Phenethyl Isothiocyanate: A comprehensive review of anti-cancer mechanisms

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Abstract

The epidemiological evidence suggests a strong inverse relationship between dietary intake of cruciferous vegetables and the incidence of cancer. Among other constituents of cruciferous vegetables, isothiocyanates (ITC) are the main bioactive chemicals present. Phenethyl isothiocyanate (PEITC) is present as gluconasturtiin in many cruciferous vegetables with remarkable anti-cancer effects. PEITC is known to not only prevent the initiation phase of carcinogenesis process but also to inhibit the progression of tumorigenesis. PEITC targets multiple proteins to suppress various cancer-promoting mechanisms such as cell proliferation, progression and metastasis. Pre-clinical evidence suggests that combination of PEITC with conventional anti-cancer agents is also highly effective in improving overall efficacy. Based on accumulating evidence, PEITC appears to be a promising agent for cancer therapy and is already under clinical trials for leukemia and lung cancer. This is the first review which provides a comprehensive analysis of known targets and mechanisms along with a critical evaluation of PEITC as a future anti-cancer agent.

Keywords

cruciferous vegetables; isothiocyanates; PEITC; reactive oxygen species; chemoprevention

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Conflict of Interest

Authors declare that there are no competing interests.

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1. Introduction

Since prehistoric times, nature has been a rich source of many drugs and drug leads, which are currently being used to treat various ailments, including cancer. According to Newman and Cragg, 74.8% of present anticancer agents originated from natural sources, with almost 48.6% being the actual natural compound or a direct derivative [1]. These include several widely used anticancer agents like taxanes, vinca alkaloids and camptothecin class of compounds. Interest in natural products as an alternative to synthetic drugs for cancer treatment is largely due to low cost, established historical use in traditional medicinal systems, easy availability and minimal or no toxicity. Also, the potential for targeting multiple pathways represents an advantage of natural compounds over synthetic agents, the latter being more target-specific.

Cancer is a complex manifestation of genomic instability in cells caused by environmental or genetic factors [2]. Genomic instability leads to disruption of basic biological functions, such as cell division, differentiation, angiogenesis and migration, which promote cancer [2]. The involvement of multiple mechanisms in cancer development raises an urgent need for multi-targeted therapy. The current anti-cancer agents primarily focus on specific targets. The suppression of unique targets usually leads to activation of compensatory mechanisms resulting in failure of therapy or development of resistance to drugs. To circumvent these problems, combination therapy is currently employed by the oncologists. Nonetheless, combination approach has also shown marginal advantage in the overall therapy due to development of resistance or associated toxicity. We have reached a threshold in terms of clinical benefit and tolerance in patients.

Of all the isothiocyanates (ITCs), phenethylisothiocyanate (PEITC) is the only one that has reached the clinical stage of testing. This review provides a critical and comprehensive analysis of the outcomes of studies showing the anti-cancer effects of PEITC.

2. Epidemiological evidence for chemo-preventive effects of dietary intake of cruciferous vegetables

Several epidemiological studies from different parts of the world provide a strong evidence for the reduced risk of cancer with higher intake of cruciferous vegetables [3–7]. Although none of these studies provide a direct correlation between cancer incidence and intake of a specific ITC, many studies do identify a correlation between the total ITC intake in the form of cruciferous vegetables and reduced risk of several types of cancers in lung, breast, gastric, bladder, colorectal, pancreatic, prostate and kidney (Table 1) [5, 8–27]. Interestingly, there are also few studies where the results obtained were opposite to the preventive effects of cruciferous vegetables. For example, depending on the duration of intake and age of subjects, one of the studies has shown differential effects of cruciferous vegetables [20]. In a prospective study, Giovannucci et al. did not observe any significant correlation between prostate cancer risk and a short-term intake of cruciferous vegetables [20]. However, long-term intake of cruciferous diet showed a stronger inverse correlation with initial stages of prostate cancer and this effect was stronger in men less than 65 years of age [20]. In contrast, a few other studies reported no correlation between the intake of cruciferous...
vegetables and cancer incidence [27]. The differences in the outcome showing no correlation
with dietary intake of cruciferous vegetables could be due to several confounding factors
such as, differences in the subject population, duration of intake of cruciferous vegetables in
subject’s lifetime, age of subjects, variation in the isothiocyanate content in cruciferous
vegetables based on geographic locations and whether the consumed vegetables were raw or
cooked. Out of 22 studies performed, one study showed increased risk of thyroid cancer
with ITC intake, while three studies (urinary, prostate and breast cancers) showed no
correlation of ITC intake with cancer incidence. Interestingly all the studies on stomach,
colon and lung cancers showed reduced cancer incidence with ITC intake. A detailed
analysis revealed that studies showing no correlation between ITC intake and cancer
incidence typically had wider trial durations or greater age differences for the subjects.
Interestingly, a study performed in 2001 showed a significant change in peoples food habits
and lifestyle over the last decade [28]. Hence, the outcomes from studies performed in the
last decade might be affected due to these confounding factors. Furthermore, the majority of
the findings were based on questionnaires for the intake of cruciferous vegetables, which
can have lot of variability. In most of the studies, questionnaires were not specific in asking
whether the vegetables were raw or cooked.

However, based on the majority of the studied outcomes, we can conclude that an inverse
relationship exists between intake of ITCs in the form of cruciferous vegetables and the
overall incidence of cancer. But no conclusion can be drawn with respect to any specific
isothiocyanate at this time.

3. PEITC source and pharmacokinetics

3.1 Cruciferous vegetables – Source of PEITC

The isothiocyanates (R-N=C=S) (ITC) are known to be the major bioactive compounds
present in cruciferous vegetables and responsible for anti-cancer activity. ITCs are released
from glucosinolates by the action of the enzyme myrosinase. The enzyme myrosinase can be
activated by cutting or chewing the vegetables, but heating can destroy its activity [29].
However, microbial myrosinase from gut can also release ITCs in the stomach after
ingestion of cruciferous vegetables [30, 31]. Studies show that myrosinase as well as
isothiocyanates are thermolabile [32]. Hence ingestion of only raw vegetables can release
ITCs and cooking of the vegetables can reduce ITC content [32]. Although water cress and
broccoli are known to be the richest source, PEITC can also be obtained from turnips and
radish. PEITC is present as gluconasturtiin in cruciferous plants. Like other ITCs, PEITC
can be released from gluconasturtiin by the action of myrosinase [30, 31]. In a study
conducted with human volunteers, approximately 2 to 6 mg of PEITC was found to be
released by the consumption of one ounce of watercress [33, 34]. Similarly, ingestion of
100g of broccoli and watercress can release up to 200μmol of PEITC in humans [29, 33].
Interestingly, several pre-clinical studies have shown that significant anti-cancer effects can
be achieved at micromolar concentrations of PEITC.
3.2 Pharmacokinetics and metabolism

A significant number of studies have shown a positive pharmacokinetic profile for orally administered PEITC (Table 2). PEITC is highly bioavailable after oral administration. A single dose of 10–100 μmol/kg PEITC in rats resulted in bioavailability ranging between 90–114% [35]. The high bioavailability was accompanied by low clearance as well as high protein binding [35]. Increased bioavailability of PEITC was also observed in another study with repeated dosing of PEITC [36]. Furthermore, about 928.5±250nM peak plasma concentration of PEITC was achieved in human subjects, after the consumption of 100g watercress. The time to reach peak plasma concentration was observed to be 2.6h±1.1h with a $t_{1/2}$ 4.9±1.1h [37]. This indicates that oral administration of PEITC can result in effective concentrations in blood plasma. However, long term studies are required to establish the safety profile of PEITC, since regular intake of PEITC can cause its accumulation resulting in cumulative effects, which could be toxic.

PEITC metabolism has been reviewed in detail by two groups of investigators [38, 39]. PEITC is primarily removed as mercapturic acid from the body (Fig. 2). After oral ingestion, PEITC is converted into glutathione conjugates by the action of enzyme glutathione S-transferases (GST). Several epidemiological as well as pre-clinical studies showed that the anti-cancer effects of ITC can vary significantly with GST polymorphism (Table 3) [15–17, 21, 40–63]. These glutathione conjugates are exported out of the cells by membrane bound transporter proteins and are cleaved by γ-glutamyl transferase and dipeptidase. The conjugation of PEITC with intracellular glutathione and the subsequent removal of the conjugate result in depletion of glutathione and alteration in redox homeostasis leading to oxidative stress, as explained in detail in section 4.3 of this review. Cysteine conjugated PEITC is acetylated by N-acetyl transferase in liver and eliminated from the body as N-acetyl cysteine (NAC) conjugates or mercapturic acid (Fig 2) [64]. Interestingly, Tang et al. reported that NAC conjugates are also biologically active and have similar activity to parent compounds, suggesting prolonged effects of ITCs [64].

PEITC and/or glutathione conjugates were found to be substrates of important membrane bound transporter proteins like breast cancer resistance protein (BCRP), multidrug resistance- associated proteins 1 (MRP1) and 2 (MRP2) [65–67]. BCRP plays an important role in absorption and disposition of many drugs. Hence, PEITC being a substrate of BCRP can potentially affect the pharmacokinetics and pharmacodynamics of other BCRP substrates. In addition, PEITC can modulate other important transporter and metabolic proteins like P-glycoprotein, [67] and cytochrome P450 enzymes [68, 69]. PEITC-mediated modulation of transporter proteins could be an important factor for the chemopreventive effects of PEITC. These effects are explained in section 4.1 of this review. Nevertheless, transporter proteins are important determinants of the efficacy and toxicity of many clinical drugs and by modulating these proteins, PEITC can affect the efficacy as well as toxicity of some other drugs.

3.3 GST polymorphism and anticancer activity of PEITC

GST is a polymorphic enzyme which promotes conjugation of glutathione (GSH) with a wide variety of electrophilic xenobiotics including carcinogens. The conjugation of GSH
with carcinogens catalyzed by GST and subsequent removal of the conjugate results in reduced carcinogenic effects [70, 71]. Increasing evidence from epidemiological and pre-clinical studies suggest a significant impact of GST polymorphism on the outcomes of dietary ITC intake (Table 3A). A majority of these studies indicates an important effect of GSTT1, GSTM1 and GSTP1 polymorphism in ITC mediated anti-cancer effects. The presence of GSTT1 or GSTM1 or both have been strongly correlated with reduced or no effects of dietary ITCs on cancer risk. Interestingly, pre-clinical studies have shown ITC-mediated induction of GST enzymes, suggesting GST-mediated metabolism and inactivation of ITCs (Table 3B). Interestingly, a study indicates concentration dependent effect of specific GST polymorphism in catalyzing glutathione conjugation with PEITC [56]. The GSTA1-1 enzyme reacts with micromolar concentrations of PEITC, while GSTM1a-1a exhibits a significantly lower affinity for PEITC. On the contrary, Meyer et al. suggested that GST catalyzes forward as well as reverse conjugation of PEITC with GSH [56]. Studies also showed PEITC mediated tissue specific induction of GST isoforms [55, 57, 60, 63, 72, 73]. Van et al. showed significant induction of GST by PEITC in esophagus [57]. Furthermore, PEITC mediated induction of GSTA in liver, GSTM in stomach and liver as well as GSTP in colon was observed. PEITC treatment also induced glutathione levels by about 1.6 fold in colon [57]. PEITC-mediated GSTP modulation was observed in few other studies as well [58, 59]. The induction of GST and glutathione appears to be an important pathway for PEITC-mediated chemopreventive effects. In a chemical carcinogen-induced multi-organ cancer model, differential effects of PEITC were observed in pre- and post-tumor phases. PEITC treatment during pre-initiation period caused reduced hyperplasia in esophagus and lungs accompanied with reduction of GSTP in kidney and liver [58]. However, PEITC treatment during post-initiation phase increased liver GSTP positive foci along with increased incidence of urinary bladder hyperplasia and tumors [58]. This suggests preventive effects of PEITC in preinitiation conditions, while during post-initiation, PEITC treatment increases tumor incidence. However, it is important to note that 0.1% PEITC was administered in the diet in this study, whereas, in most of the studies, a pure and higher concentration of PEITC was administered.

The role of GST in chemoprevention has been reported to be dependent on the polymorphic status of GST as well as the source of induction [74]. This implies that GST polymorphism and its tissue specific differential effects are important determinants to correlate cancer risk and ITC intake. Many studies have also reported the outcomes of ITC intake and cancer risk with no consideration of GST status in the subjects. Hence, outcomes from these studies need to be re-confirmed using better designed studies.

4. Anti-cancer activity of PEITC

Several researchers have demonstrated the anticancer properties of isothiocyanates in various cancer types [75–81]. The anti-cancer activity of PEITC can be divided into chemopreventive and chemotherapeutic effects. Although chemopreventive effects were identified through epidemiological evidence, chemical carcinogenesis models and transgenic mouse models, more extensive mechanistic studies were performed to evaluate its therapeutic potential in various pre-clinical models.
4.1 Chemopreventive effects of PEITC

The diverse epidemiological studies provided the first evidence of anticancer activity of ITCs (Table 1). The preventive effects of ITCs were evaluated in multiple chemically-induced cancer models. For example Morse et al. and others, demonstrated that administration of ITCs including PEITC prevented the carcinogenic effects of chemical carcinogens in rodents [82–86]. In general, carcinogenesis occurs due to the bio-activation of carcinogens by oxidation, reduction or hydrolysis via phase I drug metabolizing enzymes. Hence, modulation of phase I enzymes can affect the carcinogen activation process. PEITC shows a dual activity on phase I enzymes. For example, PEITC on one hand causes induction of CYP1A1 and CYP1A2; however, it inhibits activity of certain CytP450 enzymes, such as CYP2E1, CYP3A4 and CYP2A3 [68, 87–91]. Detailed studies are required to elucidate the differential effects of PEITC on CYP enzymes and their mechanistic implications in cancer prevention.

Phase II enzymes, which include mainly transferases, play an important role in detoxifying xenobiotics and carcinogens. As reviewed by Cheung et al., various ITCs including PEITC have been shown to induce phase II detoxification enzymes, which can explain their chemopreventive activity [92]. GSTs play an important role in chemopreventive effects of PEITC, as discussed earlier in section 3.3. PEITC also acts on other phase II enzymes. Saw et al. demonstrated induction of Nrfl2-antioxidant response element by a combination of ITC and phyto-indoles [93]. Although in general, ITCs modulate phase II enzymes, human livers demonstrated a nonidentical response to PEITC [94]. Liver enzymes were found to be most sensitive to PEITC, while lung enzymes were mostly resistant [95]. Hence, based on current evidence, it can be concluded that the effects of PEITC on drug metabolizing enzymes are individual as well as tissue specific.

Continuous high levels of ROS also promote carcinogenesis by causing oxidative DNA damage leading to mutations [96]. Interestingly, PEITC treatment caused a significant increase in the activities of ROS detoxifying enzymes such as glutathione peroxidase1, superoxide dismutase 1 and 2. This was also confirmed in human study where subjects were administered watercress, a major source of PEITC [61].

4.2 Chemotherapeutic effects of PEITC

Hanahan and Weinberg have defined ten basic common hallmarks underlying cancer progression [2, 97]. All types of cancers require these basic hallmarks for development and advancement. Hence, effective inhibition of one or more of these hallmarks can potentially enhance the outcomes of current anti-cancer therapeutics. Accumulating evidence suggests that PEITC targets a broad spectrum of proteins to suppress cancer growth and progression (Figure 1). Research group led by Zigang Dong and An-Ng Tony Kong for the first time in 1998 independently demonstrated the apoptosis-inducing effects of PEITC in cultured cancer cells [98–100]. The cytotoxic concentrations of PEITC range from 120 nM to 14 μM in cancer cells [101, 102]. Tseng et al. have reported that working concentrations of PEITC are similar to chemotherapeutic drug daunomycin in human breast cancer cells as well as in mammary epithelial cells, implying PEITC’s anti-cancer potential comparable to conventional drugs [103].
Since the discovery of anticancer effects of PEITC, several mechanisms have been proposed for its activity. Two primary mechanisms that have been identified are cell cycle arrest and induction of apoptosis \[104–106\]. PEITC-mediated generation of reactive oxygen species (ROS) is known to be a general mechanism of action leading to cytotoxic effects, especially specific to cancer cells \[107, 108\]. Some studies have also shown significant inhibition of other important cancer promoting mechanisms like angiogenesis and metastasis \[109–113\]. PEITC targets specific regulatory proteins to inhibit cancer-related oncogenes. Mi et. al. have identified more than 30 different biological targets of PEITC \[114\]. There are about 350 articles available so far on the anti-cancer effects of PEITC. Table 4 summarizes the targets and major mechanisms of PEITC that have been studied in different cancer models by various investigators during recent years (2011–2014).

4.2.1 Anti-proliferative effects—Sustained proliferative signaling is a major characteristic of cancer cells. Ras activation in cancer by different oncogenes is a common mechanism to sustain proliferation. Ras further activates various pathways like RAF/MAPK and PI3K/Akt that are crucial for cell proliferation, and their inhibition is known to suppress cancer growth. It was observed that Ras mutation did not correlate with PEITC-mediated inhibition of tumorigenesis, indicating that Ras may not be a target of PEITC \[115\]. However, PEITC inhibits Akt, a component of Ras signaling to inhibit tumor growth in several cancer types \[116–118\]. Akt (protein kinase B) is over-activated in many types of cancers and promotes multiple cell survival mechanisms. PEITC is also known to inhibit EGFR and HER2, which are important growth factors and regulators of Akt in different cancer models \[102, 117, 119\]. Hence, Akt inhibition can be due to the suppression of EGFR or HER2. Overall, it is evident from these examples that even though PEITC is unable to suppress Ras, tumor cell growth suppression is achieved through an alternative mechanism.

4.2.2 Effects on cell division and cell cycle—Cell proliferation is a tightly regulated process, orchestrated by pro- and anti-proliferative signals. Generally, the pro-proliferative signals are activated when new cells are required to replace damaged cells. At other times, the anti-proliferative signals maintain cells in a quiescent state and also keep cell division under control. However, cancer cells lock in the pro-proliferative signals, while suppressing tumor suppressor genes responsible for anti-proliferative effects. Cells have multiple check points for proliferation, which are regulated by tumor suppressor genes such as p53, Retinoblastoma protein (Rb) and PTEN. PEITC causes activation of Rb protein at the cellular level in prostate cancer cells, leading to attenuation of cell cycle progression \[100, 120\]. Interestingly, metabolic conjugates of PEITC with N-acetyl cysteine (NAC) also inhibit the phosphorylation of Rb leading to cell cycle arrest \[67\]. PEITC-mediated activation of another tumor suppressor, p53 was observed in oral squamous cell carcinoma, causing G0/G1 phase arrest in multiple myeloma, osteogenic sarcoma, breast cancer and prostate cancer, along with inhibition of other proteins regulating G2/M phase \[93, 121–125\]. It was also observed that lung carcinoma cells with wild type p53 were relatively less sensitive to PEITC as compared to cells with mutated p53 \[100, 114, 126, 127\]. Although, a direct effect on another important tumor suppressor PTEN has not been reported, PEITC inhibits Akt, which is overexpressed when PTEN is mutated.
Cells have a capability to keep track of the number of divisions undergone so that cell death can be initiated after a certain number of divisions. This phenomenon uses the shortening of telomere length with every division. This can be reversed by enzyme telomerase. Often, cancer cells exploit telomerase enzyme to alter the length of telomeres rendering them immortal [128]. PEITC has been shown to inhibit telomerase activity in prostate and cervical cancer cells [129, 130]. Furthermore, it was observed that pre-treatment with PEITC induced apoptosis as well as increased the efficacy of adriamycin and etoposide by inhibiting protein kinase C and telomerase [130].

4.2.3 Pro-apoptotic effects—Cells usually undergo apoptosis when proliferation cannot be controlled by cell cycle check points. However, cancer cells develop anti-apoptotic mechanisms to enable survival. Mounting evidence suggests strong pro-apoptotic activity of PEITC by diverse mechanisms. Most of these mechanisms are affected by generation of reactive oxygen species (ROS), which also has been shown to be the basis of selectivity of PEITC toward cancer cells leaving normal cells undamaged [107]. A detailed account of PEITC mediated ROS generation is provided in section 4.3. ROS generation by PEITC leads to mitochondrial deregulation and modulation of proteins like Bcl2, BID, BIM and BAX, causing the release of cytochrome c into cytosol leading to apoptosis [102, 121, 124, 131–136]. However, Wu et. al., suggested that cytochrome c does not play a role in PEITC-induced apoptosis [137, 138]. Roy et. al. have shown an interesting tumor regression due to PEITC mediated inhibition of DDB2 through ROS generation [139]. In addition, PEITC also induces apoptosis by activation of extrinsic apoptotic pathway in oral and cervical cancer cells through the induction of death receptors and Fas-mediated apoptosis [140–143]. Some studies also show PEITC-mediated suppression of anti-apoptotic proteins like XIAP and survivin, which are up-regulated in cancer cells [144]. Taken together, a strong cytotoxic potential of PEITC cannot be denied, although differential roles have been described.

4.2.4. Autophagy—Autophagy is a stress response mechanism, which leads to degradation of cellular organelles to preserve cell energy. Autophagy is generally considered as cell survival mechanism. However, it is also a unique form of cell death, occurring under special circumstances [145]. Investigations show that PEITC induces autophagic cell death in cancer cells [146–148]. Interestingly, Mi et. al. showed the formation of aggresomes by PEITC treatment [149], indicating induction of autophagy, since these structures are formed due to proteasome failure and are degraded by autophagic pathway. Bommareddy et. al. showed induction of autophagy by PEITC in prostate cancer cells [147]. Treatment with 3-methyladenine, an autophagy inhibitor, provided evidence that autophagy induced by PEITC was unable to protect the cells from apoptosis. It was observed that PEITC-induced autophagy was mediated by Atg5 [147]. A partial role of the inhibition of mTOR/Akt signaling was also observed in PEITC-induced autophagy. Later PEITC-mediated induction of autophagy was shown in a transgenic mice model of prostate cancer [148]. Taken together, autophagy can be one important anti-cancer mechanisms of PEITC. Nevertheless, further study is required to determine whether or not PEITC induces autophagy in other in vivo cancer models.
4.2.5. Anti-angiogenic effects—Rapidly proliferating cancer cells have increased demand for nutrients and oxygen. Hence, these cells enhance the growth of new blood vessels (angiogenesis) to meet the increasing nutritional demand. Targeting vascular endothelial growth factor (VEGF), a major promoter of angiogenesis, has been an important mechanism of cancer treatment for the past few decades. As reviewed by Loureiro and D’Amore, VEGF is regulated by two major mechanisms: hypoxic regulation and non-hypoxic regulation [150]. Importantly, cancer cells are constantly under hypoxia due to limited oxygen supply. During hypoxia, hypoxia-inducible factor (HIF1α) accumulation stimulates secretion of VEGF leading to angiogenesis [150]. A study by Xiao and Singh provided evidence on the anti-angiogenic effects of PEITC through VEGF suppression, but the exact mechanism of this inhibition was not clear [113]. It was shown later that PEITC directly or indirectly suppresses HIF1α [151–154]. Besides hypoxic regulation, VEGF is also regulated by growth factors released by cancer cells (heregulin, EGF and TGF), hormones (estrogen, testosterone and insulin) as well as oncogenes Wnt and Ras [150]. In addition, VEGF is negatively regulated by tumor suppressor genes such as p53 and p73. Based on the effects of PEITC on proliferation and cell cycle described in sections 4.2.1 and 4.2.2, it is possible that PEITC can block angiogenesis by non-hypoxic mechanisms also.

4.2.6. Anti-metastatic effects—In malignant cancer, tumor cells tend to invade blood circulation to reach other organs of the body leading to spread of cancer. Under normal conditions, non-cancerous cells undergo apoptosis under anchorage-independent conditions due to the absence of extracellular matrix, which provides survival signals. In contrast, cancer cells develop survival mechanisms that allow them to survive during circulation under anchorage-independent conditions and spread to distant organs. This process, referred to as “invasion and metastasis”, is a major reason for the poor prognosis in majority of cancers. It leads to relapse of cancer, which is mostly resistant to conventional chemotherapy. There are few studies which suggest anti-invasive and anti-metastatic effects of PEITC. Various studies with PEITC have shown suppression of invasion through inhibition of matrix metalloproteinases along with anti-metastatic effects caused by suppression of ERK kinase activity and transcriptional activity of NFkB [109, 111]. PEITC was also known to inhibit processes, such as epithelial to mesenchymal transition (EMT), cell invasion and migration, which are essential pre-requisites for metastasis [155–157]. A recent study by Gupta et. al. demonstrated anti-metastatic potential of PEITC in a novel in vivo model of breast cancer metastasis [119]. In this model, when MDA-MB-231 brain seeking luciferase breast cancer cells were injected into the left ventricle of the heart of female athymic nude mice, a small percentage of tumor cells lodge in the brain via blood circulation and grow there as metastatic tumors. Observations from a pretreatment model suggested that oral administration of 10μmol PEITC significantly prevented the migration of breast cancer cell to the brain in vivo. In another experiment, PEITC administration after tumor cell implantation not only suppressed the growth of metastasized tumors in brain but also prolonged the survival of tumor bearing mice [119]. Although these studies reveal the anti-metastatic efficacy of PEITC in a breast cancer model, more evidence is required to establish similar anti-metastatic effects in other cancer models.
4.2.7. Anti-inflammatory effects—The tumor microenvironment is similar to inflammatory lesions and plays a very important role in carcinogenesis. Inflammation promotes cancer cells to grow in a bimodal way. Firstly, pre-existing inflammation promotes carcinogenesis by stimulating cells through several chemokines, cytokines and growth factors. Secondly, the same factors can be secreted by established cancer cells to re-enforce tumor growth and development. Hence, cancer growth can be inhibited by controlling the inflammatory process. Several studies have demonstrated direct modulation of inflammatory process by PEITC. It has been shown that chemically-induced inflammatory responses in mice were suppressed by PEITC [141, 158]. The mechanistic studies have shown suppression of inflammatory mediators like nitric oxide, TNF-α, and IL-10 in LPS-stimulated macrophages, as well as impairment of macrophage migration inhibitory factor (MIF) through covalent modification by PEITC [159, 160]. A few reports also connect the immunomodulatory action with anti-cancer activity of PEITC. For example, PEITC has been shown to protect mouse liver and lung from changes caused by cigarette smoke [153]. A bio-informatic analysis of PEITC treated animal tumors showed modulation of genes involved in inflammation and extracellular matrix pathways [161]. Taken together, these findings suggest anti-inflammatory effect as one of the anticancer effects of PEITC. However, further comprehensive studies between cancer and inflammation are still required to elucidate the anti-cancer mechanisms of PEITC.

4.2.8. Immunomodulatory effects—The human body’s integral defense mechanisms serve as a major host for future therapeutic opportunities and can help in the fight against cancer. The immune system is equipped with several check points to scan and selectively eliminate malignant cells. Interestingly, cancer cells develop sophisticated mechanisms to evade the immune system. There is a need for extensive and rigorous studies to discover agents that can prevent cancer cells from evading the immune system. Tsou et al. demonstrated the immunomodulatory activities of PEITC [162]. PEITC treatment caused activation of macrophages and NK cells in a mouse leukemia model. PEITC treatment also promoted the differentiation of B cells but the proliferation remained unaffected. Conversely, it was observed that PEITC increased the proliferation of T cells while inhibiting the differentiation of the precursor cells of T cells and macrophages. PEITC treatment caused reduction in weight of the spleen and liver of leukemic mice, although no reduction in the overall weight of mice was observed [162]. The reduction of the size of the spleen and liver is more likely a result of killing of the leukemia cells in those organs, with a concomitant reduction in weight of those organs, rather than a detrimental effect on the immune system. Another study reported \textit{in vitro} and \textit{ex vivo} effects of PEITC on toll-like receptor 3 (TLR3). TLR’s are considered to be an important component of innate immune system. Studies showed that PEITC treatment inhibited TLR3 signaling [163]. PEITC treatment also inhibited the dimerization of TLR3 receptors leading to inhibition of IRF3 signaling. These effects were accompanied by inhibition of anchorage-independent growth and colony formation of cancer cells. Of note, PEITC also inhibits lipopolysaccharide induced TLR/IRF3 signaling in leukemia cells [164]. Thus, while theoretically the inhibition of TLR3 might be considered a negative effect on the immune system, the resulting inhibition of IRF3 signaling inhibits at least two mechanisms of metastatic tumor.
4.2.9. Effect of PEITC on cancer cell energetics and metabolism—Cancer cells have a high demand of ATP, which is fulfilled by higher glycolysis rates, a phenomenon known as Warburg’s effect. The discovery of Warburg’s effect in cancer cells has given new insight into cancer cell-specific biological pathways along with the possibility of specific novel targets. Agents that can inhibit or reverse this metabolic switch can be pivotal in the advancement of cancer therapeutics with reduced side effects and improved efficacy. A recent study showed reduced rates of glycolysis in PEITC-treated cells and depletion of ATP lead to death in prostate cancer cells [165]. The study showed increased glycolysis and lactic acid production in cancer cells, as measured through estimation of oxygen consumption rate and extracellular acidification rate, respectively. PEITC (5μM) treatment suppressed glycolysis in the cancer cells, but no changes were observed in normal cells. In addition, reduced concentration of ATP was also observed in cancer cells. Although this study provides an in vitro evidence of PEITC mediated inhibition of glycolysis, it is important to confirm these observations in animal models as well as in other cancer types.

4.3 Reactive Oxygen Species, a key mechanism of PEITC’s anti-cancer effects

Reactive oxygen species (ROS) have a dual role inside cells. In normal cells, ROS production causes DNA damage that drives cells toward apoptosis. Conversely, in cancer cells, ROS promotes cell survival by inducing several survival pathways responsible for cell proliferation, apoptosis suppression, cell migration and invasion, as well as suppression of the immune system. Cancer cells generate higher ROS levels due to increased oxidative metabolism and shortage of nutrient supply. However, increasingly high ROS levels can be toxic to cancer cells and can induce cell death. The levels and duration of ROS determine the final outcome (survival or death) of ROS present in cancer cells. In other words, ROS has to cross a threshold to induce apoptosis. The threshold is usually low in cancer cells as compared to normal cells, probably due to consistently high ROS levels. This difference provides an opportunity to target cancer cells by increasing ROS generation to a level that becomes toxic to cancer cells.

Interestingly, isothiocyanates were originally identified as natural antioxidants that can reduce ROS levels to serve as a chemo-preventive component of the diet [166–168]. The antioxidant effect is achieved at very low ITC levels in normal cells as shown in various animal models. At higher concentrations, ITCs may generate ROS by depleting antioxidant levels. PEITC is known to cause ROS generation, which is the major mechanism of toxicity in cancer cells [107, 119, 138]. Several mechanisms have been explained for PEITC-induced ROS. There is a continuous leakage of electrons from the electron transport chain (ETC), which is major source of ROS production. PEITC causes generation of endogenous ROS by disrupting mitochondrial respiratory chain and PEITC-mediated degradation of NADH dehydrogenase Fe-S subunit 3 inhibits complex I functioning [116, 169]. In addition, PEITC also inhibits mitochondrial complex III activity and reduces the oxygen consumption rate in prostate cancer cells [165]. Another study showed that PEITC treatment induced the growth factor adaptor protein, p66Shc, as a mechanism of ROS generation in cancer cells [170].
These outcomes were observed in the \textit{in vitro} study using prostate cancer cells. However, no studies have yet been performed to confirm these effects \textit{in vivo}.

Another established mechanism to induce ROS toxicity is by the inhibition of ROS detoxification of the cell. Each cell has well-developed mechanisms to protect against ROS-induced damage. Primary ROS scavenging mechanisms include dismutation of superoxide anion to oxygen and H$_2$O$_2$. Further, hydrogen peroxide is converted into water by glutathione peroxidase (GPX), or to oxygen and water by the enzymatic action of catalase. Glutathione (GSH) is a substrate for GPX. Levels of GSSG (oxidized form) and GSH (reduced form) reflect the redox status of cells. PEITC binds to GSH and causes its depletion in cancer cells leading to ROS-induced cell damage [107, 165, 169]. Interestingly, the sensitivity towards PEITC correlates with constitutive GSH levels present in the cells [171, 172]. The cells with higher levels of GSH were relatively more sensitive to PEITC, which may also explain the selectivity toward cancer cells compared with normal cells, since cancer cells have higher levels of GSH in normal cells.

Based on the available data, it is well-established that PEITC acts as a pro-oxidant to initiate ROS generation, leading to apoptosis in cell culture models. However, according to a recently published article, resultant modulation of ROS from an \textit{in vitro} study can vary significantly from an \textit{in vivo} study with the same agent [173]. Thus, outcomes based on the \textit{in vitro} studies may be debatable. Hence, it is crucial to confirm ROS generation by PEITC and its manifestations in an \textit{in vivo} model, along with the mechanisms involved. In another study, capsaicin treatment was shown to increase ROS levels in implanted pancreatic tumors, suggesting the role of ROS in tumor suppression. This action, along with a decrease in SOD activity and an increase in GSSG/GSH ratio in the tumors, correlated with the overall tumor growth suppression [174].

### 4.4 Direct protein modification by PEITC

Isothiocyanates are chemically characterized by N=C=S, which imparts electrophilic properties to these compounds. As reviewed by Mi \textit{et. al.}, the electrophilic property of PEITC leads to covalent interaction with nucleophilic entities like DNA, RNA, or critical amino acids of proteins and peptides [114]. Although, DNA and RNA were not the direct targets, PEITC was found to bind directly to proteins [120, 175]. PEITC modifies the functionality of several proteins by covalently binding with their nucleophilic amino acids. Some of the most important target proteins/peptides of PEITC include glutathione via sulfhydryl group, Cyp450 and tubulin via cysteine and macrophage migration inhibitory factor via proline [120, 176–178]. The modulation of proteins undoubtedly affects several cellular processes leading to cell growth suppression.

### 4.5 Cancer Specific Biomarkers

Cancer occurs due to genetic abnormalities. Several identified cancer-specific genetic changes are individual-specific. These genetic signatures demand individualized treatment strategies for patients, resulting in better therapeutic outcomes. For example, mutations in BRCA1 and/or BRCA2 account for 5–10% of breast cancer and 10–15% of ovarian cancer. Another major gene mutation known to enhance risk of any cancer significantly is p53.
Interestingly, PEITC is beneficial in these cancers and has been shown to increase the expression of BRCA2 along with induction of tumor suppressors, such as p53 and p57 [123]. In addition, aberrant expression of several other genes is known to be responsible for poor prognosis in cancer. An example of this phenomenon is HER2 and EGFR overexpression, which leads to poor prognosis in lung, breast, and ovarian cancers. PEITC works exceptionally well in suppressing EGFR, HER2 and their oncogenic manifestations [102, 117, 119, 141]. PEITC has been shown to induce apoptosis in diverse cancer cell lines with varying levels of HER2. Integrins have recently been identified as important therapeutic targets in various cancer types. PEITC was found to inhibit major integrins, such as ITGB1, ITGA2 and ITGA6 in prostate cancer cells [179]. Based on the available data on PEITC on cancer specific biomarkers, its application can be streamlined for personalized treatment options.

### 4.6 miRNAs

miRNAs are small single-stranded RNA molecules that can regulate the function and expression of various genes. miRNAs have recently been identified to be of significant importance due to their role in tumorigenesis. miRNAs commonly act as tumor suppressors, but a few miRNAs that are overexpressed and cause tumorigenesis also have been identified. An overall loss of miRNAs has been detected in human cancers, suggesting mostly tumor suppressor effects. To date, very few studies have been performed to test miRNA modulation by PEITC. Izzotti et al. demonstrated the chemopreventive effects of PEITC, using environmental cigarette smoke (ECS)-induced miRNA modulations in rat lungs and liver [153, 180, 181]. Interestingly, it was observed that miRNAs were more susceptible to changes induced by ECS as compared to proteins. In another study, 484 miRNAs were analyzed in rat lungs exposed with ECS. About 25 miRNAs were modulated significantly by ECS. However, combination treatment with PEITC and indole 3-carbinol reversed the effects of ECS on most of these miRNAs [181]. PEITC alone or in combination with indole 3-carbinol reversed the effects of ECS in rat lungs, but the combination had a much stronger effect. The combination reversed the majority of miRNAs down-regulated by ECS, which were involved in multiple processes related to cancer progression, such as angiogenesis, cell proliferation and stress response. A similar study was performed on mouse lungs and liver, where 576 miRNAs were analyzed after exposure to ECS [180]. The effect of PEITC on miRNAs modulated by ECS was more pronounced in liver as compared to lungs. A plausible reason for this phenomenon could be the first pass effect of liver, as suggested by the authors. Conaway et al. showed significantly higher concentrations of PEITC achieved in liver as compared to lungs, also suggesting enhanced effect in liver than lungs [182]. Furthermore, another group observed that PEITC caused miR-17 mediated suppression of p300/CBP-associated factor (PCAF), a co-regulator for androgen receptors (AR) leading to inhibition AR in prostate cancer cells [183]. Juturu et al. showed the modulation of miR-27a, miR-20a and miR-17-5p by PEITC in pancreatic cancer cells, leading to apoptosis [184]. One result of reduction in some tumor suppressor miRNAs is reduction in ROS (Croce, personal communication). Taken together these observations confirm that a number of miRNAs are modulated by PEITC. However, additional animal studies are required to delineate the implications of these changes in chemoprevention or chemotherapeutics.
4.7 Stem cells

Cancer stem cells (CSC) are considered to be the central element in the process of tumorigenesis. Furthermore, stem cells are resistant to many agents used to kill cancer cells. Although intensive research in this field is ongoing, in-depth knowledge on CSCs is still limited. Based on increasing evidence, the role of CSCs as therapeutic targets cannot be ignored. There is still lack of evidence for direct activity of PEITC on cancer stem cells [109, 111]. This probably can be due to lack of precise techniques for isolation and characterization of cancer stem cells. Indirect studies indicate the effects of PEITC on stem cells. Wu et. al. demonstrated reversal of resistance by PEITC in platinum resistant cells, which are known to have stem cell properties [155]. Furthermore, PEITC inhibits developmental genes in the embryonic stem cells, suggesting its effects on stem cells development. However, this also raises a concern of potential toxicity during embryonic development [185]. The clear mechanistic evidence for effects of PEITC on the growth and proliferation of CSCs is still lacking.

4.8 Epigenetics

Epigenetic modulations have recently gained a significant importance in cancer therapy. Many new agents are being identified that can alter gene expression without causing changes in gene sequence. Although gene array data showed that PEITC caused modulation of gene expression, it is now well known for epigenetic modulations in cancer cells [123, 186–188]. As discussed in sections 4.4, PEITC has the capability to bind directly with proteins to alter their function. Moreover, PEITC also affects a number of miRNAs which can play a critical role in cancer progression (section 4.6). As reviewed by Wang et. al., PEITC acts as an inhibitor of CpG methylation and histone deacetylation to manipulate gene expression [187]. Liu et. al. demonstrated that epigenetic changes like histone tail modifications occur at low non-cytotoxic concentrations of PEITC, which can play an important role in chemoprevention [189]. Furthermore, two other studies show synergism of PEITC with paclitaxel through epigenetic mechanisms [190, 191]. The hyperacetylation of α-tubulin induced by the combination of PEITC and paclitaxel was identified as the major mechanism of synergism in cancer cells. PEITC also modulates proteins like heat shock proteins (HSP) and proteases, which play a pivotal role in protein maturation and folding. For example, inhibition of HSP90, 70, 60, GRP78, ADAM 17 or topoisomerase IIα by PEITC results in epigenetic modifications [114, 121, 122, 124, 175, 192–194]. In line with this, PEITC-induced post-translational modification was observed in p53 mutant lung cancer cells, but the precise mechanism was not clear [195].

5.0 Combination therapy with PEITC

Drug resistance is a major obstacle in successful cancer therapeutics leading to poor clinical outcome. The therapy failure can occur due to different mechanisms, including reduced drug uptake, increased efflux of drug, induction of anti-apoptotic mechanism, inactivation of the drug through metabolic enzymes or reversal of DNA damage by induction of DNA repair pathways. As discussed earlier in this article, PEITC has been observed to modify several of these mechanisms. The outcomes of the studies where PEITC was used to potentiate the effects of conventional anti-cancer drugs or new anti-cancer agents have been detailed in our
previous review [196]. The proposed mechanisms by which PEITC enhances the effects of other agents or drugs are: reduction in the availability of GSH for conjugation leading to efflux and inactivation, inhibition of drug transporter proteins like MRP and PgP, hence improving drug availability to the cells and induction of pro-apoptotic pathways to counteract anti-apoptotic mechanisms. Using pre-clinical studies, improved outcomes were observed when the conventional agents, such as docetaxel, metformin, vinblastine, doxorubicin and HDAC inhibitors were combined with PEITC [102, 165, 197, 198] (Table 4). PEITC treatment sensitized glioma cells to TRAIL via ROS generation [199]. Interestingly, cisplatin also showed synergism with PEITC in cervical and breast cancer cells, which was mediated by modulation of MAPkinases, NFkB and death receptors [200]. PEITC treatment also reversed resistance to cisplatin in gastric cancer cells by inhibition of transporter proteins PgP and MRP1[201]. Considering the above facts, it can be concluded that PEITC can potentiate the effects of classical chemotherapeutic agents and therefore could be beneficial for drug-resistant cancers.

6.0 PEITC and tobacco smoke induced changes

Most of the initial studies used chemicals present in tobacco smoke induced tumor models to demonstrate efficacy of PEITC against lung tumors. Studies have shown that dietary administration of PEITC significantly reduces tumor incidence induced by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and benzopyrene (BaP) in mouse models [202–204]. Importantly, these agents are the major carcinogens of the tobacco smoke. Interestingly, studies showed PEITC mediated inhibition of the metabolic activation of these carcinogens leading to reduced tumorigenesis [205]. Furthermore, studies also showed that PEITC inhibits the formation of 4-hydroxy-1-(3-pyridyl)-1-butanone (HPB) releasing DNA adducts of NNK and BaP [206–209]. The inhibition of adduct formation was observed in whole lung tissue and in lung cells except macrophages [209]. Recently, various studies also showed stronger chemopreventive effects of PEITC in combination with myoinositol or aspirin, in NNK and BaP-induced lung tumor models [210–212]. Interestingly, PEITC suppressed cell proliferation in cigarette smoke treated human bronchial epithelial cells (HBEC) as well as lung cancer A549 cells but not in DMSO treated HBEC cells [210]. Furthermore, administration of dietary PEITC also reduced the DNA damage and molecular changes caused by cigarette smoke, along with reduction in tumor incidence in lung tumor model [213–215]. Taken together, these observations demonstrate potentially strong anti-cancer effects of PEITC for smokers and tobacco consumers.

7.0 Clinical advancement

Copious evidence from preclinical studies indicates efficacy of PEITC against cancer and supports its further development for clinical applications. Interestingly, four clinical studies for human testing of PEITC were initiated, out of which one study (NCT00968461) was withdrawn for unexplained reasons. Out of three, one study (NCT00005883) has been completed with a goal of multi-dose testing of PEITC in lung cancer patients. Although this phase I study is completed, the results have not been published yet. Another Phase I clinical study (NCT01790204) is planned at Georgetown University, with the goal of testing the beneficial effects of PEITC in oral carcinoma with mutant p53. A Phase I study (NCI
CN-55120) has confirmed that after oral intake of 200mg PEITC by human volunteers, 10μM PEITC can be achieved in plasma, as mentioned by Mi et. al. [216]. Interestingly, many studies have shown the anti-cancer efficacy of PEITC at concentrations less than 10μM. The Masonic Cancer Center at the University of Minnesota, in collaboration with National Cancer Institute, is currently conducting a phase II study with PEITC in lung cancer patients. The results of these studies will provide a clear idea of the efficacy and toxicity of PEITC in humans and will be instrumental in designing plans for further development of PEITC.

8.0 Summary

PEITC is a multifaceted agent that targets multiple processes required for cancer growth and development. It modulates many proteins to suppress survival and proliferation of cancer cells. Identification of specific targets paved ways to develop individualized patient treatment strategies. In addition, modification of proteins through covalent binding with PEITC opens avenues to develop this compound based on its affinity or selectivity towards specific cellular proteins. Based on the mechanisms of action of PEITC, such as ROS generation and inhibition of cell growth with mutant p53, there is significant evidence to demonstrate selectivity of PEITC towards cancer cells. Organ selective effects of PEITC are also evident by studies showing enhanced modulation of enzymes, protein targets and miRNAs observed in liver as compared to lungs, probably explained by the fact that PEITC levels achieved in liver were significantly higher than in lungs. PEITC exhibits strong anti-migratory and anti-invasive effects in different cancer forms, suggesting its anti-metastatic potential. Current evidence on these aspects relies mainly on in vitro studies. Due to the lack of ideal animal models, in vivo evidence is limited to breast cancer. Combination therapeutics is a common mode of intervention to circumvent problems due to drug resistance. Although, some evidence exists for increased efficacy of conventional drugs when used in combination with PEITC, major advancement is still awaited in this area. Moreover, being a natural compound, PEITC is not expected to present any severe toxicity, unlike many traditional anti-cancer agents. However, due to the likelihood of drug interactions as a result of its effects on drug metabolizing and detoxifying enzymes, more evidence is required for its practical clinical application in patients. Nonetheless, phase I and phase II clinical trials are in process. At the same time, missing information in the areas mentioned above is needed to fill in the gaps in our knowledge. Nonetheless, broad spectrum of PEITC justifies the rationale for its clinical development.

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Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>BaP</td>
<td>Benzo(a)pyrene</td>
</tr>
<tr>
<td>BCRP</td>
<td>Breast Cancer Resistance Protein</td>
</tr>
</tbody>
</table>
CSC  Cancer Stem cells
ECS  Environmental Cigarette Smoke
GPX  Glutathione peroxidase
GSH  Glutathione
GST  Glutathione S-transferase
HBEC Human Bronchial Epithelial Cells
HBP  4-hydroxy-1-(3-pyridyl)-1-butanone
ITC  Isothiocyanate
NAC  N-acetyl cysteine
NNK  4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone
PEITC Phenethylisothiocyanate
Rb  Retinoblastoma protein
ROS  Reactive oxygen species
DDB2  DNA-damage binding protein 2

References


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Figure 1.
PEITC and hallmarks of cancer
Figure 2.
General metabolic pathway for PEITC
Table 1

<table>
<thead>
<tr>
<th>Cancer form</th>
<th>Subjects</th>
<th>Year Conducted</th>
<th>Sample Size</th>
<th>Geographical Location</th>
<th>ITC quantitation method</th>
<th>Mechanism Proposed</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mixed population with mean ages 63.3 and 62.5 for cases and controls respectively</td>
<td>1999</td>
<td>697 cases &amp; 708 controls</td>
<td>Texas, USA</td>
<td>Dietary cruciferous vegetables intake based on patient questionnaire</td>
<td>GSTM1 and GSTT1 null</td>
<td>Reduced cancer risk</td>
<td>[23]</td>
</tr>
<tr>
<td></td>
<td>Cases with ages 25–86 years and controls with ages 21–52 years</td>
<td>1982–1998</td>
<td>275 cases &amp; 825 controls</td>
<td>USA</td>
<td>Dietary cruciferous vegetables intake based on patient questionnaire</td>
<td>-</td>
<td>Reduced cancer risk</td>
<td>[24]</td>
</tr>
<tr>
<td></td>
<td>Chinese women Age 20–70 years</td>
<td>1996–2005</td>
<td>3035 cases &amp; 3037 controls</td>
<td>Shanghai, China</td>
<td>Dietary ITC intake based on patient questionnaire</td>
<td>GSTP1 polymorphism</td>
<td>Reduced cancer risk</td>
<td>[14]</td>
</tr>
<tr>
<td>Breast Cancer</td>
<td>Breast cancer survivors</td>
<td>-</td>
<td>536 intervention &amp; 620 comparison</td>
<td>USA</td>
<td>Dietary cruciferous vegetables intake based on patient questionnaire</td>
<td>-</td>
<td>Reduced cancer recurrence</td>
<td>[25]</td>
</tr>
<tr>
<td></td>
<td>US and Chinese breast cancer survivors</td>
<td>1990–2006</td>
<td>11390 subjects</td>
<td>USA and China</td>
<td>Dietary cruciferous vegetables intake based on patient questionnaire</td>
<td>-</td>
<td>No association with cancer mortality or recurrence</td>
<td>[27]</td>
</tr>
<tr>
<td>Colorectal Cancer</td>
<td>Compiled from published literature</td>
<td>-</td>
<td>24case control studies and 11 prospective studies</td>
<td>-</td>
<td>Dietary intake of cruciferous vegetables</td>
<td>-</td>
<td>Reduced cancer risk</td>
<td>[12]</td>
</tr>
<tr>
<td>Gastric Cancer</td>
<td>Middle aged men ; mean age ±65</td>
<td>1986–1989</td>
<td>307 Cases and 911 Control</td>
<td>Shanghai, China</td>
<td>Urinary ITC</td>
<td>GSTM1 and GSTT1 deletion enhanced protective effect</td>
<td>Reduced cancer incidence</td>
<td>[8]</td>
</tr>
<tr>
<td></td>
<td>Age 20–79 years</td>
<td>1999–2003</td>
<td>1097 cases &amp; 1555 controls</td>
<td>Europe (multicenter)</td>
<td>Dietary ITC intake based on patient questionnaire</td>
<td>GSTM1 and GSTT1 polymorphism</td>
<td>Reduced cancer risk</td>
<td>[15]</td>
</tr>
<tr>
<td>Kidney Cancer</td>
<td>Non-Asians</td>
<td>1986–1994</td>
<td>1204 cases and 1204 controls</td>
<td>California, USA</td>
<td>Dietary cruciferous vegetables intake based on patient questionnaire</td>
<td>-</td>
<td>Reduced cancer risk</td>
<td>[26]</td>
</tr>
<tr>
<td>Lung Cancer</td>
<td>African Americans &amp; Caucasians; Age 40–84 years</td>
<td>1991–1994</td>
<td>311 Cases &amp; 922 Control</td>
<td>Los Angeles, California</td>
<td>Dietary ITC intake based on patient questionnaire</td>
<td>GSTM1 deletion enhanced protective effect</td>
<td>Reduced cancer incidence</td>
<td>[9]</td>
</tr>
<tr>
<td></td>
<td>Chinese women Age 40–70 years</td>
<td>1997–2009</td>
<td>74 914 women</td>
<td>Shanghai, China</td>
<td>Dietary intake of cruciferous vegetables</td>
<td>-</td>
<td>Reduced cancer risk</td>
<td>[13]</td>
</tr>
<tr>
<td></td>
<td>Men and women with average ages 62.3 and 60.9 years respectively</td>
<td>-</td>
<td>503 cases &amp; 465 controls</td>
<td>Texas, USA</td>
<td>Dietary ITC intake based on patient questionnaire</td>
<td>GSTM1 and GSTT1 polymorphism</td>
<td>Reduced cancer risk</td>
<td>[16]</td>
</tr>
<tr>
<td>Cancer form</td>
<td>Subjects</td>
<td>Year Conducted</td>
<td>Sample Size</td>
<td>Geographical Location</td>
<td>ITC quantitation method</td>
<td>Mechanism Proposed</td>
<td>Outcome</td>
<td>Reference</td>
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</tr>
<tr>
<td>Chinese women</td>
<td>1996–1998</td>
<td>233 cases &amp; 187 controls</td>
<td>Singapore</td>
<td>Dietary ITC intake based on patient questionnaire</td>
<td>GSTM1 and GSTT1 polymorphism</td>
<td>Reduced cancer risk</td>
<td>[17]</td>
<td></td>
</tr>
<tr>
<td>Chinese women</td>
<td>1996–1998</td>
<td>303 cases &amp; 765 controls</td>
<td>Singapore</td>
<td>Dietary ITC intake based on patient questionnaire</td>
<td>-</td>
<td>Reduced cancer risk</td>
<td>[18]</td>
<td></td>
</tr>
<tr>
<td>Hospital patients</td>
<td>1992–2000</td>
<td>716 cases and 939 controls</td>
<td>Massachusetts, USA</td>
<td>Dietary cruciferous vegetables intake based on patient questionnaire</td>
<td>GSTM1 presence</td>
<td>Reduced cancer risk</td>
<td>[21]</td>
<td></td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>Men Age 40–75 years</td>
<td>1986–2000</td>
<td>47365 subjects</td>
<td>USA</td>
<td>Dietary cruciferous vegetables intake based on patient questionnaire</td>
<td>-</td>
<td>Non-significant correlation with cancer risk</td>
<td>[20]</td>
</tr>
<tr>
<td>Stomach and colorectal cancer</td>
<td>Japanese population</td>
<td>-</td>
<td>149 cases &amp; 287 controls for stomach cancer and 115 cases and 230 controls for colorectal cancer</td>
<td>Japan</td>
<td>Dietary cruciferous vegetables intake based on patient questionnaire</td>
<td>-</td>
<td>Reduced cancer risk</td>
<td>[19]</td>
</tr>
<tr>
<td>Thyroid cancer</td>
<td>Melanesian women</td>
<td>1993–1999</td>
<td>293 Cases &amp; 354 Control</td>
<td>New Caledonia</td>
<td>Dietary ITC intake based on patient questionnaire</td>
<td>Low dietary intake of iodine</td>
<td>Increased risk of thyroid cancer</td>
<td>[10]</td>
</tr>
<tr>
<td>Urinary carcinoma</td>
<td>Scottish Terriers</td>
<td>-</td>
<td>92 cases &amp; 83 controls</td>
<td>-</td>
<td>Dietary cruciferous vegetables intake based on owner questionnaire</td>
<td>-</td>
<td>Non-significant correlation with cancer risk</td>
<td>[22]</td>
</tr>
</tbody>
</table>
## Pharmacokinetic parameters of PEITC

<table>
<thead>
<tr>
<th>Route of admin</th>
<th>Dose</th>
<th>V drug</th>
<th>Mo del</th>
<th>Nu mber of animals</th>
<th>Org an</th>
<th>AUC (h^4/μM) (SE)</th>
<th>Cmax (μM) (SE)</th>
<th>Tmax (h)</th>
<th>T1/2α (h) (SE)</th>
<th>T1/2β (h) (SE)</th>
<th>Vd (μl/kg) (SE)</th>
<th>F (%) (SE)</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral</td>
<td>10μmol (50μM/kg) (single)</td>
<td>Corn Oil</td>
<td>Male F344 rat</td>
<td>3</td>
<td>Blood</td>
<td>328.1 ± 14 ml (100.93)</td>
<td>1877 ± 43 ml (2.9)</td>
<td>2.94 ± 0.36</td>
<td>1.34 ± 0.15</td>
<td>α = 2.43 ± 0.86</td>
<td>[0.21 ± 0.77]</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
|               |      |        |        |                   | Liver | 127.57 ± 0.01 ml (201.76) | 3979 ± 39 ml (3.29) | 2.76 ± 0.56 | 0.76 ± 0.14 | 2.18 ± 0.20 | - | - |-
|               |      |        |        |                   | Lung  | 384.32 ± 0.01 ml (50.51) | 973 ± 39 ml (3.39) | 4.59 ± 0.04 | 0.79 ± 0.14 | 2.18 ± 0.20 | - | - |-
|               |      |        |        |                   | Kidney | 987.94 ± 0.01 ml (11.08) | 46.20 ± 2.06 ml (2.43) | 3.03 ± 0.05 | 1.60 ± 0.20 | 1.17 ± 0.11 | - | - |-
|               |      |        |        |                   | Nanda massa | 378.50 ± 0.01 ml (5.41) | 204 ± 37 ml (7.31) | 3.52 ± 0.06 | 0.99 ± 0.12 | 1.05 ± 0.20 | - | - |-
|               |      |        |        |                   | Esophagus | 140.19 ± 0.01 ml (11.09) | 8.2 ± 0.02 ml (2.14) | 2.79 ± 1.20 | 0.67 ± 1.20 | 9.29 ± 0.40 | - | - |-
|               |      |        |        |                   | Stomach | 149.57 ± 0.01 ml (57.62) | 211.19 ± 26.44 ml (1.05) | 1.35 ± 0.20 | 0.70 ± 0.20 | 3.57 ± 1.02 | - | - |-
|               |      |        |        |                   | Small intestine | 109.21 ± 0.01 ml (29.29) | 138.38 ± 27.66 ml (6.64) | 4.07 ± 0.04 | 1.69 ± 0.46 | 5.22 ± 0.22 | - | - |-
|               |      |        |        |                   | Cancers | 102.22 ± 0.01 ml (42.49) | 1597 ± 185 ml (13.15) | 1.90 ± 1.20 | 4.47 ± 0.42 | 2.52 ± 0.47 | - | - |-
|               |      |        |        |                   | Colon | 361.39 ± 0.01 ml (47.32) | 16.45 ± 1.22 ml (0.91) | 0.80 ± 0.15 | 5.91 ± 0.52 | 5.4 ± 0.52 | - | - |-
|               |      |        |        |                   | Pancreas | 176.09 ± 0.01 ml (20.66) | 973 ± 39 ml (0.39) | 4.58 ± 0.04 | 5.90 ± 1.12 | 0.99 ± 0.16 | - | - |-
|               |      |        |        |                   | Spleen | 57.11 ± 0.01 ml (0.55) | 964 ± 37 ml (2.00) | 1.20 ± 0.34 | 3.20 ± 0.50 | 0.30 ± 0.57 | - | - |-
|               |      |        |        |                   | Heart | 94.75 ± 0.01 ml (22.99) | 571 ± 35 ml (0.51) | 4.30 ± 0.56 | 3.78 ± 0.81 | 1.40 ± 0.61 | - | - |-
|               |      |        |        |                   | Brain | 53.71 ± 0.01 ml (5.4) | 247 ± 35 ml (0.1) | 6.46 ± 0.20 | 1.20 ± 0.77 | 2.05 ± 0.54 | - | - |-
| Oral (film-coated tablets) | 0.5, 5, 50 μg/g (single) | DMSO | Male Wistar albino rats | 4 | Blood | 100.5 ± 0.10 ml (378 mg/L) | 354 ± 12 ml (3.43) | 0.74 ± 0.08 | 0.20 ± 0.12 | 2.62 ± 0.78 | 1.57 ± 1.35 | 7.5 ± 1.3 |-
|               |      |        |        |                   | Liver | 164.6 ± 0.10 ml (378 mg/L) | 387 ± 75 ml (1.38) | 0.15 ± 0.04 | 0.90 ± 0.08 | 2.96 ± 0.20 | 1.75 ± 0.29 | 6.14 ± 1 |-
|               |      |        |        |                   | Lung  | 247 ± 90 ml (378 mg/L) | 700 ± 97 ml (1.00) | 0.70 ± 0.08 | 0.19 ± 0.08 | 3.31 ± 0.07 | 2.15 ± 0.40 | 2.5 ± 0.5 |-
|               |      |        |        |                   | Kidney | 111 ± 30 ml (378 mg/L) | 406 ± 90 ml (3.02) | 0.70 ± 0.08 | 0.10 ± 0.08 | 2.56 ± 0.20 | 1.56 ± 1.48 | 8.3 ± 0.2 |[
|               |      |        |        |                   | Nanda massa | 231 ± 22 ml (378 mg/L) | 647 ± 26 ml (6.88) | 0.00 ± 0.03 | 0.02 ± 0.01 | 1.86 ± 0.20 | 1.05 ± 0.10 | 86.40 |-
|               |      |        |        |                   | Esophagus | 579 ± 0.01 ml (378 mg/L) | 215 ± 40 ml (0.95) | 0.95 ± 0.04 | 0.20 ± 0.02 | 1.62 ± 0.40 | 0.95 ± 0.20 | 4.37 |-
| Intravenous | 0.5 μg/day (4 days) | 15% hydroxypropyl-β-cyclodextrin | Male Wistar albino rats | 4 | Blood | 100.5 ± 0.10 ml (378 mg/L) | 354 ± 12 ml (3.43) | 0.74 ± 0.08 | 0.20 ± 0.12 | 2.62 ± 0.78 | 1.57 ± 1.35 | 7.5 ± 1.3 |-
| Intravenous | 2 μg/kg | 15% hydroxypropyl-β-cyclodextrin | Male Sprague-Dawley rats | 3-4 | Blood | 2.80 ± 0.20 ml (80 mg/kg) | - | - | - | 3.5 ± 0.10 | 1.96 ± 0.27 | 4.0 ± 0.10 |-
| 100μg/kg | - | - | - | - | 17.5 ± 0.04 | - | - | - | 6.08 ± 0.70 | 3.73 | - |-
| Oral | 10μmol/kg | - | - | - | - | 18.0 ± 0.65 | 0.44 ± 0.04 | - | - | 11.5 | - | - |-

[^36]: [36]
[^37]: [37]
<table>
<thead>
<tr>
<th>Route of administration</th>
<th>Dose</th>
<th>Vehicle</th>
<th>Model</th>
<th>Number of samples</th>
<th>Organ</th>
<th>AUC ±SD or (SE)</th>
<th>Cmax ±SD or (SE)</th>
<th>Tmax ±SD or (SE)</th>
<th>T1/2a ±SD or (SE)</th>
<th>Vd ±SD or (SE)</th>
<th>F % ±SD or (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100μmol/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>298±130.4</td>
<td>42±11.4</td>
<td>2±1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
### Table 3A

Epidemiologic evidence for the effects of GST polymorphism on ITC uptake/metabolism and cancer risk

<table>
<thead>
<tr>
<th>Cancer</th>
<th>Design</th>
<th>Population</th>
<th>Location</th>
<th>Method of analysis</th>
<th>ITC intake/levels</th>
<th>GST studied</th>
<th>Status</th>
<th>Outcomes</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenoma</td>
<td>Case control (459 cases; 507 control)</td>
<td>Mixed</td>
<td>USA</td>
<td>Dietary intake</td>
<td>High</td>
<td>GSTM1</td>
<td>Null</td>
<td>Reduced risk</td>
<td>P=0.001 for trend and 0.1 for interaction</td>
</tr>
<tr>
<td>Breast</td>
<td>Case control (1052 cases and 1098 control)</td>
<td>Women (65 years age)</td>
<td>USA</td>
<td>Dietary intake</td>
<td>High</td>
<td>GSTM1/T1</td>
<td>Null/positive</td>
<td>Reduced risk</td>
<td>OR=0.6 (0.4–1.01)</td>
</tr>
<tr>
<td>Colon</td>
<td>Case control (213 cases and 1194 control)</td>
<td>Chinese men and women</td>
<td>Singapore</td>
<td>Dietary intake</td>
<td>High</td>
<td>GSTM1/T1</td>
<td>Null</td>
<td>Reduced risk</td>
<td>OR=0.43 (0.2–0.96)</td>
</tr>
<tr>
<td>Kidney</td>
<td>Case control (1097 cases and 1555 control)</td>
<td>Mixed subjects from Houston</td>
<td>USA</td>
<td>Dietary intake</td>
<td>Low</td>
<td>GSTM1</td>
<td>Null/Current smoker</td>
<td>Increased risk</td>
<td>OR=2.22 (1.2–4.1)</td>
</tr>
<tr>
<td>Lung</td>
<td>Case control (233 cases; 187 control)</td>
<td>Chinese women</td>
<td>-</td>
<td>Dietary intake</td>
<td>High</td>
<td>GSTM1</td>
<td>Null</td>
<td>Reduced risk</td>
<td>OR=0.54 (0.3–0.95)</td>
</tr>
<tr>
<td>Lung</td>
<td>Case control (233 cases; 710 control)</td>
<td>Chinese men</td>
<td>China</td>
<td>Dietary intake and urinary ITC analysis</td>
<td>Detectable</td>
<td>GSTM1</td>
<td>Null</td>
<td>Reduced risk</td>
<td>Relative risk=0.36 (0.2–0.63)</td>
</tr>
<tr>
<td>Lung</td>
<td>Case control (716 cases; 939 control)</td>
<td>Caucasian women and men</td>
<td>USA</td>
<td>Dietary intake</td>
<td>High</td>
<td>GSTM1</td>
<td>Positive</td>
<td>Reduced risk</td>
<td>OR=0.61 (0.39–0.95)</td>
</tr>
<tr>
<td>Cancer</td>
<td>Design</td>
<td>Population</td>
<td>Location</td>
<td>Method of analysis</td>
<td>ITC intake/levels</td>
<td>GST studied</td>
<td>Status</td>
<td>Outcomes</td>
<td>Statistics</td>
</tr>
<tr>
<td>--------</td>
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<td>------------</td>
</tr>
<tr>
<td></td>
<td>Literature review</td>
<td>30 studies</td>
<td>-</td>
<td>Dietary intake</td>
<td>High</td>
<td>GSTM1</td>
<td>Null</td>
<td>No correlation</td>
<td>OR=1.15 (0.78–1.68)</td>
</tr>
<tr>
<td></td>
<td>Cohort</td>
<td>Chinese (45–74 years age)</td>
<td>Singapore</td>
<td>Diet and urinary ITC analysis</td>
<td>High</td>
<td>GSTT1/M1</td>
<td>Null</td>
<td>Reduced risk</td>
<td>OR=0.41 (0.26–0.65)</td>
</tr>
<tr>
<td></td>
<td>Cohort (114 subjects) 18–50 years</td>
<td>Mixed</td>
<td>USA</td>
<td>Urinary ITC and metabolites</td>
<td>Single intake</td>
<td>GSTM1</td>
<td>Null</td>
<td>High excretion in 62% subjects</td>
<td>ITC (mol/24h)=9.9±1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>GSTT1</td>
<td>Null</td>
<td>High excretion in 39% subjects</td>
<td>ITC (mol/24h)=14.0±1.3 (P&lt;0.01)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>GSTM1</td>
<td>Positive</td>
<td>Marginally significant</td>
<td>ITC (mol/24h)=9.5±2.7 (P=0.05)</td>
</tr>
<tr>
<td></td>
<td>Cross-over intervention study</td>
<td>20 healthy subjects</td>
<td>-</td>
<td>Plasma ITC</td>
<td>-</td>
<td>GSTM1</td>
<td>Null/Positive</td>
<td>No correlation</td>
<td>OR=1.33 (0.33–4.36)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>GSTT1</td>
<td>Null/Positive</td>
<td>No correlation</td>
<td>OR=1.42 (0.45–4.41)</td>
</tr>
<tr>
<td></td>
<td>48 subjects, 28 GSTT1 and M1 positive and 20 null genotypes</td>
<td>Healthy volunteers</td>
<td>-</td>
<td>Urinary ITC</td>
<td>Watercress juice</td>
<td>GSTT1/M1</td>
<td>Null/Positive</td>
<td>No correlation</td>
<td>-</td>
</tr>
</tbody>
</table>

Reference: [47] [40] [45] [49] [51]
Table 3B

Pre-clinical evidence for effect of GST polymorphism on isothiocyanate efficacy

<table>
<thead>
<tr>
<th>Model</th>
<th>Agent used</th>
<th>GST polymorph</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male Wistar rats</td>
<td>BITC</td>
<td>Subunit 3 &amp; 2</td>
<td>Increased</td>
<td>[52]</td>
</tr>
<tr>
<td>Rat T9 Glioma cells</td>
<td>AITC, BITC, AITC, BITC + dibutyryl cAMP</td>
<td>GSTP</td>
<td>Increased marginally</td>
<td>[53]</td>
</tr>
<tr>
<td>Male ACI/N rats</td>
<td>BITC, BTC</td>
<td>GSTP</td>
<td>Increased</td>
<td>[54]</td>
</tr>
<tr>
<td>Kinetic study</td>
<td>ITC</td>
<td>GSTP1-1 and M1-1</td>
<td>Most efficient catalysis</td>
<td>[55]</td>
</tr>
<tr>
<td>Kinetic study</td>
<td>PEITC, BITC</td>
<td>GSTA1, A2, P1</td>
<td>Catalyze forward and reverse reactions in a concentration dependent manner</td>
<td>[56]</td>
</tr>
<tr>
<td>Male Wistar rats</td>
<td>PEITC</td>
<td>GSTA</td>
<td>Increased in liver</td>
<td>[57]</td>
</tr>
<tr>
<td>Male F344 rats</td>
<td>PEITC</td>
<td>GSTP</td>
<td>Reduced in liver (Pre-initiation period of tumors) with reduced hyperplasia in esophagus and kidney</td>
<td>[58]</td>
</tr>
<tr>
<td>RL34 cells (Rat liver epithelial)</td>
<td>BITC, PEITC</td>
<td>GSTP1</td>
<td>Increased</td>
<td>[59]</td>
</tr>
<tr>
<td>RL34 cells</td>
<td>ITC</td>
<td>GSTA1/2</td>
<td>Nrf2 dependent increase</td>
<td>[60]</td>
</tr>
<tr>
<td>PBMC cells</td>
<td>Watercress/PEITC</td>
<td>GSTM1-0</td>
<td>Increased GPX and SOD</td>
<td>[61]</td>
</tr>
<tr>
<td>HL60 cells</td>
<td>ITC</td>
<td>GSTA (Overexpression)</td>
<td>Reduced efficacy</td>
<td>[62]</td>
</tr>
<tr>
<td>Male Wistar rats</td>
<td>ITC</td>
<td>GSTM</td>
<td>Increased in liver</td>
<td>[63]</td>
</tr>
<tr>
<td></td>
<td>ITC</td>
<td>GSTA</td>
<td>Increased in Liver</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ITC</td>
<td>GSTT</td>
<td>Increased in kidney</td>
<td></td>
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Table 4

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>Mechanism</th>
<th>Molecular Targets</th>
<th>Outcome</th>
<th>Concentration and duration</th>
<th>Model used</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>Epigenetic</td>
<td>CYP2E1</td>
<td>Reduced activation of carcinogens</td>
<td>25, 50μM</td>
<td>Escherichia coli MV1304 (c DNA CYP2E1)</td>
<td>[89]</td>
</tr>
<tr>
<td>-</td>
<td>Third modification</td>
<td>Topoisomerase Il</td>
<td>Apoptosis</td>
<td>2.5, 5, 10μM (6h)</td>
<td>Top2[38]; ap2[39] primary MEFs</td>
<td>[179]</td>
</tr>
<tr>
<td>Breast</td>
<td>Protein suppression</td>
<td>BIM, PUMA, BCL-2, BCL-xl, BAX, Bak</td>
<td>Apoptosis</td>
<td>2.5, 5μM (36h)</td>
<td>BRI-JM04; MDA-MB-231; MCF-7; HCT-116; HCT-116 (PUMA −/−)</td>
<td>[136]</td>
</tr>
<tr>
<td></td>
<td>Synergism with paclitaxel</td>
<td>-</td>
<td>Apoptosis; G2/M cell cycle arrest</td>
<td>1–10μM (24, 48h)</td>
<td>MCF7; MDA-MB-231</td>
<td>[190]</td>
</tr>
<tr>
<td></td>
<td>Protein suppression</td>
<td>HSP90, HSF1, Cyclin B1, Cdc2, Cdc25, PLK-1, and p21, caspase 3, 9</td>
<td>Apoptosis; G2/M cell cycle arrest</td>
<td>0.1, 0.5, 2.5, 5μM (24h)</td>
<td>MCF7; MDA-MB-231</td>
<td>[192]</td>
</tr>
<tr>
<td></td>
<td>Protein suppression</td>
<td>BCL-2, BAX, NFR2, GSH, caspase 3, 8 and 9</td>
<td>Apoptosis; G2/M cell cycle arrest</td>
<td>20μM (2, 4, 6, 24, 48h)</td>
<td>MCF7; BT549; MDA-MB-231; ZR-75-1; SKBR3; T47D</td>
<td>[171]</td>
</tr>
<tr>
<td></td>
<td>Protein suppression</td>
<td>p30S6K, Akt, NDRG1, HSP70, ERK, AMPK, 4E-BP1, mTORC1</td>
<td>Proliferation inhibition</td>
<td>0–20μM (3,4h)</td>
<td>MCF7; PTEN deficient MEF; TSC2 deficient MEF</td>
<td>[194]</td>
</tr>
<tr>
<td></td>
<td>Chemoprevention</td>
<td>-</td>
<td>Reduced tumor incidence and tumor free survival</td>
<td>50 μmol/kg or 150 μmol/kg every 2 days (pretumor initiation); PEITC continued (18 weeks)</td>
<td>NMU induced breast cancer in Sprague-Dawley rats</td>
<td>[85]</td>
</tr>
<tr>
<td></td>
<td>Chemoprevention</td>
<td>-</td>
<td>Apoptogenic inhibition and tumor growth inhibition</td>
<td>3 μmol/g diet (29 weeks)</td>
<td>MMTV-neu mice</td>
<td>[86]</td>
</tr>
<tr>
<td></td>
<td>Epigenetic changes</td>
<td>PARP-1, BCL-2, Bax, Cdc1, Cyclin B1; c-tubulin, β-tubulin, acetyl-α-tubulin</td>
<td>Increase in Taxol (10, 100μM) sensitivity</td>
<td>5, 10μM (24h)</td>
<td>MCF7; MDA-MB-231</td>
<td>[191]</td>
</tr>
<tr>
<td></td>
<td>Genetic expression</td>
<td>P37, CYP19, BRCA2, IL2, ATF2, p53, Hsp27</td>
<td>-</td>
<td>3μM (48h)</td>
<td>MCF7</td>
<td>[123]</td>
</tr>
<tr>
<td></td>
<td>ROS</td>
<td>HER2, EGFR, Akt, STAT3, BIM, BAX, BCL-xl, XIAP</td>
<td>Proliferation inhibition; apoptosis; reduced tumor growth</td>
<td>5, 10, 15μM (24h)</td>
<td>MCF7; MCF-7 high HER2; MDA-MB-231; MD-A-MB-231 high HER2; MDA-MB-231 high HER2 xenograft in athymic nude mice</td>
<td>[102]</td>
</tr>
<tr>
<td>breathed and prostate</td>
<td>Protein suppression</td>
<td>Vimentin</td>
<td>Migration inhibition; apoptosis; tumor growth inhibition</td>
<td>2.5, 5μM (6, 12, 24h); 3μmol PEITC/μg diet (29 weeks)</td>
<td>MDA-MB-231; PC-3; DU145; MTTV-neu mice</td>
<td>[136]</td>
</tr>
<tr>
<td>Cervical</td>
<td>Protein suppression</td>
<td>DR4, D65, HO-1, c, cytochrome c, clusterin, clasin, cDAP1, cDAP2, catalase, Bcl2, Bcl-xl, BAX, BAD, BID, ERK, JNK, MEK</td>
<td>Apoptosis</td>
<td>2.5, 5, 10μM (48h)</td>
<td>HEP-2; KB</td>
<td>[141]</td>
</tr>
<tr>
<td>Cervical, Breast</td>
<td>Synergism with ciplatin</td>
<td>MAPKs, MEK1/2, NFIB, Bcl2, Bcl-xl, BAX, NOXA, PUMA, DR4, DR5, TRAIL</td>
<td>Apoptosis</td>
<td>5μM (2, 4, 8, 12, 24h)</td>
<td>Hela, C33A: MCF-7</td>
<td>[200]</td>
</tr>
<tr>
<td>Cancer Type</td>
<td>Mechanism</td>
<td>Molecular Targets</td>
<td>Outcome</td>
<td>Concentration and duration</td>
<td>Model used</td>
<td>Reference</td>
</tr>
<tr>
<td>---------------------</td>
<td>------------------------------------------</td>
<td>----------------------------------</td>
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<td>-----------------------------</td>
<td>------------------------------------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Cholangio carcinoma</td>
<td>Increase of free calcium level in cytosol</td>
<td>Cyt c, BCL-XL, BAX, AIF</td>
<td>Apoptosis</td>
<td>1, 3, 9μM (3, 6h)</td>
<td>KKU-M214; Chang cells (reference)</td>
<td>[133]</td>
</tr>
<tr>
<td>ROS</td>
<td></td>
<td>Cyt c, BCL-2, caspases 3, 8, 9, AIF</td>
<td>Apoptosis</td>
<td>3, 10μM (3, 6, 24h)</td>
<td>KKU-300</td>
<td>[135]</td>
</tr>
<tr>
<td>Colon</td>
<td>ROS</td>
<td>DDB2</td>
<td>Tumor regression</td>
<td>10μM (0.5, 1, 2, 4.6, 12h)</td>
<td>HCT156; HCT16 expressing control shRNA or DDB2 shRNA xenografts</td>
<td>[139]</td>
</tr>
<tr>
<td>Colon and blood</td>
<td>Epigenetic (Histone modification and</td>
<td>NFKB, STAT1, chemokines, MMPs</td>
<td>Proliferation inhibition</td>
<td>2.5, 5, 10, 15μM (5, 8, 12, 18h)</td>
<td>SW480, HT-29, THP-1</td>
<td>[189]</td>
</tr>
<tr>
<td>Gliotic</td>
<td>ROS, Rhodamine-123; Caspase resistance</td>
<td>ERCC1, survivin, and Mad2, Akt,</td>
<td>Apoptosis; cell cycle arrest,</td>
<td>1, 5μM (24h)</td>
<td>SOC7901/DOP</td>
<td>[201]</td>
</tr>
<tr>
<td>Gliona</td>
<td>ROS, Sensitization to TRAIL</td>
<td>DRS5, p53, caspase 3, 8, and 9</td>
<td>Apoptosis</td>
<td>2.5μM (24h)</td>
<td>U87MG, A172, U343, T98G</td>
<td>[199]</td>
</tr>
<tr>
<td>Gliona, prostate,</td>
<td>Sensitivity to hypoxia</td>
<td>HBFI1, VEGF, ERK, Akt, MMP2,</td>
<td>Apoptosis; tumor growth</td>
<td>1, 2.5, 5μM (6, 16h)</td>
<td>U87, DU145; HCT16; HepG2, SKBR3</td>
<td>[154]</td>
</tr>
<tr>
<td>Colon and blood</td>
<td>Protein suppression</td>
<td>Akt, JNK, XIAP, MCH1, BCL2, BCL-</td>
<td>Apoptosis; tumor growth suppression</td>
<td>2, 4, 6, 8μM (0–24h); 50mg/kg, i.p (20 days)</td>
<td>U937; HL-60; Jurkat cells</td>
<td>[116]</td>
</tr>
<tr>
<td>Leukemia</td>
<td>Immune-modulation</td>
<td>CD3, CD19, Mac-3</td>
<td>Enhanced phagocytic activity of</td>
<td>80 &amp; 160 mg/kg (2 weeks)</td>
<td>WEHI-3; BALB/c mice</td>
<td>[162]</td>
</tr>
<tr>
<td>Lung</td>
<td>ROS, nitric oxide</td>
<td>Mitochondrial complex, peroxiredoxin III (Prx III), superoxide dismutase-2, (SOD-2), Bcl-2, Glutathione peroxidase 4, NDUF3</td>
<td>Apoptosis</td>
<td>10μM (3, 6, 12h)</td>
<td>HL-60, Raji</td>
<td>[169]</td>
</tr>
<tr>
<td>Lung</td>
<td>Epigenetic (Covalent protein binding)</td>
<td>Actin, Tubulin, Tropomyosin, HSPs',</td>
<td>-</td>
<td>20μM (1h)</td>
<td>A549</td>
<td>[114]</td>
</tr>
<tr>
<td>Lung</td>
<td>Cytoskeletal changes</td>
<td>F-actin, α-tubulin,</td>
<td>Apoptosis; G2/M cell cycle arrest</td>
<td>1–10μM (24 &amp; 72h)</td>
<td>A549, H1299</td>
<td>[126]</td>
</tr>
<tr>
<td>Lung</td>
<td>Epigenetic</td>
<td>Tubulin</td>
<td>Cell growth inhibition and apoptosis</td>
<td>5, 10, 20μM (4h)</td>
<td>A549</td>
<td>[176]</td>
</tr>
<tr>
<td>Lung</td>
<td>Genetic expression</td>
<td>AP1, JNK, p38 MAPK, p44/42 MAPK,</td>
<td>Apoptosis; G2/M cell cycle arrest</td>
<td>12.5, 20μM (24h)</td>
<td>L9981</td>
<td>[188]</td>
</tr>
<tr>
<td>Lung</td>
<td>Post translational modification</td>
<td>p53 (mutant)</td>
<td>Apoptosis</td>
<td>15, 20μM (0–24h)</td>
<td>H596; MDA-A-MB-231</td>
<td>[195]</td>
</tr>
<tr>
<td>Lung, colon, ovarian</td>
<td>Sensitization of platinum resistant cells</td>
<td>GSH</td>
<td>Reduced cell survival</td>
<td>5, 7.5μM (6, 12, 24h); 25mg/kg, i.d, once a week (3 weeks)</td>
<td>A549; A590/DDP; THCS007; THCS07L-OHP; HCT156; A2780; A549/ CDDP xenografts in BALB/c nude mice</td>
<td>[155]</td>
</tr>
<tr>
<td>Myeloid leukemia</td>
<td>ROS</td>
<td>Fas, Fas ligand, caspase 9 and 3</td>
<td>Apoptosis</td>
<td>5, 10, 15, 20μM (24, 48, 72h)</td>
<td>K562</td>
<td>[143]</td>
</tr>
<tr>
<td>Melanoma</td>
<td>ROS, ER stress</td>
<td>CDC25C, CDK1, Chkl, Chk2, cyclin A, Wnt1, p533 and p21 (a); Bax, Bcl-2 and Bcl-6 (b); Apaf-1, caspase-9 and -3 (c), PARP, AIF and Endo G (c), GRP 78 and GADD153 (d); caspase-8, Fas and FasL, (e); nNOS, catalase, SOD (Cu/Zn) and SOD (Mn)</td>
<td>Apoptosis; G2/M cell cycle arrest</td>
<td>5–20μM (34–48h)</td>
<td>A175S2</td>
<td>[193]</td>
</tr>
<tr>
<td>Cancer Type</td>
<td>Mechanism</td>
<td>Molecular Targets</td>
<td>Outcome</td>
<td>Concentration and duration</td>
<td>Model used</td>
<td>Reference</td>
</tr>
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<tr>
<td>Multiple Myeloma (MM)</td>
<td>Protein suppression</td>
<td>AIF, XIAP, MCL1, Survivin, Hsp70, Hsp90, XIAP, XIAP, MCL1, Survivin, Hsp70, Hsp90, AIF, XIAP, MCL1, Survivin, Hsp70, Hsp90, AIF, XIAP, MCL1, Survivin, Hsp70, Hsp90,</td>
<td>Apoptosis; G2/M cell cycle arrest; tumor growth suppression</td>
<td>1.56–20μM (12, 24, 48h); 60mg/kg (34 days)</td>
<td>RPMI 8226-S; RPMI-Dox40; RPMI-MR20; RPMI-LR5; OPM-1; OPM-2; MM-1S; MM-1R cells; the non-transformed human liver epithelial cell line THLE-3; the human bone marrow stromal cell line HS-55; primary MM cells purified from bone marrow aspirates of MM patients or healthy donors</td>
<td>[122]</td>
</tr>
<tr>
<td>Myeloma</td>
<td>ROS; Proteasomal activity inhibition</td>
<td>p53, H2Aa</td>
<td>Proliferation inhibition</td>
<td>10, 20, 40μM (0–24h)</td>
<td>U266; RPMI-8226; A549</td>
<td>[127]</td>
</tr>
<tr>
<td>Oral</td>
<td>ROS</td>
<td>p53, p27, p21, CDK2, CyclinE, p15, p26, cyclin D, AIF, NFκB, GRP78</td>
<td>Proliferation inhibition; apoptosis</td>
<td>0.5, 1, 2, 2.5, and 5 μM (24-48h)</td>
<td>HSC-3</td>
<td>[121]</td>
</tr>
<tr>
<td>Oral squamous carcinoma</td>
<td>Protein suppression</td>
<td>EGF receptor signaling, MMP2, 9, Akt, ERK, PKD1, PKJ, Bcl0, p38</td>
<td>Invasion inhibition</td>
<td>1.2μM (24h)</td>
<td>OSCC S AS</td>
<td>[157]</td>
</tr>
<tr>
<td>Ovarian</td>
<td>Protein suppression</td>
<td>EGFR, Akt, mTOR, complex,</td>
<td>Apoptosis; tumor growth inhibition</td>
<td>2.5–40μM (24h); 12μmol/mouse</td>
<td>SKOV-3; OVCAR-3; TOV-21G</td>
<td>[117]</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>Protein suppression</td>
<td>Bcl-2 and Bcl-XL, upregulated the proapoptotic protein Bak, and suppressed Notch 1 and 2 levels</td>
<td>Apoptosis; G2/M cell cycle arrest; tumor growth delayed</td>
<td>2.5, 5, 10μM (24h); 12 μmol/day (1 wk pre-inoculation)</td>
<td>MIA PaCa-2, PL-45, B-PC3, MEA PaCa2 xenograft</td>
<td>[134]</td>
</tr>
<tr>
<td>Prostate</td>
<td>Protein suppression</td>
<td>ITGβ1, ITGα2, ITGα6</td>
<td>Proliferation inhibition, tumor growth inhibition</td>
<td>0.1, 1, 5μM (24h); 3 μmol/g diet (2 weeks)</td>
<td>LNCaP, xenograft</td>
<td>[179]</td>
</tr>
<tr>
<td>Prostate</td>
<td>Autophagy</td>
<td>p62, LC3</td>
<td>Apoptosis; Tumor growth suppression</td>
<td>3 μmol PEITC/g diet (19 weeks)</td>
<td>Mouse model</td>
<td>[148]</td>
</tr>
<tr>
<td>Prostate, head and neck</td>
<td>Protein suppression</td>
<td>TLR3, IFNα,</td>
<td>Inhibition of anchorage-independent growth and colony formation; tumor growth inhibition</td>
<td>2.5, 5, 10μM; 3μmol/g diet (19 weeks)</td>
<td>HED293; HED293 derivedTLR3 expressing stable (Wt11); RL24 cells; HT1080 cells; RAW264.7 cells; SV40 T</td>
<td>[163]</td>
</tr>
<tr>
<td>Cancer Type</td>
<td>Mechanism</td>
<td>Molecular Targets</td>
<td>Outcome</td>
<td>Concentration and duration</td>
<td>Model used</td>
<td>Reference</td>
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<tr>
<td>Sarcoma</td>
<td>ROS, DNA damage</td>
<td>Cyclin A, Cyclin B1, Chk1, p53, catalase, iNOS, Mn-SOD, AIF, cytochrome c, caspase 9 and 1</td>
<td>Apoptosis, G2/M cell cycle arrest</td>
<td>5, 7.5, 10, 15μM (12, 18, 24h)</td>
<td>antigen expressing FVB MEF; PCI15A, LNCaP; C57/B16 mice</td>
<td>[125]</td>
</tr>
</tbody>
</table>