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Mini Review

Phloretin: An Apple Polyphenol with Cancer Chemopreventive Potential

Kundu J[a], Chun K.S[a], Chae IG and Kundu J.K[*]

College of Pharmacy, Keimyung University, Daegu, 704-701, Republic of Korea

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Abstract

Cancer still exists as a major global health concern. Because of the limited clinical success with chemotherapy, there is now a paradigm shift towards chemoprevention for the eradication of cancer. Numerous dietary phytochemicals, especially those present in fruits and vegetables, have been reported to reduce the risk of many cancers. Multiple lines of evidence suggest that phenolic compounds present in apple fruits hold the promise of preventing certain types of cancer. Phloretin [2',4',6'-trihydroxy-3-(4-hydroxyphenyl)-propiophenone], is one of the major chemopreventive phytochemicals present in apple fruits. Over the last several years, remarkable advances have been made in delineating the biochemical mechanisms underlying the chemopreventive effects of phloretin. Results from *in vitro* cell culture studies as well as animal model experiments have revealed that phloretin inhibits experimentally induced carcinogenesis and exhibits potent anti-oxidative, anti-inflammatory, antiproliferative and apoptosis inducing properties. Moreover, phloretin enhances the sensitivity of chemoresistant cancer cells to conventional chemotherapeutic agents. The compound also interferes with cancer cell metabolism by targeting glucose transporter proteins and can induce antitumor immunity by stimulating the proliferation of γδ T cells. The purpose of this review is to shed light on the detailed mechanistic perspective of cancer chemoprevention with phloretin.

Keywords: Phloretin, Chemoprevention, Inflammation, Apoptosis, Glucose uptake

Chemoprevention– a realistic approach to eradicate cancer

The ever-increasing incidence of and mortality from cancer suggest that cancer still remains as a global health challenge. According to an estimate, the number of cancer-related deaths are expected to be doubled in the next 50 years (Mann *et al*, 2005). Although a large spectrum of anticancer agents have been developed over the last several decades, the ultimate success of chemotherapy in reducing the global cancer burden remains questionable. The growing challenges of chemotherapy resistance, recurrence of certain cancers and the poor quality of life issues set back the clinical efficacy of many chemotherapeutic agents (Baer-Dubowska, 2006). Recent advances in the molecular basis of carcinogenesis as well as results of a vast array of epidemiological studies suggest that cancer is largely a preventable disease. About 90% of cancers are of epithelial origin and are linked to life style factors, such as exposure to dietary carcinogens and solar radiation, smoking, increased alcohol intake, etc. Thus, cancer can be avoided by changing these life style factors. Realizing these facts, Michael B. Sporn first coined the term 'chemoprevention', which refers to the use of non-toxic chemical substances of natural or synthetic origin to inhibit, retard or even reverse the course of neoplastic transformation of cells (Sporn, 1976).

Carcinogenesis is a multi-step process that involves apparently three distinct phases: initiation, promotion and progression (Moolgavkar, 1978; Surh, 2003). The initiation stage is the first and irreversible process that begins with the damage of cellular DNA upon exposure to genotoxic carcinogens. Many environmental carcinogens are inactive *per see*, but can be converted to active carcinogens through biotransformation process. The metabolically activated carcinogens initiate tumorigenesis through the covalent modification of the genomic DNA, thereby leading to activation of oncogenes and inactivation of tumor suppressor genes. In addition, microbial infection and persistent local tissue inflammation, through the generation of highly reactive inflammatory mediators and reactive oxygen species (ROS), cause oxidative or covalent modification of cellular macromolecules, such as proteins, lipids and nucleic acids, thereby inciting tumorigenesis (Bartsch and Nair, 2004; Surh *et al*, 2005). The promotion phase of carcinogenesis is reversible long-lasting stage, when aberrant alteration of intracellular signal transduction pathways promotes clonal expansion of transformed cells to develop localized solid tumor. This stage involves increased cell proliferation and neovascularization. The final stage of carcinogenesis is known as tumor progression, which involves the dissemination of primary tumors to multi-organ sites, known as tumor invasion and metastasis (Moolgavkar, 1978; Surh, 2003). Thus, cancer can be prevented by blocking the metabolic activation and enhancing detoxification of carcinogens, reversing or halting oncogenic cell signaling

[a] *These two authors contributed equally to this work*

**Author for correspondence* - kundujk@yahoo.com; kundujk@kmu.ac.kr

pathways, inhibiting tumor cell proliferation and suppressing tumor angiogenesis.

From the clinical point of view, chemoprevention strategies have recently been classified as primary, secondary and tertiary prevention. The prevention of carcinogenesis among the healthy individuals, commonly known as the low risk group population, is termed as primary chemoprevention. The secondary chemoprevention strategy involves the blockade of the premalignant lesions to progress into complete neoplasia. Tertiary chemoprevention refers to the blockade of cancer recurrence after successful eradication of primary tumors (De Flora and Ferguson, 2005; Mann *et al*, 2005; Tsuda *et al*, 2004). Over the last several decades, extensive research has been done in the field of cancer chemoprevention. Based on the success of a wide variety of preclinical and human intervention studies, chemoprevention is considered as an alternative, realistic and fundamental strategy to fight against cancer (Aggarwal and Shishodia, 2006; Baer-Dubowska, 2006).

Biochemical basis of cancer chemoprevention

As has been mentioned above, cancer begins its journey through the transformation of normal cells caused by oxidative and/or covalent modifications of important cellular macromolecules, especially the DNA. The clonal expansion of these transformed cells involves extensive alterations in normal cellular biochemical pathways. Whereas the initiation of cancer can be prevented by reducing the oxidative stress load and inducing the cellular detoxification machinery to eliminate oxidants, free radicals and electrophilic toxicants, tumor promotion and progression can be halted or reversed by switching altered biochemical pathways back to function as normal. Distinctly different from normal cells, the tumor cells exhibit features of altered biochemical events, such as loss of density-dependent inhibition of growth, independence of external growth stimuli for cell proliferation, defective cell cycle control, evasion from apoptosis, enhanced angiogenesis, host tissue invasion and metastasis, altered metabolism, and the ability to escape host immune response. Collectively these features constitute the hallmarks of cancer (Hanahan and Weinberg, 2000). A key pathologic event that drives tumor cells to attain all these characteristic features is the chronic or persistent local tissue inflammation. Moreover, recent findings suggest that tumors grow as a conglomerate of various host-derived cells, such as inflammatory immune cells and host stromal cells, thereby creating a tumor microenvironment. A persistent inflammatory condition prevails within the tumor microenvironment (Kundu and Surh, 2012). Thus, inflammation is also considered as a hallmark of cancer (Hanahan and Weinberg, 2011). Chemoprevention can, hence, be achieved through the intervention with these tumor-specific biochemical process. These include: (1) reinforcement of cellular antioxidant and/or detoxification system, (2) blocking of bioactivation of carcinogens, (3) suppressing tissue inflammation, (4) inhibiting cell proliferation selectively in cancer cells, (5) inducing tumor cell apoptosis, (6) interfering with the angiogenic process, (7) blocking epithelial to mesenchymal transition and tumor invasion, and (8) suppressing tumor metastasis. A wide variety of chemopreventive phytochemicals follow one or more of these biochemical mechanisms, thereby preventing carcinogenesis (Kundu and Surh, 2005; Surh, 2003).

Phloretin– an apple polyphenol with cancer chemopreventive potential

Multiple lines of evidence suggest that regular intake of fruits and vegetables is inversely proportional to carcinogenesis. Besides their nutritive value, fruits and vegetables are enriched with a large number of non-nutritive constituents, generally called phytochemicals, which elicit cancer chemopreventive properties (Surh, 2003). Among the commonly consumed fruits, apples (MALUS sp., Rosaceae) contain a variety of secondary metabolites, such as chalcones, flavonoids, procyanidins and terpenoids (Gerhauser, 2008). A large number of epidemiological and laboratory-based studies have shown the cancer chemopreventive activity of different apple products (Gerhauser, 2008; Zessner *et al*, 2008). For instance, administration of apple polyphenol extract in drinking water for 12 weeks significantly reduced the number and the size of polyp in the colon and intestine of *adenomatous polyposis* $(APC)^{min+/+}$ mice, an animal model that spontaneously develops colorectal tumors (Fini *et al*, 2011). One of the major constituent of apple polyphenols is phloretin [2',4',6'-trihydroxy-3-(4-hydroxyphenyl)-propiophenone], which is a dihydrochalcone characterized by the presence of two benzene rings joined by a saturated three carbon bridge (Nakamura *et al*, 2003) (Fig.1).

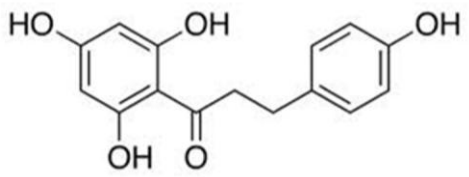

Figure 1.
Chemical structure of phloretin

Review of literature indicates that phloretin is an emerging natural compound to fight against cancer. An expanding body of research has demonstrated that phloretin possesses antioxidant, anti-inflammatory, antiproliferative and apoptosis inducing properties. Moreover, phloretin alone or in combination with chemotherapeutic agents inhibited the *in vivo* growth of various tumor cell xenograft in athymic nude mice (Chang *et al*, 2012; Nakamura *et al*, 2003; Park *et al*, 2007; Yang *et al*, 2009). In a rat model of colitis, administration of phloretin significantly ameliorated trinitrobenzene sulfonic acid (TNBS)-induced colon inflammation and the loss of body weight (Lee *et al*, 2011). Since colitis is a condition characterized by persistent colonic mucosal inflammation that often progresses to colorectal cancer (Clevers, 2004), the inhibitory effect of phloretin on TNBS-induced colitis indicate the potential of this compound to prevent colorectal carcinogenesis. This hypothesis is supported by the fact that the management of colitis with anti-inflammatory therapy reduces the risk of colorectal cancer (Eaden *et al*, 2000). Phloretin completely inhibited the transformation of BALB/3T3 cells and its promotion by 12-*O*-tetradecanoyl phorbol-13 acetate (TPA), which is a prototypic tumor promoting agent (Tanaka *et al*, 1986). We have recently demonstrated that topical application of phloretin significantly reduced the average number of papillomas in 7,12,-dimethylbenz*(a)*anthracene (DMBA)-

initiated and TPA-promoted mouse skin carcinogenesis study (Shin *et al*, 2012). Exposure to ultraviolet radiation (UV) is a major cause of skin carcinogenesis. Topical application of an antioxidant formulation containing vitamin C, ferrulic acid and phloretin suppressed ultraviolet (UV) radiation-induced increases in sunburn cells, thymine dimer formation, and matrix metalloproteinase-9 (MMP-9) expression in skins of healthy human volunteers (Oresajo *et al*, 2008), suggesting that the compound holds the potential to inhibit UV-induced skin carcinogenesis.

Although there is a paucity of information about the *in vivo* antitumor activity of phloretin, several studies have demonstrated the inhibitory effect of the compound on the growth of cancer cell xenograft tumors. For instance, intraperitoneal administration of phloretin significantly reduced the volume and weight of human liver cancer (HepG2) cell tumor xenograft in severe combined immunodeficiency (SCID) mice (Wu *et al*, 2009). Yang *et al.* further demonstrated that co-treatment with phloretin potentiated the inhibitory effect of paclitaxel on the growth of HepG2 cell tumor xenograft in SCID mice (Yang *et al*, 2009). These findings indicate that it would be worthwhile to examine the effect of phloretin on multi-organ carcinogenesis in different animal models.

Molecular mechanisms of anticancer effects of phloretin

It has been reported that phloretin attenuates oxidative stress, ameliorates inflammatory responses, inhibits tumor cell proliferation and induces apoptosis in various cancer cells (Chang *et al*, 2012; Nakamura *et al*, 2003; Park *et al*, 2007; Yang *et al*, 2009). Moreover, phloretin sensitizes chemoresistant cancer cells by modulating the expression and/or the activity of different drug transporter proteins and can play role in cancer immunotherapy (Molnar *et al*, 2010; Nguyen *et al*, 2003). The mechanistic basis of cancer chemoprevention with phloretin (**Fig. 2**) has been discussed in the following sections.

Antioxidant activity of phloretin

Several studies have demonstrated the anti-oxidative effect of phloretin. Nakamura *et al.* (Nakamura *et al*, 2003) examined the antioxidant activities of a series of dihydrochalcones against the stable free radical (1,1-diphenyl-2-picrylhydrazyl; DPPH) and lipid peroxidation in the erythrocyte membrane, and showed that all dihydrochalcones including phloretin exhibited high antioxidant activity. Further structure-activity analysis revealed that the presence of a hydroxyl group at 2'-position of the dihydrochalcone is an essential pharmacophore for the radical scavenging potential (Nakamura *et al*, 2003). According to a previous study, phloretin exhibited antioxidant activity through peroxynitrite scavenging and the inhibition of lipid peroxidation (Rezk *et al*, 2002). Comparison of these effects of phloretin with several other structurally related compounds showed that 2,6-dihydroxyacetone moiety serves as the antioxidant pharmacophore (Rezk *et al*, 2002). In an *in vitro* assay, vitamin C equivalent antioxidant capacity of phloretin was found to be 1.63 (Lee *et al*, 2003). Schaefer and colleagues (Schaefer et al 2006) reported that phloretin inhibited oxidative DNA damage and restored cellular glutathione level in human colon cancer (Caco-2 and HT-29) cells. Yang *et al*. (2011) demonstrated that phloretin induced the expression of hemeoxygenase-1 (HO-1) and γ-glutamyl cysteine ligase (GCL), which are representative antioxidant enzymes, and increased cellular glutathione level in rat hepatocytes by inducing the nuclear factor-erythroid related factor-2 (Nrf2) signaling via activation of extracellular signal regulated kinase (ERK).

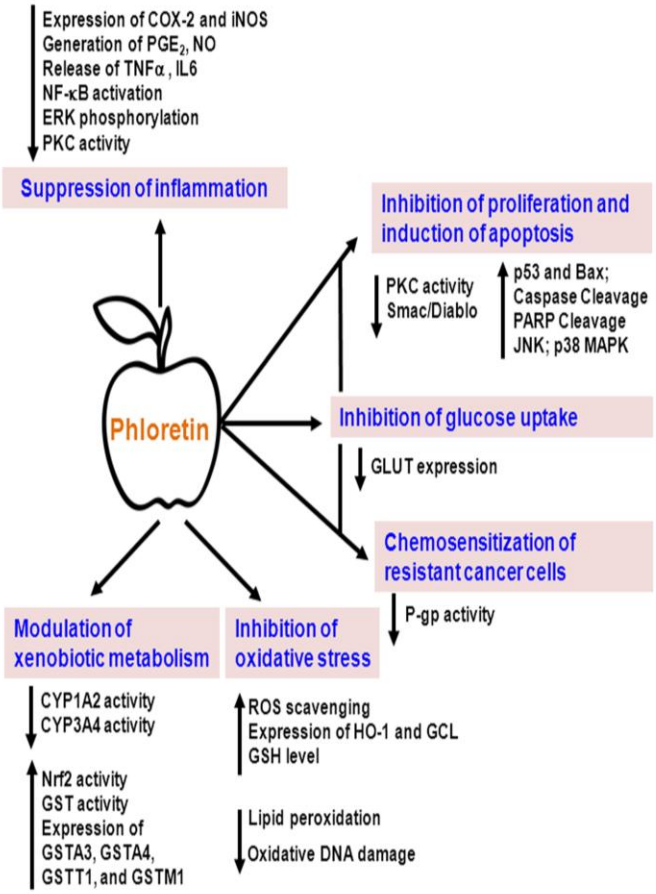

Figure 2.
Biochemical basis of cancer chemoprevention with phloretin

Inhibition of carcinogen activation and induction of detoxification

As has been mentioned earlier, chemopreventive agents are expected to inhibit metabolic activation of procarcinogens and to promote the detoxification of metabolically activated carcinogens. Whereas the members of the hepatic cytochrome (CYP) p450 enzyme family are engaged in the phase I biotransformation of procarcinogens to generate highly reactive intermediates, a series of detoxification enzymes, such as glutathione-*S*-transferase (GST), glucuronyl transferase, sulfotransferase, etc. carry out phase II biotransformation and enhance the rapid elimination of metabolically active carcinogens. A recent study by Gao *et al.* (2012) demonstrated that phloretin inhibited the metabolic activation of aflatoxin B1 (AFB1), a fungal mycotoxin that causes liver cancer, and accelerated its detoxification. According to this study, phloretin attenuated the activities of CYP1A2 and CYP3A4, there by blocking the generation of AFB1-8,9-epoxide (AFBO), an active metabolite of AFB1. Moreover, phloretin induced the activity of GST and

increased the expression of GSTA3, GSTA4, GSTM1, GSTP1 and GSTT1 via activation of Nrf2.

Phloretin inhibits inflammatory responses

Since chronic inflammation triggers all stages of tumor development, the anti-inflammatory effects of phloretin appear as a biochemical basis of its chemoprevention potential. Two representative pro-inflammatory enzymes, such as inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) are overexpressed in many cancers (Surh *et al*, 2001). Studies from genetically engineered mouse models demonstrated that transgenic overexpression of *cox-2* in stomach (Oshima *et al*, 2004) and skin (Muller-Decker *et al*, 2002) of mice enhances the susceptibility of developing tumors in these organs, while *cox-2* knockout mice are less prone to intestinal tumorigenesis (Oshima *et al*, 1996) and skin papillomagenesis (Tiano *et al*, 2002). Likewise overexpression of iNOS enhances dextran sulfate sodium-induced colon carcinogenesis in APC^{min+} mice as compared to $APC^{+/+}$ mice (Tanaka *et al*, 2006). Pretreatment of mouse skin with aminoguanidine, an inhibitor of iNOS, attenuated chemically induced mouse skin papilloma formation (Chun *et al*, 2004). While COX-2 is a rate-limiting enzyme in the synthesis of prostaglandins (PGs), iNOS catalyzes the oxidative deamination of L-arginine to produce nitric oxide (NO). Both PGs, especially PGE_2, and NO play a critical role in carcinogenesis (Kundu and Surh, 2008). Besides these proinflammatory mediators, a series of cytokines, such as interleukins (IL) and tumor necrosis factor-α (TNFα) and chemokines are important inflammatory mediators that promote tumorigenesis. Biochemical mechanisms of aberrant expression of COX-2, iNOS, various cytokines and chemokines involve inappropriate amplification various upstream kinases, such as ERK, p38 mitogen-activated protein (MAP) kinase, c-Jun-N-terminal kinase (JNK), phosphatidylisonositol-3-kinase (PI3K), Akt, and Janus-activated kinase (JAK), which transmit activating signal to downstream transcription factors, such as nuclear factor-kappaB (NF-κB), activator protein-1 (AP-1), signal transducer and activator of transcription (STAT) (Kundu and Surh, 2008; Kundu and Surh, 2012). Blockade of the activation of these inflammatory signaling molecules constitute the biochemical basis of cancer prevention with various anti-inflammatory agents.

Pretreatment of murine macrophage RAW264.7 cells with phloretin significantly attenuated lipopolysaccharide (LPS)-induced production of NO, PGE_2, IL-6, and TNFα as well as the expression of iNOS and COX-2. This study also reported that phloretin inhibited the nuclear translocation of NF-κB subunit p65 protein, and decreased the phosphorylation in MAP kinases (Chang *et al*, 2012). We have recently demonstrated that treatment of mouse skin with phloretin diminished TPA-induced expression of COX-2 by blocking the DNA binding of NF-κB and phosphorylation of ERK (Shin *et al*, 2012).

Inflammatory bowel disease (IBD), which is characterized by an excessive release of several pro-inflammatory cytokines and chemokines by different cell types, is a chronic inflammatory disease of the colon that often progress to colon cancer. Treatment of immunorelevant human cell lines (DLD-1, T84, MonoMac6, Jurkat) with phloretin revealed that the compound significantly inhibited pro-inflammatory gene expression and repressed NF-kappaB-, IL-8-promoter-, and STAT1-dependent signal transduction in a concentration-dependent manner (Jung *et al*, 2009). Phloretin markedly reduced the biofilm formation by pathogenic E. coli O157:H7 by blocking the attachment of this bacterial strain to colonic epithelium, whereas the compound did not affect the growth of planktonic cells and the formation of biofilm by commensal E. coli K-12 strain. This was further confirmed by the reduced expression of several toxin genes as assessed by global transcriptome analysis and electron microscopy. Moreover, phloretin attenuated TNFα-induced inflammatory response in human colonic epithelial cells in culture (Lee *et al*, 2011). Since IBD increases the risk of colorectal cancer by 10-fold (Prior *et al*, 1982), this study indicates that phloretin may elicit chemopreventive effect in experimentally induced colon carcinogenesis.

Phloretin inhibits proliferation and induces apoptosis in cancer cells

Phloretin significantly decreased cell viability and induced apoptosis in human colon cancer (HT-29) cells in a dose-dependent manner. Western blot analysis of total cell lysates revealed that phloretin increased the protein levels of Bax without affecting that of Bcl-2. In addition, phloretin induced the cleavage of caspase-8, -9, -7, and -3 and poly(ADP-ribose) polymerase (PARP), which are critical markers of apoptosis. Furthermore, phloretin increased the levels of cytochrome c and Smac/Diablo in the cytosol. (Park *et al*, 2007). In another study, phloretin suppressed the proliferation and induced apoptosis in H-*ras*-transformed human mammary epithelial (MCF-10A) cells in a concentration-dependent manner and induced nuclear condensation in the cells, indicating that phloretin-induced cell death occurs mainly via the induction of apoptosis. Prominent upregulation of p53 and Bax and cleavage of PARP were also detected in the phloretin-treated cells. Finally, phloretin markedly increased the caspase-3 activity as well as JNK and p38 mitogen-activated protein kinase signaling. (Kim *et al*, 2009). Phloretin markedly augmented TNFα-related apoptosis-inducing ligand (TRAIL)-induced apoptosis and cytotoxicity in human prostate cancer (LNCaP) cells and confirmed the significant role of chalcones in chemoprevention of prostate cancer (Szliszka *et al*, 2010). Phloretin-induced apoptosis in B16 melanoma cells (Kobori *et al*, 1997) and human leukemia HL60 cells (Kobori *et al*, 1999) was associated with decreased protein kinase C (PKC) activity. In addition, the induction of apoptosis in B16 melanoma cells by phloretin was associated with the induction Bax and caspase activation. However, phloretin did not affect the expression of p53 or that of antiapoptotic protein Bcl-2 and Bcl-xl in these cells (Kobori *et al*, 1999).

Potential of phloretin in chemosensitization

The development of resistance to chemotherapeutic agents and subsequent increasing trend in chemotherapy failure is an emerging challenge in reducing the global burden of cancer. It has been reported that co-administration of dietary phytochemicals can sensitize resistant cancer cells towards chemotherapeutic agents. One of the major biochemical mechanisms associated with chemoresistance is the ability of cancer cells to promote efflux of chemotherapeutic agents, thereby reducing the intracellular level of the drug. This is

primarily operated by the overexpression of a drug transporter protein p-glycoprotein (P-gp), alternatively known as multidrug resistance protein-1 (MRP1 or MDR1), in cancer cell membrane. Selective inhibition of the MDR-efflux proteins may improve the effectiveness of cancer chemotherapy. Nguyen *et al.* examined the effect of 22 flavonoids including phloretin on the accumulation of daunomycin and vinblastine in human pancreatic carcinoma Panc-1 cells. This study demonstrated that treatment with phloretin significantly increased the intracellular accumulation of daunomycin and vinblastine in Panc-1 cells through the inhibition of MRP1-mediated drug transport (Nguyen *et al*, 2003). Moreover, incubation of human MDR1 gene-transfected mouse lymphoma cells (L1210) and human breast cancer cells MDA-MB-231 expressing the MRP1 pump (HTB26) with phloretin showed that the compound reduced the P-gp activity as revealed by rhodamine 123 uptake by these cells (Molnar *et al*, 2010).

Inhibition of glucose uptake by phloretin

A high-rate of glycolysis, which is associated with elevated expression of glucose transporter proteins (GLUT) and glycolytic enzymes, is a fundamental feature of many solid tumors. Incubation of B16 melanoma cells with phloretin resulted in increased intranucleosomal DNA fragmentation and apoptosis, which was abrogated by the addition of extracellular glucose (Kobori *et al*, 1997). Intratumoral environment is always hypoxic, and the expression of different GLUT proteins has been found to be elevated in the hypoxic region of human colon cancer (SW620) and leukemia (K562) cells. Hypoxia contributes to two to five fold increase in chemotherapy resistance of these cells to daunorubicin. Treatment with phloretin reduced glucose uptake by 60% and sensitized these cells to daunorubicin (Cao *et al*, 2007). Likewise, more than five-fold increase in GLUT2 mRNA expression has been noted in human hepatoma (HepG2) cells as compared to nonmalignant hepatocytes. Inhibition of GLUT2 expression by phloretin resensitizes these cells to paclitaxel-induced apoptosis, as revealed by the activation of caspase-9,-8 and-3. Moreover, co-treatment of mice bearing HepG2 cell xenograft tumor with phloretin and paclitaxel significantly reduced the tumor volume as compared to treatment with paclitaxel alone (Yang *et al*, 2009). Wu *et al.* (2009) also demonstrated that GLUT2 expression is elevated in HepG2 cells and that the siRNA-mediated knockdown of GLUT2 induced apoptosis in these cells. Treatment of HepG2 cells with phloretin induced apoptosis, which was reversed by pretreatment with glucose. Moreover, co-treatment of these cells with a glucose uptake inhibitor cytochalasin B potentiated phloretin-induced apoptosis, suggesting that induction of apoptosis in these cells by phloretin is mediated through inhibition of GLUT2 expression and glucose uptake. Phloretin also reduced the proliferation of rat mammary adenocarcinoma and Fischer bladder cell carcinoma cells in culture by blocking glucose transmembrane transport and inhibited the in vivo growth of these cells as xenograft tumors (Nelson and Falk, 1993).

Role of phloretin in cancer immunotherapy

One of the hallmarks of cancer is the avoidance of host immune response by the cancer cells. While substantial progress has made in developing cancer immunotherapies, such as monoclonal antibodies, the potential of natural compounds in antitumor immunity has not been studied in large scale. A recent study by Zhu *et al* (2013) reported that phloretin can enhance the tumoricidal effect of γδ T cells on human colon cancer (SW-1116) cells, possibly by stimulating the proliferation of γδ T cells.

Conclusion

Over the last several decades, an expanding body of research has discovered a wide variety of phytochemicals as effective cancer chemopreventive agents. While dietary carcinogens are the major risk factors for cancer, diet contains a bounty of anticarcinogenic substances. The search for dietary anticancer principles and elucidation of their biochemical mechanisms is practical approach to establish molecular target-based cancer chemoprevention. Through systematic research carried over last several years, the apple polyphenol phloretin has been emerged as a promising anticancer agent. However, additional studies are necessary to develop this molecule as a candidate chemopreventive agent. In spite of current progress, there is dearth of knowledge about the effect of phloretin on several hallmarks of cancers, such as cell cycle regulation, angiogenesis, invasion and metastasis. Moreover, future chemoprevention research with phloretin would be focused on its *in vivo* effects, especially experimentally induced multi-organ carcinogenesis and pharmacokinetic properties using different animal models.

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