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Review

Anticancer Properties of Capsaicin Against Human Cancer

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Abstract. *There is persuasive epidemiological and experimental evidence that dietary phytochemicals have anticancer activity. Capsaicin is a bioactive phytochemical abundant in red and chili peppers. While the preponderance of the data strongly indicates significant anticancer benefits of capsaicin, more information to highlight molecular mechanisms of its action is required to improve our knowledge to be able to propose a potential therapeutic strategy for use of capsaicin against cancer. Capsaicin has been shown to alter the expression of several genes involved in cancer cell survival, growth arrest, angiogenesis and metastasis. Recently, many research groups, including ours, found that capsaicin targets multiple signaling pathways, oncogenes and tumor-suppressor genes in various types of cancer models. In this review article, we highlight multiple molecular targets responsible for the anticancer mechanism of capsaicin. In addition, we deal with the benefits of combinational use of capsaicin with other dietary or chemotherapeutic compounds, focusing on synergistic anticancer activities.*

Despite recent advances in therapies, cancer is still the second leading cause of death in the Unites States and a major cause of morbidity and mortality worldwide (1, 2). Tumorigenesis is a multi-stage process that generally occurs over an extended period of time. Cancer cells acquire unique capabilities that most healthy cells do not possess. For example, cancer cells become resistant to growth-inhibitory signals, proliferate without dependence on growth-stimulatory factors, replicate without limit, evade apoptosis and acquire invasive and angiogenic properties (3). Cancer is initiated and progresses by multiple genetic alterations and aberrant signaling pathways. Identification of molecular targets involved in the steps of tumor development will provide opportunities to establish a promising strategy to fight against cancer. Due to the invasiveness, toxicity and ineffectiveness of current therapeutic approaches, there has been intense interest in using natural and dietary compounds for prevention and treatment of cancer. It has been proposed that 35% of all human cancers could be prevented by changing diet and lifestyle patterns. Large numbers of epidemiological studies have shown that individuals consuming a diet high in fruits and vegetables dramatically reduce their lifetime risk of developing cancer (4). Given the strong evidence of research showing the ability of phytochemicals to suppress all stages of cancer (from initiation to metastasis), a number of national nutrition programs in the United States have been created to educate the population on the importance of eating a variety of fruits, vegetables and whole grains for optimum health.

For the past several decades, phytochemicals found in fruits, vegetables, whole grains, spices and teas have been hot topics in the area of chemoprevention because they exhibit diverse inhibitory effects against cancer initiation, promotion, progression and metastasis (5). Moreover, phytochemicals are readily available, inexpensive and generally non-toxic, which sets them apart from current treatment methods that are indiscriminately toxic and result in many unpleasant side-effects and drug resistance for patients undergoing treatment.

Capsaicin (*trans*-8-methyl-*N*-vanillyl-6-nonenamide) is a homovanillic acid derivative and the major spicy component in chili peppers that are consumed as a spice in many cultures worldwide. It has been used medicinally for centuries, but recently it has been extensively studied for its analgesic (6, 7), antioxidant (8), anti-inflammatory (9) and anti-obesity (10) properties. The anticancer activity of capsaicin has been broadly reviewed for a variety of cancer types (11), however, the effect on carcinogenesis remains

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Table I. *Summary of proposed anticancer mechanisms for capsaicin in various types of cancer.*

	Cancer type	Anticancer mechanism	Reference
Apoptosis	Colon, lung	Stabilizes p53 *via* modulation of p53 ubiquitination and acetylation	30, 43
	Bladder, glioma	TRPV1-dependent.	12, 35-38
	Stomach, lung	TRPV6-dependent.	39, 40
	Colon	Reduces β-catenin and TCF4 expression, and their interaction	44
	Colon	Increases proapoptotic NAG1 expression	45
	Nasopharynx	Induces ER stress through promoting IRE1 and GADD153	73
	Glioma	Increases expression of beclin1, p62, puma-α and autophage	74
	Myeloma	Down-regulates *STAT3* target genes (BCL2, survivin)	17
	Osteosarcoma	Activates AMPK pathway	46
	Colon	Activates PPARγ-induced apoptosis	75
	Liver	Inhibits *de novo* fatty acid synthesis	47
Cell cycle	Colon, bladder	Inhibits CDK2, CDK4 and CDK6 and induces cell-cycle arrest	27, 44, 50
	Breast	Insensitizes EGFR/HER2 and enhances p27	52
	Prostate	Downregulates androgen receptor by restoring miR449a	53
	Stomach	Downregulates tumor-associated NADH oxidase	54
Angiogenesis	Lung, myeloma	Increases HIF1α degradation and reduces VEGF	17, 55
Metastasis	Colon, bile, bladder	Inhibits MMPs and EMT	21, 38, 61
	Melanoma	Inhibits PI3K/AKT/RAC1 signal pathway and cell invasion	58
	Breast CSC	Inhibits NOTCH pathway	71

TRPV: Transient receptor potential vanilloid; TCF4: T-cell factor 4; NAG1: NSAID-activated gene 1; ER: estrogen receptor; IRE1: inositol-requiring enzyme 1; GADD153: growth arrest and DNA-damage-inducible protein; *STAT3*: signal transducer and activator of transcription 3; BCL2: B-cell lymphoma 2; AMPK: AMP-activated protein kinase; PPARγ: peroxisome proliferator-activated receptor gamma; CDK: cyclin-dependent kinases; EGFR: epidermal growth factor receptor; HER2: human epidermal growth factor receptor 2; NADH: nicotinamide adenine dinucleotide phosphate; H1F1: hypoxia-inducible factor-1; VEGF: vascular endothelial growth factor; MMPs: matrix metalloproteinases; EMT: epithelial mesenchymal transition; PI3K: phosphoinositide 3-kinase; AKT: v-Akt murine thymoma viral oncogene; RAC1: RAS-related C3 botulinum toxin substrate 1.

controversial due to conflicting results in epidemiological and basic research studies. Many laboratories have reported that capsaicin possesses chemopreventive and chemotherapeutic effects (12-15), and several *in vivo* studies using rodent models support the antitumorigenic activity of capsaicin (16-20). However, capsaicin may act as a carcinogen or co-carcinogen (21, 22). The proposed anticancer mechanisms of capsaicin include an increase of cell-cycle arrest and apoptosis, but the exact cellular mechanisms are still not completely understood. This review highlights the impact of capsaicin as a chemopreventive dietary factor and summarizes the proposed mechanisms of anticancer activity of capsaicin focusing on signaling pathways and target genes involved in the main hallmarks of cancer (Table I).

Capsaicin and Apoptosis

Apoptosis is an essential barrier against cancer development and progression and loss of apoptotic signaling is highly associated with malignancy (23). Many types of cancer disrupt apoptotic pathways and enhance anti-apoptotic ones that make cancer cells resistant to apoptosis.

Capsaicin has been shown to induce apoptosis in many different types of cancer cell lines including pancreatic (19) colonic (24), prostatic (25), liver (26), esophagieal (27), bladder (28), skin (29), leukemia (30), lung (31), and endothelial cells (32), while leaving normal cells unharmed (11, 13, 19, 30). A recent review by Bley *et al*. noted that capsaicin appears to induce apoptosis in over 40 distinct cancer cell lines (11).

The intrinsic mitochondrial death pathway and the extrinsic death receptor pathway are two major signaling systems that activate the executioner/effector caspases and lead to programmed cell death. In particular, the mitochondrial pathway is engaged in full execution of apoptosis, therefore, the mitochondrion has been termed the 'gatekeeper' of the apoptotic mechanism and the proteins and pathways of the mitochondrial death pathway have become promising targets for novel therapeutic treatments (33). Capsaicin has been shown to target several proteins involved in the mitochondrial death pathway to initiate apoptosis in different cancer cell lines. For example, treatment of capsaicin activated the cluster of differentiation 95 (CD95)-mediated intrinsic and extrinsic apoptotic pathways (12) and suppressed expression of anti-apoptotic protein, B-cell lymphoma 2 which led to caspase-9 and -3 activation, loss of mitochondrial membrane potential, and subsequent increases of cytochrome *c* release (34).

Transient receptor potential vanilloids (TRPVs) are receptors of capsaicin which lead to Ca^{2+}-mediated

mitochondrial damage and cytochrome *c* release. Proapoptotic activity of capsaicin is mediated *via* TRPV1 in many types of cancers (12, 35-38) and *via* TRPV6 (39, 40).

The p53 tumor suppressor is a well-known anticancer mechanism that is frequently mutated in many carcinomas (41). Activation of p53 in response to a variety of stress signals or DNA damage results in cell death. The role of p53 in apoptosis has been intensely studied for decades (42). Capsaicin was found to induce p53 phosphorylation at the Ser-15 residue (30) and enhanced p53 acetylation through down-regulation of sirtuin 1 (43), which is responsible for activation of apoptosis. In contrast, Mori *et al*. found that capsaicin induced apoptosis in a p53-independent manner in both *in vitro* and *in vivo* xenograft models using prostatic cancer cells (25). Treatment of urothelial cancer cells with capsaicin resulted in increased expression of p53 and phosphorylation at Ser-15, -20 and -392, which was accompanied by increased apoptosis (12). These results suggest that p53 is a target of the anticancer mechanism of capsaicin.

Deregulation of β-catenin-dependent signaling is a significant event in the development of malignancies. In many types of cancer cell, β-catenin is constitutively active. In a previous study, we found that capsaicin down-regulated β-catenin transcription, as well as reducing its protein stability, and induced apoptosis of colorectal cancer cells (44). We also reported that capsaicin increases apoptosis through activating a novel pro-apoptotic gene, NSAID-activated gene-1 and proposed a novel pathway associated with phosphorylation of CCAAT/enhancer binding protein β by glycogen synthase kinase 3β and protein kinase C in human colorectal cancer cells (45). Other proposed pro-apoptotic mechanisms of capsaicin include AMP-activated protein kinase (24, 46), reactive oxygen species (14, 30, 47), prohibitin 2 (48) and fatty acid synthase (47). Taken together, these studies support the hypothesis that capsaicin induces apoptosis in cancer cells through activation of multiple pathways.

Capsaicin and the Cell Cycle

Cells proliferate through the cell cycle, which is divided into G_0/G_1, S and G_2/M phases, and which has DNA checkpoints to ensure the integrity of DNA replication. The checkpoint and repair pathways facilitate cellular responses to DNA damage. Any alteration in these pathways can increase the risk of cancer. The cell cycle and growth arrest has gained much attention as a defense mechanism against cancer and target for cancer prevention and therapy, as well as tissue homeostasis (49). Essential parts of the cell-cycle machinery are the cyclins, cyclin-dependent kinases (CDKs) and the CDK inhibitors. Once activated, the CDKs provide a driving force for the cells to move from one phase to the next. In particular, the CDKs and cyclins are highly activated in most cancer types. Therefore, suppression of CDK activity results in cell-cycle arrest and has been identified as a target for cancer therapy to prevent uncontrolled proliferation of cancer cells.

Capsaicin induced G_0/G_1 phase arrest in human esophageal carcinoma cells with an increase of p21 and a decrease of CDK4, CDK6 and cyclin E (27, 50). Treatment with capsaicin also inhibited proliferation and induced cell-cycle arrest in human cancer KB cells (51). In a previous *in vitro* study, we observed a dose-dependent reduction in cyclin D1 in colon cancer cell lines following treatment with capsaicin (44). Capsaicin is a novel modulator of the epithelial growth factor receptor pathway and strong growth suppressor of both estrogen receptor-positive and -negative breast cancer cells (52). Capsaicin is considered a novel anti-androgenic receptor drug in prostate cancer (53). On the other hand, capsaicin was found to have divergent effects on the growth of gastric cancer cells depending on the status of tumor-associated NADH oxidase expression (54). Taken together, these data show that capsaicin may halt growth and division of cancer cells by targeting cell-cycle regulators.

Capsaicin and Angiogenesis

Angiogenesis is the creation of new blood vessels and an essential homeostatic process for normal wound healing and embryonic development. However, angiogenesis is a prerequisite for cancer progression because cancer tissues cannot grow beyond a certain size (generally 1- to 2-mm in diameter), due to lack of oxygen and other essential nutrients. Angiogenesis is a complex process that involves extracellular matrix degradation and proliferation, migration and morphological differentiation of endothelial cells to form tubes. Many factors control the process such as growth factors and cytokines, but vascular endothelial growth factor has been the particular focus of research due to its key role in angiogenesis.

Capsaicin has anti-angiogenic properties both *in vitro* and *in vivo* (32). Treatment of endothelial cells with capsaicin suppressed VEGF-induced proliferation, migration and tube formation *in vivo*. Capsaicin also inhibited VEGF-induced vessel sprouting and formation in a mouse Matrigel assay, which was associated with down-regulation of p38 mitogen-activated protein kinases (MAPK), protein kinase B (PKB) and focal adhesion kinase (FAK) activation (17, 55). In addition, capsaicin increased degradation of hypoxia inducible factor 1α, which is key transcription factor increasing VEGF transcription (55). Therefore, capsaicin interferes with common angiogenic signaling pathways and may have the potential to prevent cancer from becoming malignant.

Capsaicin and Metastasis

Metastasis is a complex and multistep process that occurs when cancer cells acquire the ability to invade the vasculature and migrate to distant organs. Metastatic cancer is resistant to therapy and causes 80% of cancer-related deaths and remains a major challenge in cancer therapy (56). Tumor cell invasion and migration involve the proteolytic degradation of extracellular matrix components by tumor cell-secreted proteases, including serine proteases, plasminogen activators, and matrix metalloproteinases (MMPs).

Capsaicin showed anti-invasive and anti-migratory activity through modulating signaling pathways involved in cell invasion and migration, and suppressed advanced stages of cancer. Capsaicin treatment significantly reduced the metastatic burden in transgenic adenocarcinoma of the mouse prostate (TRAMP) mice (57). Capsaicin significantly inhibited the migration of melanoma cells without leading to obvious cellular cytotoxicity (58). This effect was correlated with down-regulation of the phosphoinositide 3-kinase (PI3K) signaling cascade as well as a reduction in RAS-related c3 botulinum toxin substrate 1 (RAC1) which is key kinase regulating cell motility and migration (59). Capsaicin inhibited the EGF-induced invasion and migration of human fibrosarcoma cells *via* down-regulation of AKT/FAK, extracellular signal-regulated kinases and p38 MAPK signaling and subsequent down-regulation of MMP9 in invasive fibrosarcoma cells (60, 61). Capsaicin-stimulated migration and MMP9 activation are TRPV1-dependent in bladder cancer (38). However, a low dose of capsaicin promoted metastasis of colonic cancer (21).

Synergistic Anticancer Activity of Capsaicin with Other Compounds

Recently, there has been a growing interest in combinations of low doses of chemopreventive agents in both prevention and treatment of cancer. Due to their synergistic effect and use of low doses, such combinations enhance the efficacy and reduce the risk of toxicity and resistance caused by use of higher doses of single compounds. Numerous studies highlighted that novel combination treatments with various phytochemicals and other chemopreventive agents may exert enhanced antitumor activity through additive or synergic action (62, 63). The mechanisms include complementary action on cancer inhibition through modulating carcinogen de-toxification and hormone metabolism, scavenging oxidative stress, and enhancing immunity (64-66). In fact, thousands of phytochemicals have different chemical properties such as polarity and solubility, and bioavailability, which target different signaling pathways and transcription factors determining cancer phenotype. Therefore, the use of multiple phytochemicals with distinct anticancer mechanisms could be a promising approach for efficient cancer prevention and therapy.

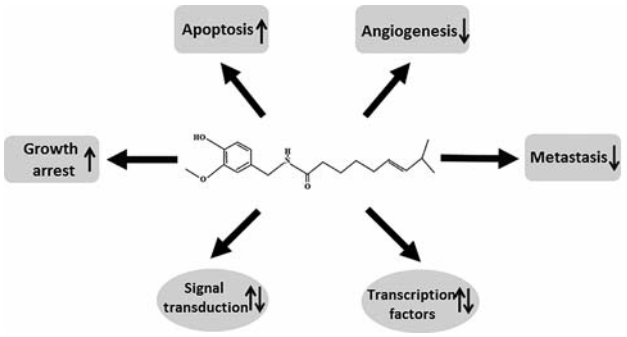

Figure 1. *Outline of anticancer mechanisms of capsaicin in cancer.*

Capsaicin is known to have synergistic anticancer activities with other agents. Capsaicin combined with resveratrol promoted apoptosis by the elevation of NO *via* a p53-dependent manner (34). Capsaicin had synergistic anticancer activity with pirarubicin, an anthracycline drug, through TRPV1 activation in bladder cancer (37). The dietary phytoestrogen, genistein also had synergistic anticancer activity in combination with capsaicin through modulation of AMPK and cyclo-oxygenase 2 in breast cancer cells (67). Recently, we reported that capsaicin and 3,3'-diindolylmethane, a major *in vivo* metabolite of indole-3 carbinol, abundant in cruciferous vegetables, work synergistically to induce apoptosis in colorectal cancer through modulating transcriptional activity of nuclear factor kappa B, p53 and target genes associated with apoptosis (68). Kim *et al*. reported that brassinin, a type of indole derived from cruciferous vegetables, exerted synergistic anticancer activity in combination with capsaicin by suppressing MMP2 and -9 expression and enzymatic activities, and migration and invasion of prostate carcinoma cells (69). Capsaicin also interacts with chemotherapeutic agents. Capsaicin improved the therapeutic activity of 12-O-tetradecanoylphorbol-13-acetate in some myeloid leukemia cells (70). Interestingly, capsaicin may influence the viability of cancer stem cells. Capsaicin was found to induce death of cancer stem cells through inhibiting the NOTCH signaling pathway in breast cancer stem cells (71). However, antagonistic activity of capsaicin with chemotherapeutic agents was also reported. Capsaicin reversed the anticancer activity of 5-fluorouracil in cholangiocarcinoma which is resistant to chemotherapeutic agent (72).

Conclusion

Given that cancer is initiated and progresses by aberrant expression of genes and transduction pathways, an emerging

area of cancer research is to look for responsible molecular targets and effective anticancer compounds modulating these cancer targets. Capsaicin exhibits strong anticancer activity through targeting multiple signaling pathways and cancer-associated genes in different tumor stages including initiation, promotion, progression and metastasis. Overall, the anticancer mechanisms of capsaicin include activation of apoptosis, cell-growth arrest and inhibition of angiogenesis and metastasis. Capsaicin stimulates the anti-tumorigenic/tumor-suppressive signaling pathway and related transcription factors, whereas it inhibits oncogenic signaling pathways and tumor promoters (Figure 1). In addition, capsaicin interacts with other cancer-preventive agents synergistically, providing the possibility for the potential use of capsaicin in cancer therapy with other chemotherapeutic agents. Further study of the anticancer targets of capsaicin holds potential for novel therapies in the future and warrants more research to improve our understanding on its efficacy in cancer prevention and treatment.

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