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◈ Review Article ◈

Inhibition of Invasive Phenotype and Induction of Apoptosis in Human Breast Cancer Cells by Chemopreventive Agents

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The most effective way to deal with cancer would be to prevent development of the cancer. Cancer chemoprevention is the treatment to block initiation or promotion-progression phases. Cancer metastasis represents the most important cause of cancer death and anti-tumor agents that may inhibit this process have been pursued. Ras expression has been suggested as a marker for tumor aggressiveness of breast cancer, including the degrees of invasion and tumor recurrence. In this review article, we summarized several chemopreventive agents which inhibit invasive phenotype and/or induce apoptosis of H-*ras*-transformed MCF10A human breast epithelial cells. The molecular mechanism(s) underlying the cancer chemopreventive effects are also reviewed. A potential use of natural products including apicidin, *Aster,* curcumin and capsaicin for human breast cancer chemoprevention has been suggested.

Key Words: Apicidin, *Aster*, Curcumin, Capsaicin, H-ras, MCF10A cells, Invasion, Migration, MMP-2, Apoptosis

BACKGROUND

Incidence of breast cancer in Korea continues to rise year by year, and its clinical features will become closer to those now observed in Western countries mainly due to drinking and dietary habits.[1] Surgical operation, radiotherapy and chemotherapy have widely been used, but the cost of expensive hospital treatment and death as the negative side-effects of drug occurred. Cancer chemoprevention has emerged as a new strategy against cancer. Chemoprevention is the use of pharmacologic or natural agents that inhibit the development of invasive cancer either by blocking the DNA damage that initiates carcinogenesis or by arresting or reversing the progression of pre-malignant cells in which such damage has already occurred. Cancer metastasis represents the most important cause of cancer death and anti-tumor agents that may inhibit this process have been pursued.

Mutated *ras* genes are found in about 30% of all human cancers, and elevated levels of the Ras protein are detected in 60～70% of human primary breast carcinomas.[2] Ras expression has been suggested as a marker for tumor aggressiveness of breast cancer, including the degrees of invasion and tumor recur-

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rence. In order to suggest a potential use of natural products for human breast cancer chemoprevention, we reviewed chemopreventive agents including apicidin, *Aster*, curcumin and capsaicin with regard to their anti-metastatic and/or apoptosis-inducing effects on H-*ras*-transformed MCF10A breast epithelial cells.

ras Oncogenes

Ras proteins are GTP-binding switch proteins that relay signals from cell-surface receptors to the nucleus and regulators of cell proliferation and differentiation. Ras proteins are controlled by GTP or GDP binding, such that they alternate between GTP-Ras (active) and GDP-Ras (inactive) forms.[3~5] Oncogenic Ras proteins, however, cannot regulate GTPase switch, which remain in the active GTP-bound state and thus upregulate cell proliferation even in the absence of growth factor stimulation.

The *ras* gene family consists of three identified members: Harvey-*ras* (H-*ras*), Kirsten-*ras* (K-*ras*) and Neuroblastoma-*ras* (N-*ras*), encoding proteins of 188~189 amino acids with a molecular weight of 21 kDa. While the N-terminal 85 amino acids are identical and the middle 80 amino acids contain 85% homology between Ras proteins, the C-terminal sequence is highly divergent.[6,7] Expression of H-, K-, and N-*ras* is regulated in a tissue-specific manner and during development, indicating that Ras proteins may have different cellular functions. Unique functions of *ras* family members are also supported by the demonstration that there are differences in the signal transduction pathways induced by Ras proteins.[8] To examine the roles of H- and N-*ras* on the induction of invasive phenotypes, we established MCF10A human breast epithelial cell lines in which H- and N-*ras* are constitutively activated using retroviral vectors containing a mutation of gly to asp in amino acid codon 12 in H- and N-*ras*.[9]

H-*ras* Specific Invasion and Migration in MCF10A Cells

In vitro invasion assay and *in vitro* migration assay revealed that H-*ras*, but not N-*ras*, induces invasive[9] and migrative[10] phenotypes in MCF10A cells (Fig. 1A). Invasion and metastasis of malignantly transformed cells involve degradation of the extracellular matrix (ECM) components by matrix metalloproteinases (MMP), especially MMP-2 (72 kDa type IV collagenase) and MMP-9 (92 kDa type IV collagenase). MMP-9 has been suggested to be critical for the induction of an invasive phenotype in rat embryonic fibroblast cells.[11,12] H-*ras*-induced invasive phenotype is associated more closely with the expression of MMP-2 in human breast epithelial cells, rather than the induction of MMP-9 expression shown previously in rat embryonic fibroblasts demonstrating that the role of MMPs in invasive phenotype may be cell type-specific.[9] These studies revealed differential regulation of *ras* signaling pathways by H-*ras* and N-*ras* with a single mutation at the codon 12, providing an insight into the prospect of developing targeted therapy for mammary carcinoma.

The p38 mitogen-activated protein kinase (MAPK) has been suggested as a key signaling molecule differentially regulated by H-*ras* and N-*ras*, leading to H-*ras*-specific cell invasive and migrative phenotypes in human breast epithelial cells (Fig. 1B).[10] Treatment with SB203580, a specific inhibitor of p38, significantly inhibited invasive phenotype (Fig. 1C) and MMP-2 expression (Fig. 1D) in H-ras MCF10A cells, suggesting that the activation of p38 may contribute to a more invasive phenotype *via* the secretion of MMP-2.[10]

Effects of Apicidin on H-*ras*-induced Invasive Phenotype

Apicidin [cyclo(*N*-*O*-methyl-L-tryptophanyl-L-isoleucinyl-D-pipecolinyl-L-2-amino-8-oxodecanoyl)],

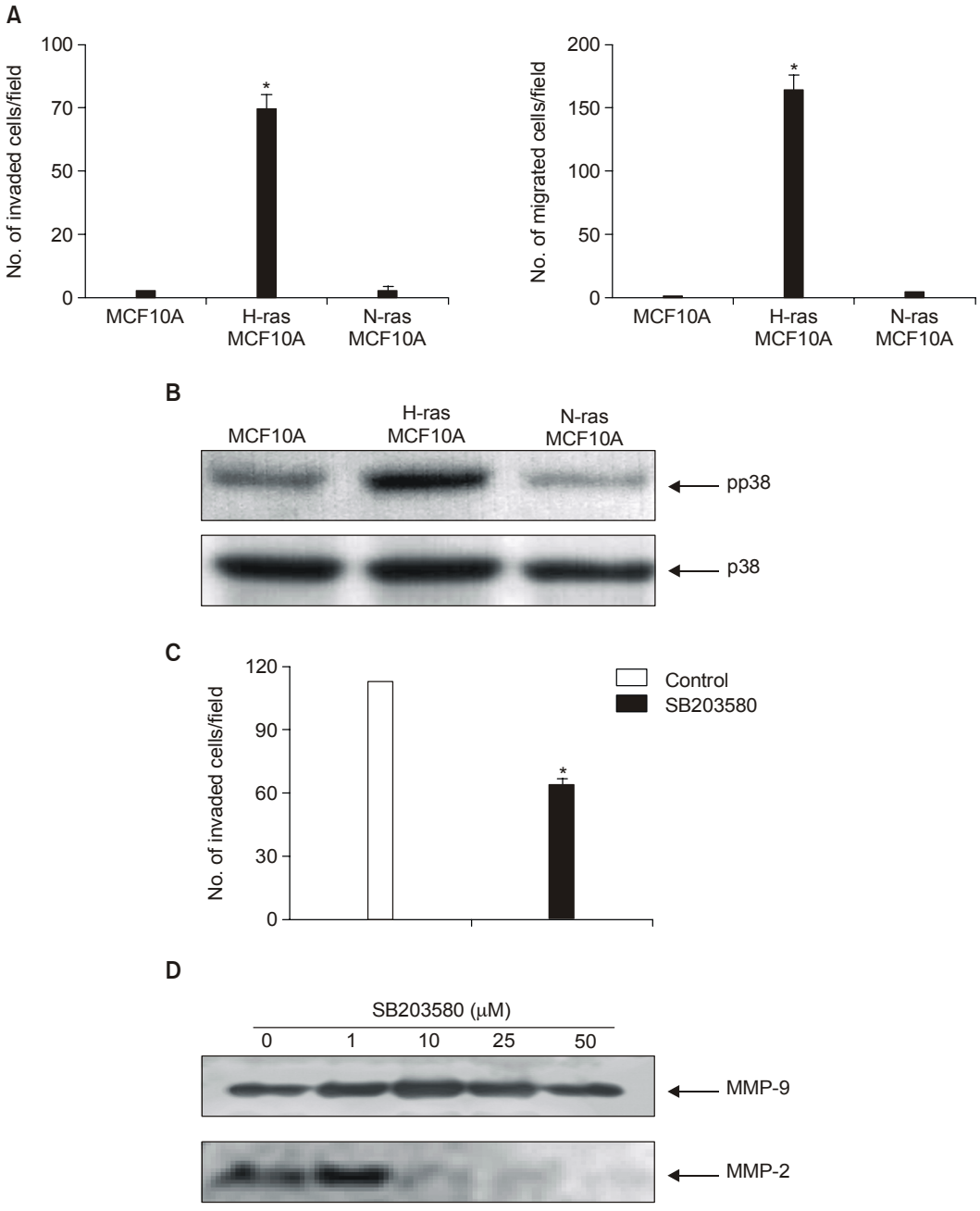

Fig. 1. H-*ras*, but not N-*ras*, induces invasive phenotypes in MCF10A cells.[9,10] (A) The *in vitro* invasion assay and *in vitro* migration assay were performed on MCF10A, H-ras MCF10A and N-ras MCF10A cells. The number of invaded or migrated cells per field was counted (×400) in thirteen fields. The results represent means±S.E. of triplicates. *Statistically different from control at $p < 0.01$. (B) The levels of activated p38 were determined by immunoblot analysis of whole cell lysates using anti-p38 antibodies. (C) H-ras MCF10A cells were pretreated with 50μM SB203580 for 30 min and subjected to *in vitro* invasion assay for 17 hr. (D) H-ras MCF10A cells were treated with various concentrations of SB203580 for 48 hr. Levels of secreted MMP-2 and MMP-9 were determined by immunoblot analysis.

a novel fungal metabolite, has been identified as an antiprotozoal agent that inhibits parasite histone deacetylase (HDAC).[13] Cellular responses by HDAC inhibitors including cell cycle arrest, alteration of gene expression, and induction of apoptosis have been demonstrated.[14~16] Apicidin induced a morphological reversal and growth inhibition of H-ras MCF10A cells similar to that induced by other HDAC inhibitors.[17] In addition, apicidin significantly inhibited H-*ras*-induced invasiveness of MCF10A cells in parallel with a specific down-regulation of MMP-2, but not MMP-9 (Fig. 2).[17] The results demonstrated that apicidin exerted anti-invasive and detransforming activities in H-*ras*-transformed MCF10A cells, suggesting a potential use of HDAC inhibitors for treatment of cancer.

Effects of *Aster* on H-*ras*-induced Invasive Phenotype

Aster species are widespread and used as culinary vegetables in Korea.[18] They have also been used in traditional Chinese medicine for treatment of bruises, snakebite, headache and dizziness.[19] Recently studies revealed that quinic acid derivatives from *Aster scaber* exhibited diverse biological activities: antiviral/anti-hepatotoxic activities and neuroprotective/neurotrophic effects.[20,21] Ahn *et al.* reported chemopreventive ef-

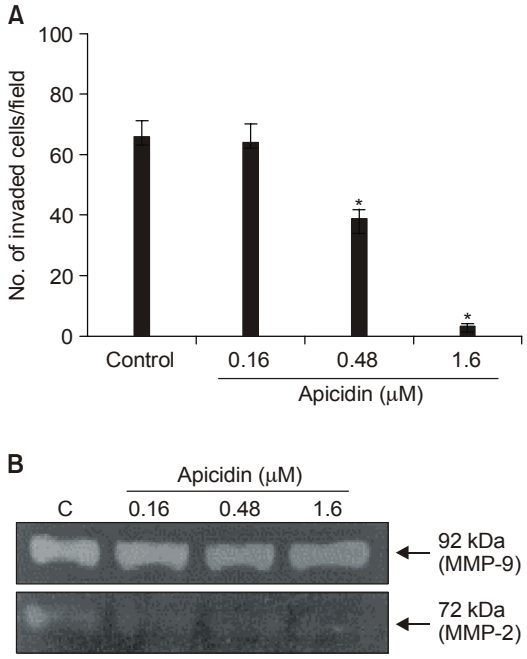

Fig. 2. Apicidin inhibits invasive phenotype and down-regulates MMP-2 in H-ras MCF10A cells[17] (A) Anti-invasive activity of apicidin was examined in H-ras MCF10A cells by *in vitro* invasion assay. The number of invaded cells per field was counted (×400) in thirteen fields. The results represent means±S.E. of triplicates. *Statistically different from control at $p<0.01$. (B) A gelatin zymogram assay was performed to detect the enzymatic activities of MMP-2 and MMP-9 secreted by H-ras MCF10A cells treated with various concentrations of apicidin for 48 hr.

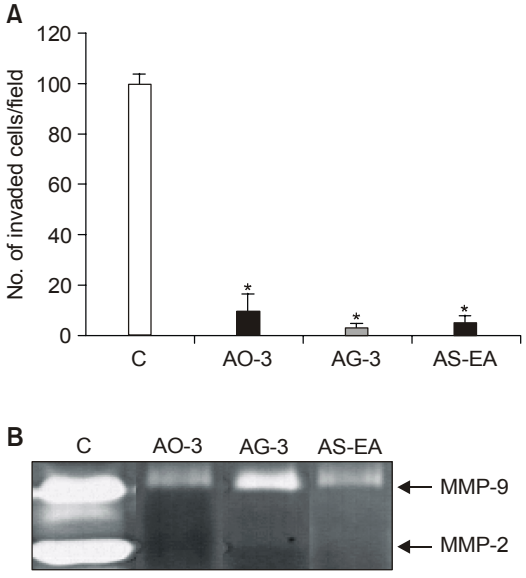

Fig. 3. *Aster* extracts inhibit invasive phenotype and down-regulated MMP-2 and MMP-9.[22] (A) H-ras MCF10A cells were incubated with 5 mg/ml *Aster* extracts (AO-3, AG-3 and AS-EA) in a Matrigel-coated transwell chamber for 17 hr. The number of invaded cells per field was counted (×400) in thirteen fields. The results represent means± S.E. of triplicates. *Statistically different from control at $p<0.01$. (B) H-ras MCF10A cells were treated with 5 mg/ml *Aster* extracts (AO-3, AG-3 and AS-EA) for 24 hr in serum-free media. Conditioned media were subsequently analyzed for secretion of MMP-2 and MMP-9 by gelatin zymogram analysis.

fects of extracts from three *Aster* species *(Aster scaber, Aster oharai* and *Aster glehn*) on H-ras MCF10A human breast epithelial cells.[22] Out of twelve extracts, three extracts (AO-3, AG-3 and AS-EA) were selected for further studies since they exerted a marked inhibition in the ratio of MMP-2 to MMP-9 (Fig. 3A). Treatment with AO-3, AG-3 and AS-EA in H-ras MCF10A cells caused a significant inhibition of invasive phenotype and migration, proving a chemopreventive potential of these extracts (Fig. 3B). The results demonstrated that extracts of *Aster* effectively inhibit invasion and migration of highly malignant human breast cells, possibly *via* downregulation of MMP-2 and MMP-9.

Inhibition of Invasion and Induction of Apoptosis and by Curcumin

Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)1,6-hepta-diene-3,5-dione], a naturally yellow coloring agent from the root of the plant *Curcuma longa Linn*, has been used as spice in foods as well as in cosmetics and drugs.[23] Curcumin is a phenolic compound possessing anti-cancer, anti-inflammatory and anti-metastatic activities.[24~27] Curcumin treatment significatly inhibited invasiveness (Fig. 4A) and MMP-2 secretion, but not that of MMP-9 (Fig. 4B) in H-ras MCF10A cells as evidenced by gelatin zymography.[28]

Efforts have been made to develop a chemoprevention strategy that selectively triggers apoptosis in malignant cancer cells. Curcumin induced apoptosis with a specific down-regulation of anti-apoptotic oncoprotein Bcl-2 and a up-regulation of the death-promoting Bax in H-ras MCF10A cells (Fig. 4C).[28] Curcumin induced reactive oxygen species (ROS) and was significantly inhibited cell-death and DNA fragmentation by pretreatment of an antioxidant N-acetyl-L-cysteine (NAC).[28] Ac-DEVD-CHO, a caspase-3-inhibitor attenuated curcumin-induced apoptosis in H-ras MCF10A cells.[29] The data demonstrate that curcumin induces apoptosis of H-ras MCF10A cells in which ROS and caspase-3 are involved. Taken together, these reports demonstrate that curcumin inhibits invasion and induces apoptosis, proving the chemopreventive potential of curcumin.

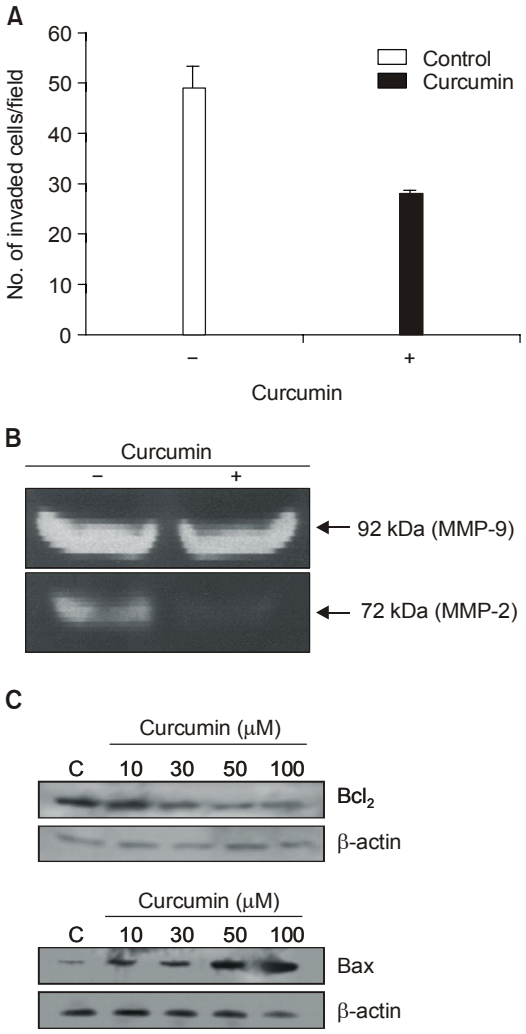

Fig. 4. Curcumin inhibits invasive phenotype and induces apoptosis in H-ras MCF10A cells.[28,29] (A) Cells treated with 50μM curcumin for 48 hr were subjected to *in vitro* invasion assay. The number of invaded cells per field was counted (×400) in thirteen fields. The results represent means±S.E. of triplicates. (B) Cells were treated with 100 μM curcumin for 48 hr and conditioned media were subjected to the gelatin zymogram assay. (C) Immunoblot analysis for Bcl-2 and Bax was performed on lysates of cells treated with various concentrations of curcumin for 24 hr.

Induction of Apoptosis by Capsaicin in H-ras MCF10A Cells

A major pungent ingredient in red pepper, capsaicin [trans-8-methyl-N-vanillyl-6-nonenamide] is widely used as a spice.[30,31] Recent studies have shown the anti-carcinogenic, anti-mutagenic or chemopreventive activities of capsaicin.[32,33] In order to determine whether capsaicin selectively induces apoptosis in

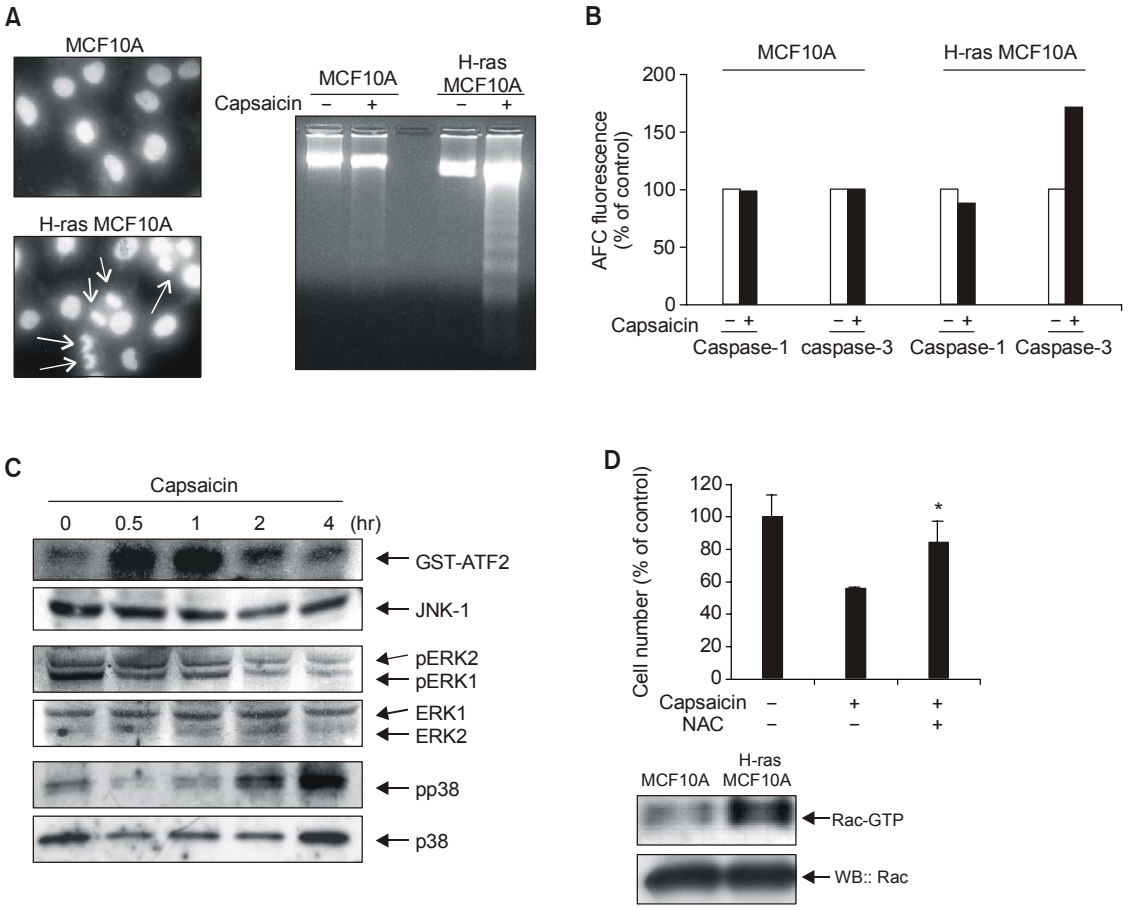

Fig. 5. Capsaicin selectively induces apoptosis in H-ras MCF10A cells.[34,35] (A) Nuclear morphological changes were examined on MCF10A and H-ras MCF10A cells treated with 25μM capsaicin for 48 hr by staining with bis-benzimide. Internucleosomal DNA fragmentation of the cells treated with 50μM capsaicin for 48 hr was analyzed by 1.8% agarose gel electrophoresis. (B) Activation of MAPKs in H-ras MCF10A cells treated with 50μM capsaicin for indicated times was determined by immunocomplex kinase assay using GST-ATF2 fusion protein as a protein substrate for JNK-1 and immunoblot analysis for ERKs and p38. (C) Capsaicin selectively increases caspase-3 activity in H-ras MCF10A cells. MCF10A cells and H-ras MCF10A cells were treated with 50μM capsaicin for 1 hr and caspase-1 and -3 activities were measured by using a fluorometric assay kit. (D) Left: ROS is required for capsaicin-induced growth inhibition of H-ras MCF10A cells. Cells were incubated with 50μM capsaicin for 28 hr with or without pretreatment with 2 mM NAC for 30 min. The percentage of cell survival was normalized to the control cells. *Statistically different from control at $p<0.05$. Right: Rac is activated by H-*ras* in MCF10A cells. Equal amounts of cell lysates were incubated with GST-PBD fusion protein and the bound active Rac-GTP molecules were analyzed by immunoblotting using anti-Rac antibody.

transformed cells, Kang *et al.* investigated the effect of capsaicin in non-transformed and *ras*-transformed cells of a common origin: parental (MCF10A) and H-*ras*-transformed (H-ras MCF10A) human breast epithelial cells.[34] Capsaicin selectively induced apoptosis in H-ras-transformed cells, but not in their normal cell counterparts as evidenced by nuclear morphological changes and DNA fragmentation (Fig. 5A). The capsaicin-induced apoptosis involved the activity of caspase-3 (Fig. 5B). As shown in Fig. 5C, capsaicin treatment markedly activated c-Jun N-terminal protein kinase (JNK)-1 and p38 MAPK while it deactivated extracellular signal-regulated protein kinases (ERKs) only in H-ras MCF10A cells. Functional significance of capsaicin-activated JNK-1 and p38 in *ras*-specific apoptosis of MCF10A cells was demonstrated by using chemical inhibitors and dominant negative (DN) mutants while inhibition of ERK activity was not effective in this regard.[34]

It has been demonstrated that *ras*-transformed fibroblasts produce reactive oxygen species (ROS) through a mechanism which is dependent of Rac1.[35] Possible roles of ROS and Rac1 in capsaicin-induced apoptosis of H-ras MCF10A cells have been investigated.[36] Pretreatment of H-ras MCF10A cells with an antioxidant N-acetylcysteine (NAC) significantly reversed capsaicin-induced growth inhibition, suggesting that ROS may mediate the apoptosis of H-*ras*-transformed cells induced by capsaicin (Fig. 5D). Rac1 was prominently activated by H-*ras* in MCF10A cells (Fig. 5D). Based on the studies using a wild type Rac1 and a dominant negative Rac1 constructs, it has been proposed that Rac1 activity is critical for inhibitory effect of capsaicin on growth of H-*ras*-transformed MCF10A cells possibly through ROS generation.[36]

CONCLUSIONS

Molecular biology of cancer is beginning to contribute to the development of new approaches for the prevention and treatment of cancer, which may ultimately yield major advances in dealing with this disease. The most effective way to deal with cancer would be to prevent development of the cancer. Most of drugs currently used in cancer treatment either damage DNA or inhibit DNA replication. Consequently, these drugs can be detrimental not only to malignant cells but also to normal cells. The action of anti-cancer drugs against these normal cell populations accounts for most of the toxicity associated with these drugs and limits their effective use in cancer treatment. Therefore, efforts have been made to develop a chemoprevention strategy that selectively triggers apoptosis in malignant cells but not in the normal cells.[37] This review article summarized that curcumin and capsaicin induced apoptosis in H-*ras*-transformed malignant breast epithelial cells.

Since cancer metastasis represents the most significant cause of cancer death, anti-tumor agents that may inhibit metastatic process have been extensively pursued. A wide variety of natural products derived from plants was reported to retain marked anti-invasive and anti-migrative activities in various malignant cell systems. In the present study, we reviewed that apicidin, *Aster* and curcumin exerted inhibitory effects on the metastatic properties of highly invasive H-ras MCF10A cells. Taken in conjunction with the fact that uncontrolled *ras* activation is probably the most common genetic defect in human cancer cells, these findings may be critical to the chemopreventive potential of several natural products, and for developing a strategy to inhibit invasion and induce tumor cell-specific apoptosis.

ACKNOWLEGEMENT

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REFERENCES

1) Ahn SH. Clinical characteristics of breast cancer patients in Korea in 2000. Korean Breast Cancer

Society. *Arch Surg* 2004; 139: 27-30.
2) Clair T, Miller W, Cho-Chung Y. Prognostic significance of the expression of the ras protein with a molecular weight of 21,000 by human breast cancer. *Cancer Res* 1987; 49: 5290-5293.
3) Bar-Sagi D, Hall A. Ras and Rho GTPases: a family reunion. *Cell* 2000; 103: 227-238.
4) Shields JM, Pruitt K, McFall A, Shaub A, Der CJ. Understanding Ras: 'it ain't over 'til it's over'. *Trends Cell Biol* 2000; 10: 147-154.
5) Downward J. Targeting RAS signalling pathways in cancer therapy. *Nat Rev Cancer* 2003; 3: 11-22.
6) Barbacid M. ras genes. *Annu Rev Biochem* 1987; 56: 779-827.
7) Boguski MS, McCormick F. Protein regulating ras and its relative. *Nature* 1995; 663: 643-654.
8) Carbone A, Gusella GL, Radzioch D, Varesio L. Human Harvey-ras is biochemically different from Kirsten- or N-ras. *Oncogene* 1991; 6: 731-735.
9) Moon A, Kim MS, Kim TG, Kim SH, Kim HE, Chen YQ, Kim HRC. H-ras, but not N-ras, induces an invasive phenotype in human breast epithelial cells: A role for MMP-2 in the H-ras induced invasive phenotype. *Int J Cancer* 2000; 85:176-181.
10) Kim MS, Lee EJ, Kim HRC, Moon A. p38 kinase is a key signaling molecule for H-ras-induced cell motility and invasive phenotype in human breast epithelial cells. *Cancer Res* 2003; 63: 5454-5461.
11) Bernhard EJ, Muschel RJ, Hughes EN. Mr 92,000 gelatinase release correlates with the metastatic phenotype in transformed rat embryo cells. *Cancer Res* 1990; 50: 3872-3877.
12) Bernhard EJ, Gruber SB, Muschel RJ. Direct evidence linking expression of matrix metalloproteinase 9 (92 kDa gelatinase/collagenase) to the metastatic phenotype in transformed rat embryo cells. *Proc Natl Acad Sci* 1994; 91: 4293-4297.
13) Darkin-Rattray S, Gurnett AM, Myres RW, Dulski PM, Crumely KM, Allocco JJ, Cannova C, Meinke PT, Colletti SL, Bednarek MA, Singh SB, Goetz MA, Dombrowski AW, Polishook JD, Schimatz DM. Apicidin: a novel antiprotozoal agent that inhibits parasite histone deacetylase. *Proc Natl Acad Sci* 1996; 13143-13147.
14) Kim YB, Lee KH, Sugita K, Yoshida M, Horinouchi S. Oxamflatin is a novel antitumor compound that inhibits mammalian histone deacetylase. *Oncogene* 1999; 18: 2461-2470.
15) Arts J, Lansink M, Grimbergen J, Toet KH, Kooistra T. Stimulation of tissue type plaminogen activator gene expression by sodium butyrate and TSA in human endothelial cells involves histone acetylation. *Biochem J* 1995; 310: 171-176.
16) Vicente M, Belinda E, Graeme PY, Robert J, Sarah A, Peter DZ. Induction of caspase-3 protease activity and apoptosis by butyrate and trichostatin A (Inhibitors of histone deacetylase): Dependence on protein synthesis and synergy with a mitochondrial/cytochrome c-dependent pathway. *Cancer Res* 1997; 57: 3697-3707.
17) Kim MS, Son MW, Kim WB, In Park Y, Moon A. Apicidin, an inhibitor of histone deacetylase, prevents H-ras-induced invasive phenotype. *Cancer Lett* 2000; 157: 23-30.
18) Lee TB. Illustrated Flora of Korea. Seoul, HyangMun, 1989; pp 760.
19) Kim CM, Sin MK, An TK, Lee KS, Lee KS. Dictionary of Chinese Herb. Seoul, JungDam, 1997; pp 1431.
20) Gesa M, Anthony DW, Gabriele MK. HIV reverse transcriptase inhibitors of natural origin. *Planta Medica* 1999; 65: 493-506.
21) Hur JY, Soh Y, Kim BH, Suk K, Sohn NW, Kim HC, Kwon HC, Lee KR, Kim SY. Neuroprotective and neurotrophic effects of quinic acids fromAster scaber in PC12 cells. *Biol Pharm Bull* 2001; 24: 921-924.
22) Ahn SM, Lee KR, Moon A. Extracts of aster species inhibit invasive phenotype and motility of H-ras MCF10A human breast epithelial cells possibly via downregulation of MMP-2 and MMP-9. *J Applied Pharmacol* 2002; 10: 240-245.
23) Wattenberg LW. Inhibition of carcinogenesis by minor anutrient constituents of the diet. *Proc Natl Acad Sci* 1990; 49: 173-83.
24) Pereira MA, Grubbs CJ, Barnes LH, Li H, Olson GR, Eto I, Juliana M, Whitaker LM, Kelloff GJ, Steele VE, Lubet RA. Effects of the phytochemicals, curcumin and quercetin, upon azoxymethane-induced colon cancer and 7,12-dimethylbenz[α] anthracene-induced mammary cancer in rats. *Carcinogenesis* 1996; 17: 1305-1311.
25) Chun KS, Shon Y, Kim HS, Kim OH, Park KK, Lee JM, Lee J, Lee JY, Moon A, Lee SS, Surh YJ. Anti-tumor promoting potential of naturally occurring diarylheptanoids structurally related to curcumin. *Mutat Res* 1999; 428: 49-57.
26) Huang MT, Lysz T, Ferraro T, Abidi TF, Laskin JD, Conney AH. Inhibitory effects of curcumin on in vitro

lipoxygenase and cyclooxygenase activities in mouse epidermis. *Cancer Res* 1991; 51: 813-819.
27) Menon LG, Kuttan R, Kuttan G. Anti-metastatic activity of curcumin and catechin. *Cancer Lett* 1999; 141: 159-165.
28) Kim MS, Kang HJ, Moon A. Inhibition of invasion and induction of apoptosis by curcumin in H-ras-transformed MCF10A human breast epithelial cells. *Arch Pharm Res* 2001; 24: 349-354.
29) Kim MS, Moon A. Curcumin inhibits invasive phenotype of H-ras transformed human breast epithelial cells and suppresses matrix metalloproteinase-2 activity. *J Kor Assoc Cancer Prevention* 1999; 4: 119-126.
30) Suzuki T, Iwai K. Constitution of red pepper species: chemistry, biochemistry, pharmacology, and food science of the pungent principle of Capsicum species. In: eds, by A Brosi. The Alkaloides, pp 227-299, New York, Academic Press, 1984.
31) Cordell GA, Araujo OE. Capsaicin: identification, nomenclature, and pharmacotherapy. *Ann Pharmacother* 1993; 27: 330-336.
32) Ferguson LR. Antimutagens as cancer chemopreventive agents in the diet. *Mut Res* 1994; 307: 395-410.
33) Stavric B. Role of chemopreventers in human diet. *Clinical Biochem* 1994; 27: 319-332.
34) Kang HJ, Soh Y, Kim MS, Lee EJ, Surh YJ, Kim HRC, Kim SH, Moon A. Roles of JNK-1 and p38 in selective induction of apoptosis by capsaicin in ras-transformed human breast epithelial cells. *Int J Cancer* 2003; 103: 475-482.
35) Irani K, Xia Y, Zweier JL, Sollott SJ, Der CJ, Fearon ER, Sundaresan M, Finkel T, Goldschmidt-Clermont PJ. Mitogenic signaling mediated by oxidants in Ras-transformed fibroblasts. *Science* 1997; 275: 1649-1652.
36) Kim S, Moon A. Capsaicin-induced apoptosis of H-ras-transformed human breast epithelial cells is Rac-dependent via ROS generation. *Arch Pharm Res* 2004; 27: 845-849.
37) Kong AN, Yu R, Chen C, Mandlekar S, Primiano T. Signal transduction events elicited by natural products: role of MAPK and caspase pathways in homeostatic response and induction of apoptosis. *Arch Pharm Res* 2000; 23: 1-16.