have shown that STAT3 is a target of capsaicin in causing apoptosis in pancreatic cancer and hepatocellular carcinoma cells (69,100). Pramanik *et al.* (69) reported that capsaicin inhibited the expression of disheveled protein Dvl-1 and phosphorylation of glycogen synthase kinase-3 β (GSK-3 β), thereby blocking nuclear localization of T cell factor-1 (TCF1), β -catenin, and suppressing the expression of c-myc and cyclin D1. Moreover, capsaicin negated phosphorylation of STAT3 at tyrosine 705 residue, and its nuclear translocation in MiaPaCa-32 and PANC1 cells. Ectopic overexpression or activation of STAT3 signaling by IL-6 treatment abrogated capsaicin-mediated inhibition of β -catenin and its target gene expression and induction of apoptosis (69). In contrast, capsaicin generated ROS and induced phosphorylation of STAT3 at tyrosine 705 residue in HepG2 cells, resulting in the activation of autophagy markers such as light chain-3 (LC3)-II and beclin-1. Pharmacological or genetic interference with autophagy triggered capsaicin-induced PARP cleavage, caspase activation, and

apoptosis induction in these cells (100). Thus, the effect of capsaicin on STAT3 signaling appears to be mediated in a cell type-specific manner.

Role of intracellular kinases in capsaicin-induced cell death:

The activation of a series of intracellular kinases contributed to capsaicin-induced cell death. For instance, increased activation of MAP kinases was associated with capsaicin-induced apoptosis in PC3 cells and H-ras-transformed human mammary epithelial cells (71,79). Similarly, capsaicin-induced activation of JNK resulted in the induction of apoptosis in human pancreatic cancer AsPC-1 and BxPC-3 cells, which was abrogated by pharmacological inhibition of JNK (84). Chow *et al.* (70) also demonstrated a JNK-dependent apoptosis in human gastric cancer cells in association with decreased mitochondrial membrane permeability and elevated expression of Bax and p53 upon treatment with capsaicin. Moreover, capsaicin inhibited the growth of AsPC-1 cell xenograft in nude mice, at least in part, by blocking JNK phosphorylation, elevated Bax expression, and increased caspase-3 activation (84). Similarly, capsaicin induced apoptosis in human renal cancer cells through activation of p38 MAP kinase and JNK (101). Although activation of adenosine monophosphate-activated protein kinase (AMPK) has been attributed to capsaicin-induced apoptosis in human colon cancer (HT-29) cells (102) and osteosarcoma (U2OS and MG63) cells (103), the latter study demonstrated an additional mechanism of AMPK-independent but JNK-mediated apoptosis. According to the latter study, capsaicin-induced AMPK activation resulted in the inhibition of mTOR complex-1, Bcl2 and the activation of p53 in osteosarcoma cells. In a recent study, capsaicin was found to degrade mutant p53 and restore wild type p53 function in human non-small cell lung cancer cells (H1299) (104). These findings suggest that capsaicin can be a potential candidate for p53-targeting drug discovery.

Roles of TRPV in capsaicin-induced apoptosis: While the induction of apoptosis in capsaicin-treated HT-29 (105), glioblastoma (A172) (106), and HepG2 cells (107) was independent of VR1/TRPV1, a paradoxical role of TRPV1 in capsaicin-induced apoptosis was reported by Amantini *et al.* (108). According to the latter study, co-treatment with a VR1/TRPV1 antagonist capsazepine protected glioma U373 cells from apoptosis by reversing capsaicin-induced increased Ca²⁺ influx, p38 MAP kinase phosphorylation, and caspase-3 cleavage (108). These authors further reported that capsaicin arrested the growth of human urothelial cancer (RT4) cells at the G0/G1 phase and induced apoptosis by TRPV1-dependent activation of death receptor signaling and transcriptional activation of pro-apoptotic genes, such as Fas/CD95, and caspases (109). Besides TRPV1, TRPV6 was also reported to cause capsaicin-induced apoptosis. Tumor cells overexpressing TRPV6, but not those lacking this particular receptor, underwent apoptotic death upon treatment with capsaicin (70). A recent study reported selective and robust expression of TRPV6 in human small cell lung cancer cells, but not in normal lung epithelial cells. Although capsaicin-induced apoptosis in these cells was mediated through the activation of calpain pathway, siRNA-mediated

blockade of TRPV6 abrogated capsaicin-induced cell death (110).

Other mechanisms of capsaicin-induced apoptosis: Additional mechanisms of capsaicin-induced apoptosis involve the activation of peroxisome proliferator activated receptor (PPAR)- γ and the induction of growth arrest and DNA damage-inducible gene 153 (GADD153)/CHOP (59,105). While the former study (105) demonstrated that treatment with PPAR- γ antagonist bisphenol-A diglycidyl ether protected HT-29 cells against capsaicin-induced apoptosis, the latter study showed that inactivation of GADD153/CHOP by siRNA abrogated PC3 cells death (59). Moreover, elevated prostate apoptosis response-4 (par-4) expression and intracellular ceramide accumulation contributed to capsaicin-induced apoptosis in PC3 cells (79). Capsaicin induced phosphorylation of CCAAT/enhancer binding protein- β (CEBP β) at serine/threonine residues and subsequently enhanced the formation of a tri-protein complex between CEBP β , GSK-3 β and activating transcription factor-3, which then resulted in transcriptional activation of non-steroidal anti-inflammatory drug-activated gene-1 (NAG1) gene, thereby inducing apoptosis in human colorectal cancer cells (111). The compound also induced apoptosis in human breast cancer (MCF-7) cells and arrested the growth of breast cancer stem cells by blocking nuclear localization of Notch intracellular membrane domain (112). Capsaicin increased Bak and Bim expression and downregulated the Bcl-2 level, resulting in induction of apoptosis in human bladder cancer cells via activation of FOXO3a transcription factor (57).

Inhibitory effects of capsaicin on tumor angiogenesis Capsaicin inhibited angiogenesis in the chick chorioallantoic membrane assay (113). Mechanisms of antiangiogenic effects of capsaicin include the inhibition of VEGF-induced cell proliferation, DNA synthesis, chemotactic motility, capillary-like tube formation, and vessel sprouting (113). Vascular endothelial growth factor (VEGF) is one of the molecular switches of the angiogenic process. Although capsaicin has been reported to increase hypoxia inducible factor-1 α (HIF1 α) DNA binding and VEGF formation in human melanoma (A375P and A375SM) cells (114), a subsequent study demonstrated that capsaicin attenuated the growth of human multiple myeloma cells xenograft by down-regulating the expression of VEGF (54). The inhibition VEGF expression and antiangiogenic effects of capsaicin in human non-small cell lung cancer was mediated through stabilization of p53 via upregulation of scaffold matrix-associated region-1 (SMAR1)-binding protein, and resultant degradation of HIF1 α (115). Capsaicin-induced SMAR1 also resulted in repression of COX-2 activity and the production of PGE₂, thus blocking nuclear localization and transcriptional activation of HIF1 α (115).

Role of capsaicin in migration, invasion and metastasis of tumor cells Several studies have demonstrated the ability of capsaicin to block migration and invasion of various cancer cells. The anti-migration and anti-invasion effects of capsaicin on cholangiocarcinoma (HuCCT1) cells were mediated through the inhibition of MMP-9

expression and the NF- κ B activation via induced phosphorylation of AMPK and induction of SIRT1 activity (116). However, capsaicin treatment at a low concentration upregulated tNOX expression that contributed to increased migration of human colon cancer HCT116 cells as revealed by reduced expression of epithelial markers and increased expression of mesenchymal markers. Knockdown of tNOX reversed the capsaicin-induced proliferation and migration of these cells (117). In contrast, Chen *et al.* (57) recently reported that capsaicin inhibited the migration and invasion of gastric cancer AGS cells by down-regulating the expression of tNOX via suppression of POU3F2 binding to tNOX promoter. The inhibition of tNOX by capsaicin has recently been co-related with its anti-migratory effects in bladder cancer cells. Chen *et al.* (57) demonstrated that capsaicin diminished tNOX expression and attenuated phosphorylation of ERK, paxillin and focal adhesion kinase, thereby blocking migration of human TSGH and T24 bladder cancer cells. This study also revealed that capsaicin elevated E-cadherin and reduced the level of N-cadherin, suggesting that the compound can suppress epithelial to mesenchymal transition (57). These opposing effects of capsaicin appear to happen in a concentration-dependent manner. While at low concentration the compound exhibited increased migration of cancer cells, high concentration of the compound elicited anti-migratory effects. Venier *et al.* (118) reported that capsaicin reduced the migration and invasion of PC3 prostate cancer cells in culture and decreased the percentage of poorly differentiated cells, intraepithelial neoplasia, and metastatic tumor burden in a model of transgenic adenocarcinoma of mouse prostate, which was correlated with downregulation of p27 expression.

Modulation of chemotherapy resistance with capsaicin Chemotherapy failure is a major barrier in gaining success in anticancer therapy. P-Glycoprotein (P-gp), a product of multi drug resistant-1 (*mdr-1*) gene, plays a key role in developing chemoresistance by promoting cellular efflux of chemotherapeutic agents. Capsaicin sensitizes chemoresistant cancer cells to undergo apoptosis or growth arrest (119). Treatment with capsaicin resulted in the enhanced intracellular accumulation of daunorubicin and vinblastine in human multidrug-resistant carcinoma KB-C2 cells and increased the sensitivity of these cells to vinblastine-induced cytotoxicity (120). Capsaicin inhibited the P-gp activity in human intestinal carcinoma (Caco2) cells in a concentration- and time-dependent manner (121). Incubation of vinblastine-resistant human colon carcinoma (LS-180V) cells with capsaicin increased intracellular accumulation of rhodamine 123, without a significant change in P-gp expression or MDR1 mRNA level, but upregulated the mRNA level of multi drug resistance associated protein (MRP)-1 and MRP3 (122). While P-gp-mediated efflux of [³H]-digoxin was inhibited by capsaicin at a relatively low concentration and short exposure, prolonged incubation of Caco-2 cells with high concentration capsaicin increased the expression and the activity of P-gp (123). Thus, the long-term intake of capsaicin at high concentrations may affect the bioavailability of anticancer

drugs, which are P-gp substrates, thereby inducing chemotherapy failure. Capsaicin exhibited synergistic growth inhibitory effects with 5-fluorouracil (5FU) in cholangiocarcinoma cells in culture as well as xenograft tumor growth in nude mice by blocking 5FU-induced autophagy via blockade of 5FU-induced phosphorylation of Akt and ribosomal-S6 kinase (124). Combination of capsaicin with pirarubicin, an instillation chemotherapy for bladder cancer, augmented the growth arrest of 5637 bladder cancer cells through the activation of TRPV1 and subsequent inhibition of nuclear localization of PCNA (125).

Role of capsaicin in cancer immunotherapy Recently, the anticancer drug discovery has largely been shifted to develop cancer immunotherapeutics, which by activating antitumor immune responses eradicate cancer. Several monoclonal antibodies targeting immune checkpoint markers have been approved by the United States Food and Drug Administration (FDA) for the treatment of various cancers including melanoma. One mechanism by which tumors escape the body's immune attack is by increasing the intratumoral population of regulatory T cells (Tregs) and depleting cytotoxic CD8⁺ T cells (CTL). Intratumoral administration of capsaicin has been shown to increase CTL population while declining the numbers of Tregs. Moreover, capsaicin-induced tumor cell apoptosis resulted in increased sensitization of stromal cells as manifested by enhanced antigen presentation by stromal macrophages and their destruction by tumor specific T-cells (126,127). Thus, capsaicin or its structural analogs hold the promise for developing small molecule immune regulator for cancer immunotherapy.

Effects of capsaicin on experimental carcinogenesis *in vivo* Over the last three decades, the effects of capsaicin on various experimental models of carcinogenesis have been investigated. Jang *et al.* (128) reported the anti-tumor promoting effects of capsaicin in a chemically induced lung carcinogenesis model. This study demonstrated a significant decrease in benzo[a]pyrene (BP)-induced pulmonary adenomas and the burden of DMBA-induced lung tumors in NIH(GP) mice, which were treated with BP or DMBA within 24 h of birth and fed with either vehicle or capsaicin (0.01% in diet) for 6 weeks after weaning (128). Capsaicin (0.01%) given as part of a diet also significantly attenuated the formation of glutathione-S-transferase-positive (GST-P⁺) hepatic foci and lung tumors in mice exposed to carcinogens (129). In contrast, orally administered capsaicin failed to suppress lung carcinogenesis in female A/J mice challenged with tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) (130). However, a series of subsequent experiments in Swiss albino mice demonstrated that intraperitoneal administration of capsaicin prevented BP-induced lung tumorigenesis, partly by blocking lung mitochondrial lipid peroxidation and activating intracellular enzymatic and non-enzymatic antioxidant molecules. Moreover, capsaicin augmented the activities of the enzymes involved in citric acid cycle, such as isocitrate dehydrogenase (ICDH),

α -ketoglutarate dehydrogenase (α -KDH), succinate dehydrogenase (SDH), and malate dehydrogenase (MDH)), and that of respiratory chain enzymes such as NADH dehydrogenase and cytochrome c oxidase (24,27,131). These authors further demonstrated that capsaicin downregulated BP-induced overexpression of tumor necrosis factor- α (TNF- α), interleukin (IL)-6, cyclooxygenase-2 (COX-2) and nuclear factor-kappa B (NF- κ B) (132), and the elevated levels of extracellular matrix components, such as collagen, elastin, uronic acid, glycosaminoglycans and matrix metalloproteinases (MMP)-2 and -9 in mouse lung adenocarcinomas (133).

Park *et al.* (12) reported that capsaicin inhibited chemically induced mouse skin carcinogenesis, partly by blocking metabolic activation of chemical carcinogens through the inhibition of CYP2E1 enzyme activity (3). Contrary to these findings, a recent study by Hwang *et al.* (134) demonstrated capsaicin as a co-carcinogen that promotes TPA-induced skin carcinogenesis through EGFR-dependent mechanisms. According to this study, topical application of capsaicin to DMBA-initiated and TPA-promoted skin of mice lacking transient receptor potential of vanilloid-1 (TRPV1) showed increased skin tumor formation and elevated COX-2 expression compared with those retaining wild type TRPV1, thereby suggesting a TRPV1-independent mechanism of co-carcinogenic effect of capsaicin (134). In a subsequent study, Liu *et al.* (52) also reported the co-carcinogenic potential of capsaicin as the topical application of the compound in DMBA-initiated and TPA-promoted mouse skin further elevated the expression of COX-2 and inducible nitric oxide synthase (iNOS) and aggravated papilloma growth. Thus, defining the exact role of capsaicin in skin carcinogenesis requires additional experiments.

Besides its controversial role in skin carcinogenesis, a significant amount of experimental evidence suggesting the anticancer potential of capsaicin exists. Administration of capsaicin as a diet at the initiation stage of azoxymethane (AOM)-induced colon carcinogenesis in rats significantly reduced the incidence of colonic adenocarcinoma formation (135), as well as reduced the incidence of 4-nitroquinoline-1-oxide (4-NQO)-induced tongue carcinogenesis in male F344 rats (22) and caerulein-induced pancreatic intraepithelial neoplasia (PanIN) in LSL-K-ras(G12D)/Pdx1-Cre mice harboring mutant K-ras (136). The latter study reported that the inhibition of caerulein-induced development of PanIN-1 and its progression to grade 2 or 3 PanIN by capsaicin were mediated through the inactivation of ERK, c-Jun and Gli signaling pathway (136).

Capsaicin arrested xenograft tumor growth in mice implanted with various human cancer cells. When administered by intratumoral injection, a combination therapy with capsaicin and *tert*-butylhydroperoxide attenuated the B16 mouse melanoma xenograft tumor growth in C57BL/6 mice (74). The compound also inhibited the growth of human promyelocytic leukemia (NB4) cells transplanted subcutaneously into non-obese diabetic (NOD)/severe combined immunodeficiency (SCID) mice (80). Oral or subcutaneous administration of capsaicin reduced the growth of human prostate cancer xenograft in nude mice (45,77). Similarly, intraperitoneal

administration of capsaicin diminished the growth of human multiple myeloma cells xenograft in nude mice (54). Capsaicin also inhibited the growth of human pancreatic cancer (AsPC-1) (84,85), colorectal

cancer (COLO 205) (137) and human small cell lung cancer (H69) (58) cells tumor xenograft in athymic nude mice. The latter study further demonstrated that the inhibitory effects of capsaicin on the growth

Table 1. Anti-tumor effects of capsaicin *in vivo*

	Cancer types and Experimental models	Findings	Ref.
Skin cancer	Vinyl carbamate-induced skin arcinogenesis in ICR mice	62% inhibition of skin tumor formation; inhibited CYP 2E1 activity	(3)
	DMBA-initiated mouse skin followed by repeated use of capsaicin	No significant increase in tumor multiplicity	(12)
	Mouse skin first treated with capsaicin and followed by repeated TPA		
	DMBA-initiated mouse skin treated with TPA twice a week and capsaicin was applied prior to each TPA application	Significant inhibition of DMBA plus TPA-induced skin papillomagenesis	
	Application of capsaicin to v-Ha-ras-transgenic mouse skin	No induction of skin tumor	(14)
	Application of TPA to v-Ha-ras-transgenic (Tg.Ac) mouse skin	50% increase in tumor multiplicity	
Pancreatic cancer	Capsaicin given to nude mice bearing AsPC-1 cells xenograft	Decreased growth of xenograft tumor activation of Bax, AIF, cytochrome c, caspase-3, and JNK; inhibition of Bcl-2 and survivin	(84)
	LSL-Kras(G12D)/Pdx1-Cre mice challenged with caerulein and treated with capsaicin (10 or 20 ppm) for 8 weeks	Reduced pancreatitis and inhibited PanIN; attenuated activation of ERK, c-Jun, Gli and diminished the expression of PCNA	(136)
	Capsaicin (5 mg/kg/day p.o. for 7 weeks) given to nude mice bearing AsPC-1 cells xenograft or orthotopic tumor model of Panc-I-Luc cells	Significantly reduced the volume and weight of pancreatic tumors; decreased the thioredoxin level and, increased phosphorylation of ASK1; activated caspase-9 and -3.	(85)
Lung cancer	Capsaicin given to nude mice bearing H69 cells xenograft; Implantation of H69 cells into Chicken chorioallantonic membrane (CAM) and treatment with capsaicin	Decreased xenograft tumor growth; inhibited the expression of PCNA, cyclin E, thymidylate synthase, cdc25A, and cdc6	(58)
	Dietary administration of capsaicin in F344 rats treated sequentially with diethylnitrosamine, <i>N</i> -methylnitrosourea, and <i>N,N</i> -dibutyl nitrosamine (DBN)	Decreased lung adenoma formation	(129)
	Capsaicin (10 mg/kg body weight; once a week for 14 weeks) administered to Swiss albino mice challenged with BP (50 mg/kg body weight)	Reduced the number of lung adenomas; Downregulated the expression of MMP-2 and MMP-9; Diminished the levels of extracellular matrix components, such as collagen, uronic acids, and glycosaminoglycans.	(133)
Prostate cancer	Oral administration of capsaicin to nude mice bearing PC3 cells xenograft	Inhibited tumor growth; decreased PSA and AR; inhibited NF- κ B signaling	(45)
	Subcutaneous administration of capsaicin or capsazepine to nude mice bearing PC3 cells xenograft	Inhibited tumor growth; TRPV1-independent effect; induction of apoptosis; activation of casapase-3	(77)
Renal cancer	Administration of capsaicin to nude mice bearing T24 cells xenograft	Inhibition of tumor growth; generation of ROS; TRPV1-dependent induction of cell death	(102)
	Capsaicin given to nude mice bearing T24 cells xenograft	Inhibit tumor growth; increase expression of FOXO3a, catalase, and SOD2	(13)
	Treatment of 786-O renal cancer xenograft tumor in mice	Slow down tumor growth; increase activation of p38 MAP kinase and JNK; increase Bax and decrease Bcl-2 expression; cleavage of caspase-3, -8, and -9	(25)
Liver cancer	Dietary administration of capsaicin in F344 rats treated sequentially with diethylnitrosamine, <i>N</i> -methylnitrosourea, and <i>N,N</i> -dibutyl nitrosamine (DBN)	Inhibited carcinogen-induced GSTP(+) hepatic foci formation	(129)
Colon cancer	Administration of capsaicin to nude mice bearing Colo205 cells xenograft	Inhibited tumor growth; activation of caspase-8, -9, and -3; induction of Bax and inhibition of Bcl-2	(137)
	Dietary administration of capsaicin to F344 rats treated with AOM	Decreased colon adenocarcinomas	(135)

of H69 cells tumor xenograft was associated with reduced expression of proliferating cell nuclear antigen (PCNA) and E2F-target genes, such as cyclin E, thymidylate synthase, cell division cycle (cdc)-25A and cdc6 (58). Qian *et al.* (101) recently reported that capsaicin inhibited the growth of bladder cancer cells xenograft in nude mice by blocking G0/G1 phase cell cycle arrest, which was associated with the activation of forkhead box-O3a (FOXO3a) signaling. Table 1 provides a summary of *in vivo* antitumor effects of capsaicin.

Future perspective Although the initial dispute regarding the carcinogenic, co-carcinogenic or anti-carcinogenic effects of capsaicin have apparently resolved through an understanding that pure *trans*-capsaicin lacks carcinogenic or co-carcinogenic potential, several recent studies further ignited the initial debate. The induction of VR1/TRPV1 in epidermal HaCaT keratinocytes by capsaicin induced Ca^{2+} influx increased the expression of COX-2 and the release of PGE_2 and IL-8, which were attenuated by VR1 antagonist capsazepine (39). This finding suggests that capsaicin may provoke inflammatory responses in a VR1-dependent mechanism. A recent study by Hwang *et al.* (134) reported that topical application of capsaicin induced more skin papillomas in TRPV1 knockout (TRPV1-KO) mice compared with TRPV1 wild type (TRPV1-WT) mice following DMBA-initiation and TPA promotion. This study also demonstrated that co-treatment of mouse skin with capsaicin and TPA induced COX-2 expression more in papillomas and in MEFs from TRPV1-KO mice than those from TRPV1-WT mice. Treatment of TRPV1-KO MEFs with inhibitors of EGFR or MEK abrogated capsaicin plus TPA-induced expression of COX-2 and phosphorylation of MEK. Incubation with capsaicin alone induced COX-2 expression in MEFs harboring wild type EGFR, but not in cells lacking EGFR. Moreover, capsaicin induced EGFR-tyrosine phosphorylation. These findings suggest that capsaicin might act as a co-carcinogen in a TRPV1-independent but EGFR-dependent manner (134). Moreover, capsaicin-induced VEGF production in malignant melanoma cells suggests that the compound elicits pro-angiogenic effects (114). The lung metastasis of transplanted murine 4T1 breast cancer cells in mice occurs after sensory denervation with capsaicin (138). In addition, the expression of caspase-7, a disintegrin and matrixmetalloproteinase-10 and Elk3 was significantly decreased in primary breast tumors from mice treated with capsaicin compared with those from untreated animals, thereby turning into more aggressive phenotype of breast cancer (139). A case-control study comprising 234 cases of gastric cancer and 468 matched controls was conducted in Mexico. The study reported that the intake of capsaicin at a dose of 90–250 mg per day, which is approximately equivalent to 9–25 Jalapeno peppers, increased the risk of gastric cancer independent of *Helicobacter pylori* infection compared with subjects who consumed an average daily intake of 0–29.9 mg of capsaicin (140). Although these limited number of studies have demonstrated the carcinogenic or co-carcinogenic role of capsaicin, a vast majority of research has reported the anticancer effects of the compound. The US FDA has also defined capsaicin as “generally recognized as safe

(GRAS).” Although a number of clinical trials have been conducted to address the therapeutic use of capsaicin to relieve neuropathic pain (101), human trials with capsaicin as an anticancer agent are yet to be launched. In conclusion, further investigation of pro-carcinogenic role of capsaicin is warranted to ascertain the safety of the compound. Alternatively, structural modification of capsaicin may result in safer therapeutic molecule to be exploited for future drug development.

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