Biochimica et Biophysica Acta 1846 (2014) 405–424

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbacan

Review

Phenethyl isothiocyanate: A comprehensive review of anti-cancer mechanisms

Parul Gupta [a], Stephen E. Wright [a,b], Sung-Hoon Kim [c,*], Sanjay K. Srivastava [a,c,**]

[a] Department of Biomedical Sciences and Cancer Biology Center, Texas Tech University Health Sciences Center, Amarillo, TX 79106, USA
[b] Department of Internal Medicine, Texas Tech University Health Sciences Center, Amarillo, TX 79106, USA
[c] Cancer Preventive Material Development Research Center, College of Korean Medicine, Department of Pathology, Kyunghee University, 1 Hoegi-dong, Dongdaemun-ku, Seoul 131-701, South Korea

ARTICLE INFO

Article history:
Received 14 April 2014
Received in revised form 11 August 2014
Accepted 13 August 2014
Available online 23 August 2014

Keywords:
Cruciferous vegetables
Isothiocyanates
PEITC
Reactive oxygen species
Chemoprevention

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.

Contents

Abbreviations: BaP, Benzo(a)pyrene; BCRP, Breast Cancer Resistance Protein; 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

* Corresponding author.
** Correspondence to: S. K. Srivastava, Department of Biomedical Sciences, Texas Tech University Health Sciences Center, Suite 1103 ARB, 1406 Coulter Drive, Amarillo, TX 79106, USA. Tel.: +1 806 414 9211; fax: +1 806 356 4770.
E-mail addresses: sungkim7@khu.ac.kr (S.-H. Kim), sanjay.srivastava@ttuhsc.edu (S.K. Srivastava).

http://dx.doi.org/10.1016/j.bbcan.2014.08.003
0304-419X/

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 people's 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].

Table 1
Epidemiological evidence for the anti-cancer effects of ITCs.

Cancer form	Subjects	Year conducted	Sample size	Geographical location	ITC quantitation method	Mechanism proposed	Outcome	Reference
Bladder cancer	Bladder cancer patients	1980–1998	239 cases	Roswell Park Cancer Institute, USA	Dietary ITC intake based on patient questionnaire	–	Improved survival	[5]
	Mixed population with mean ages 63.3 and 62.5 for cases and controls respectively	1999	697 cases & 708 controls	Texas, USA	Dietary cruciferous vegetables intake based on patient questionnaire	GSTM1 and GSTT1 null	Reduced cancer risk	[23]
	Cases with ages 25–86 years and controls with ages 21–92 years	1982–1998	275 cases & 825 controls	USA	Dietary cruciferous vegetables intake based on patient questionnaire	–	Reduced cancer risk	[24]
Breast cancer	Chinese women Age 20–70 years	1996–2005	3035 cases & 3037 controls	Shanghai, China	Dietary ITC intake based on patient questionnaire	GSTP1 polymorphism	Reduced cancer risk	[14]
	Breast cancer survivors	–	536 intervention & 620 comparison	USA	Dietary cruciferous vegetables intake based on patient questionnaire	–	Reduced cancer recurrence	[25]
	US and Chinese breast cancer survivors	1990–2006	11,390 subjects	USA and China	Dietary cruciferous vegetables intake based on patient questionnaire	–	No association with cancer mortality or recurrence	[27]
Colorectal cancer	Compiled from published literature	–	24 case control studies and 11 prospective studies	–	Dietary intake of cruciferous vegetables	–	Reduced cancer risk	[12]
Gastric cancer	Middle aged men; mean age ±65	1986–1989	307 cases and 911 control	Shanghai, China	Urinary ITC	GSTM1 and GSTT1 deletion enhanced protective effect	Reduced cancer incidence	[8]
Kidney cancer	Age 20–79 years	1999–2003	1097 cases & 1555 controls	Europe (multicenter)	Dietary ITC intake based on patient questionnaire	GSTM1 and GSTT1 polymorphism	Reduced cancer risk	[15]
	Non-Asians	1986–1994	1204 cases and 1204 controls	California, USA	Dietary cruciferous vegetables intake based on patient questionnaire	–	Reduced cancer risk	[26]
Lung cancer	African Americans & Caucasians; Age 40–84 years	1991–1994	311 cases & 922 control	Los Angeles, California	Dietary ITC intake based on patient questionnaire	GSTM1 deletion enhanced protective effect	Reduced cancer incidence	[9]
	>18 year adults	1990–2005	274 cases & 1089 control	Maryland, USA	Dietary ITC intake based on patient questionnaire	_	Reduced cancer incidence	[11]
	Chinese women Age 40–70 years	1997–2009	74,914 women	Shanghai, China	Dietary intake of cruciferous vegetables	–	Reduced cancer risk	[13]
	Men and women with average ages 62.3 and 60.9 years respectively	–	503 cases & 465 controls	Texas, USA	Dietary ITC intake based on patient questionnaire	GSTM1 and GSTT1 polymorphism	Reduced cancer risk	[16]
	Chinese women	1996–1998	233 cases & 187 controls	Singapore	Dietary ITC intake based on patient questionnaire	GSTM1 and GSTT1 polymorphism	Reduced cancer risk	[17]
	Chinese women	1996–1998	303 cases & 765 controls	Singapore	Dietary ITC intake based on patient questionnaire	–	Reduced cancer risk	[18]
	Hospital patients	1992–2000	716 cases and 939 controls	Massachusetts, USA	Dietary cruciferous vegetables intake based on patient questionnaire	GSTM1 presence	Reduced cancer risk	[21]
Prostate cancer	Men Age 40–75 years	1986–2000	47,365 subjects	USA	Dietary cruciferous vegetables intake based on patient questionnaire	–	Non-significant correlation with cancer risk	[20]
Stomach and colorectal cancer	Japanese population	–	149 cases & 287 controls for stomach cancer and 115 cases and 230 controls for colorectal cancer	Japan	Dietary cruciferous vegetables intake based on patient questionnaire	–	Reduced cancer risk	[19]
Thyroid cancer	Melanesian women	1993–1999	293 cases & 354 control	New Caledonia	Dietary ITC intake based on patient questionnaire	Low dietary intake of iodine	Increased risk of thyroid cancer	[10]
Urinary carcinoma	Scottish Terriers	–	92 cases & 83 controls	–	Dietary cruciferous vegetables intake based on owner questionnaire	–	Non-significant correlation with cancer risk	[22]

Table 2
Pharmacokinetic parameters of PEITC.

Route of administration	Dose	Vehicle	Model	Number of samples (n)	Organ	AUC ± SD or (SE)
Oral	10 μmol (50 μmol/kg) (single)	Corn oil	Male F344 rats	3/time point	Blood	328.1 nmol ml^{-1} h (106.93)
					Liver	1277.57 nmol ml^{-1} h (291.76)
					Lungs	346.321 nmol ml^{-1} h (6.51)
					Kidneys	987.941 nmol ml^{-1} h (114.88)
					Nasal mucosa	378.50 pmol mg^{-1} h(341.42)
					Esophagus	140.19 pmol mg^{-1} h (111.69)
					Stomach	1409.7 pmol mg^{-1} h (457.62)
					Small intestine	1691.21 pmol mg^{-1} h (292.94)
					Cecum	102422.29 pmol mg^{-1} h (44129.9)
					Colon	361.39 pmol mg^{-1} h (47.32)
					Pancreas	176.0 pmol mg^{-1} h (29.04)
					Spleen	57.71 pmol mg^{-1} h (30.55)
					Heart	94.35 pmol mg^{-1} h (22.99)
					Brain	53.71 pmol mg^{-1} h (5.4)
Oral (Gastric-intubation)	0.5, mg/kg(single)	DMSO	Male Wistar albino rats	4	Blood	1033 ± 174378 μg L^{-1} h
	1.0 mg/kg(single)			4		1646 ± 284 378 μg L^{-1} h
	5.0 mg/kg(single)			4		2438 ± 462 μg L^{-1} h
	0.5 mg/kg/day (4 days)			4		1117 ± 282 378 μg L^{-1} h
	1.0 mg/kg/day (4 days)			4		2311 ± 258 μg L^{-1} h
	5.0 mg/kg/day (4 days)			4		5796 ± 1004 μg L^{-1} h
Intravenous	0.5 mg/kg (single)	15% hydroxypropyl-β-cyclodextrin		4		1346 ± 378 μg L^{-1} h
Intravenous	2 μmol/kg	15% hydroxypropyl-β-cyclodextrin	Male Sprague Dawley rats	3–4	Blood	2.96 ± 0.78 μM h
	10 μmol/kg					17.3 ± 9.3
	100 μmol/kg					322.1 ± 149.6
	400 μmol/kg					807.5 ± 66.9
Oral	10 μmol/kg	–				19.89 ± 3.27
	100 μmol/kg					298 ± 139.4

Similarly, ingestion of 100 g of broccoli and watercress can release up to 200 μmol 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 and 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 ± 250 nM peak plasma concentration of PEITC was achieved in human subjects, after the consumption of 100 g watercress. The time to reach peak plasma concentration was observed to be 2.6 h ± 1.1 h with a $t_{1/2}$ 4.9 ± 1.1 h [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 (Tables 3A and 3B) [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-acetylcysteine (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

C_{max} ± SD or (SE)	T_{max} ± SD or (SE)	$T_{1/2a}$ ± SD or (SE)	$T_{1/2e}$ ± SD or (SE)	V_d ± SD or (SE)	F% ± SD or (SE)	Ref
18.77 pmol/μl (2.9)	2.94 h (0.78)	1.34 h (3.15)	α = 2.41 h (6.96) β = 21.7 h (25.77)	–	–	[182]
39.79 pmol/μl (3.29)	2.5 h (0.56)	0.44 h (0.14)	20.45 h (5.84)	–	–	
9.73 pmol/μl (0.39)	4.53 h (0.4)	0.97 h (0.14)	21.3 h (2.99)	–	–	
46.29 pmol/μl (2.06)	4.31 h (0.39)	1.18 h (0.2)	11.37 h (1.99)	–	–	
20.4 pmol/mg (7.31)	3.52 (0.66)	0.93 h (1.2)	10.10 (12.16)	–	–	
8.2 pmol/bg (2.14)	2.77 h (1.78)	0.67 h (0.69)	9.72 h (9.89)	–	–	
211.19 pmol/mg (26.44)	1.35 h (0.45)	0.37 h (0.24)	3.57 h (1.93)	–	–	
130.58 pmol/mg (7.64)	4.07 h (0.41)	1.69 h (0.64)	5.23 h (2.22)	–	–	
15.07 pmol/mg (1.78)	19.09 h (11.51)	465.87 h (2061.42)	2.52 h (1.07)	–	–	
16.43 pmol/mg (1.22)	8.08 h (1.15)	5.81 h (37.92)	5.4 h (35.71)	–	–	
9.73 pmol/mg (0.39)	4.53 h (0.4)	54.96 h (11.78)	0.97 h (0.14)	–	–	
9.64 pmol/mg (2.0)	1.2 h (0.71)	3.2 h (2.83)	0.33 h (0.37)	–	–	
5.71 pmol/mg (0.51)	4.36 h (0.74)	7.75 h (3.41)	1.48 h (0.61)	–	–	
2.07 pmol/mg (0.1)	6.46 h (0.57)	12.58 h (2.27)	2.07 h (0.41)	–	–	
318 ± 62 μg/L	0.73 ± 0.15 h	0.2 h (Ka = 3.52 ± 1.58)	2.62 ± 1.37 h	1.53 ± 0.81 L/kg	77 ± 13	[37]
387 ± 73 μg/L	0.45 ± 10 h	0.13 h (Ka = 5.19 ± 2.14)	2.78 ± 0.36 h	1.75 ± 0.29 L/kg	61 ± 11	
769 ± 107 μg/L	0.78 ± 0.21 h	0.18 h (Ka = 3.88 ± 0.40)	3.19 ± 0.37 h	2.15 ± 0.40	23 ± 5	
404 ± 80 μg/L	0.7 ± 0.24 h	0.16 h (Ka = 4.26 ± 2.31)	2.55 ± 0.26 h	1.5 ± 0.14 L/kg	83 ± 21	
647 ± 38 μg/L	0.90 ± 0.12 h	0.3 h (Ka = 2.26 ± 0.48)	1.86 ± 0.2 h	1.05 ± 0.10 L/kg	86 ± 10	
2139 ± 444 μg/L	0.95 ± 0.10 h	0.22 h (Ka = 3.02 ± 2.03)	1.62 ± 0.44 h	0.88 ± 0.28 L/kg	43 ± 7	
–	–	–	1.36 ± 0.36 h	0.75 ± 0.06 L/kg	100	
–	–	–	3.52 ± 0.35 h	1.94 ± 0.42 L/kg	100	[36]
–	–	–	6.92 ± 3.73 h	3.27 ± 2.06	100	
–	–	–	9.19 ± 0.83 h	2.66 ± 1.22	100	
–	–	–	13.1 ± 2.0	5.72 ± 1.10	100	
9.2 ± 0.6 μM	0.44 ± 0.1 h	–	–	–	115	
42 ± 11.4	2 ± 1	–	–	–	93	

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 pre-initiation 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

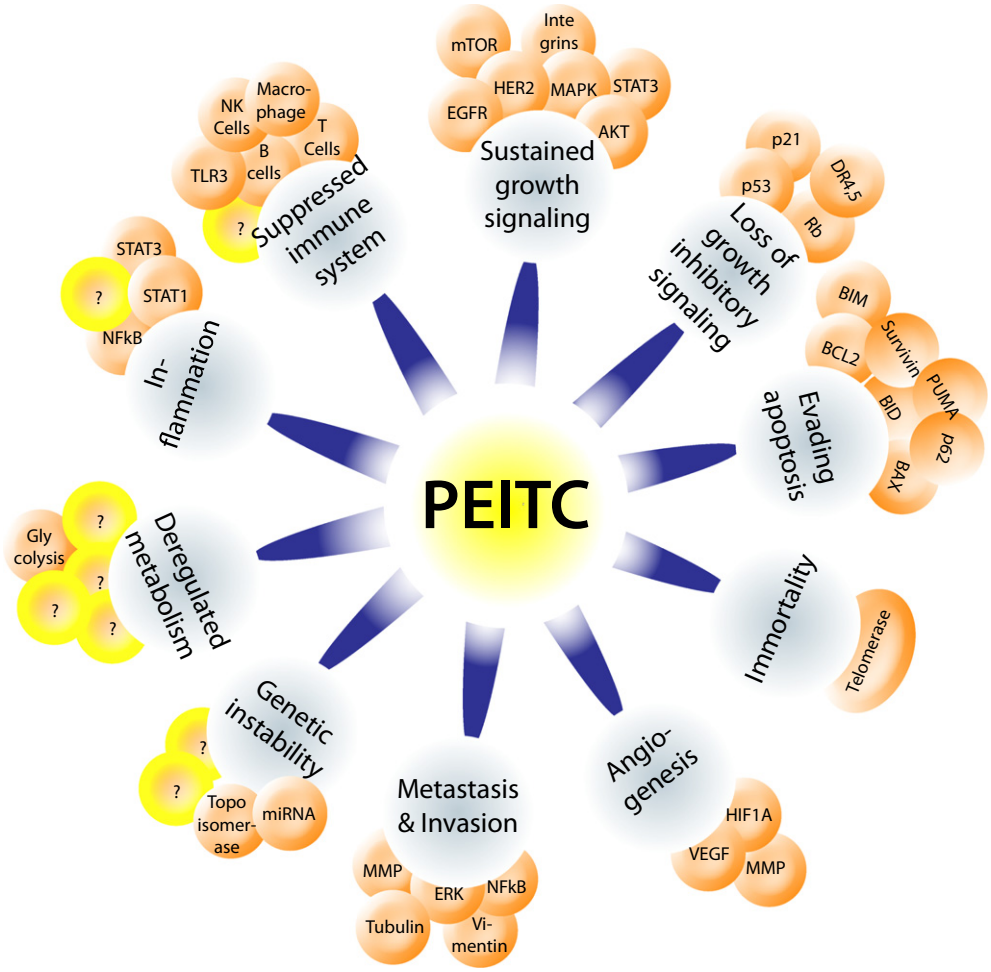

Fig. 1. PEITC and hallmarks of cancer.

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 Nrf2-antioxidant response element by a combination of ITC and phyto-indoles [93]. Although in general, ITCs modulate phase II enzymes, human livers demonstrated a non-identical 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 (Fig. 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

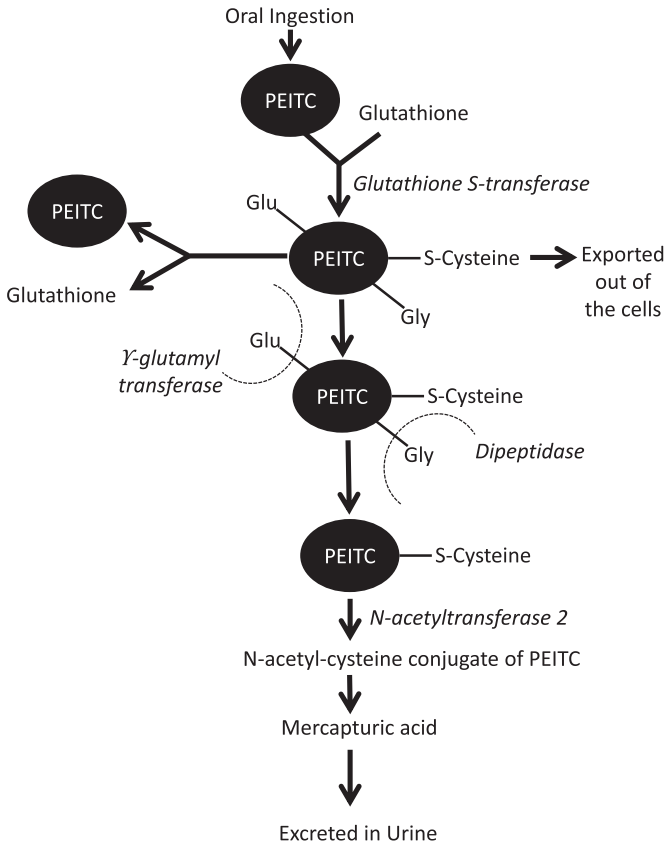

Fig. 2. General metabolic pathway for PEITC.

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/MEK-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 adriyamycin 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

Table 3A
Epidemiologic evidence for the effects of GST polymorphism on ITC uptake/metabolism and cancer risk.

Cancer	Design	Population	Location	Method of analysis	ITC intake/ levels	GST studied	Status	Outcomes	Statistics	Reference
Adenoma	Case control (459 cases; 507 control)	Mixed	USA	Dietary intake	High	GSTM1	Null	Reduced risk	P = 0.001 for trend and 0.1 for interaction	[42]
Breast	Case control (740 cases; 810 control)	Caucasian women	USA	Dietary intake	High	GSTM1/ T1	Null/positive	Reduced risk	OR = 0.6 (0.4–1.01) Premenopausal women OR = 1.0 (0.7–1.4) Postmenopausal women	[44]
	Case control (1052 cases and 1098 control)	Women (<65 years age)	USA	Dietary intake	–	GSTT1	Null	Increased risk	OR = 1.86 (1.12–3.08)	[46]
					–	GSTM1	Null	Increased risk	–	
					–	GSTP	Ile/Ile	Increased risk		
					High/Low	GSTT1/ M1/P	Null/Positive	No correlation		
Colon	Case control (213 cases; 1194 control)	Chinese men and women	Singapore	Dietary intake	High	GSTM1/ T1	Null	Reduced risk	OR = 0.43 (0.2–0.96)	[43]
Colorectal	Case control (173 cases and 313 controls)	Mixed	USA	Urinary ITC	Detectable	GSTT1	Null/Positive	No correlation	–	[48]
					Detectable	GSTM1	Null/Positive	No correlation	–	
					Detectable	GSTP1	AG or GG	Marginally reduced risk	P = 0.09	
	Case control (322 cases and 1251 controls)	Women 40–70 years	China	Urinary ITC	High	GSTT1	Null	Reduced risk	P = 0.04	[50]
					High	GSTM1	Null	Reduced risk	P = 0.07	
					High	GSTT1/ M1	Null	Reduced risk	OR = 0.51 (0.27–0.95)	
Kidney	Case control (1097 cases and 1555 control)	Mixed	Europe	Dietary intake	Low	GSTT1	Null	Increased risk	OR = 1.86 (1.07–3.23)	[15]
					Low	GSTM1/ T1	Null	Increased risk	OR = 2.49 (1.08–5.77)	
Lung	Case control (503 cases; 465 control)	Mixed subjects from Houston	USA	Dietary intake	Low	GSTM1	Null/Current smoker	Increased risk in	OR = 2.22 (1.2–4.1)	[16]
					Low	GSTT1	Null/Current smoker	Increased risk	OR = 3.19 (1.54–6.62)	
					Low	GSTM1 and T1	Null/Current smoker	Increased risk	OR = 5.45 (1.72–17.22)	
					Low	GSTM1	Null/Former smoker	No correlation	OR = 1.14 (0.66–1.96)	
					Low	GSTT1	Null/Former smoker	Increased risk	OR = 1.79 (0.95–3.37)	

	Case control (233 cases; 187 control)	Chinese women	–	Dietary intake	High	GSTM1/T1	Null	Reduced risk	OR = 0.54 (0.3–0.95)	[17]
					High	GSTM1	Positive	No effect	1.07 (0.5–2.29)	
	Case control (232 cases; 710 control)	Chinese men	China	Dietary intake and urinary ITC analysis			Detectable	GSTM1	Null	Reduced risk
					Relative risk = 0.36 (0.2–.63)	[41]				
	Detectable	GSTM1/T1	Null	Reduced risk	Relative risk = 0.28 (0.13–0.57)					
	Case control (716 cases; 939 control)	Caucasian women and men	USA	Dietary intake	High	GSTM1	Positive	Reduced risk	OR = 0.61 (0.39–0.95)	[21]
					High	GSTM1	Null	No correlation	OR = 1.15 (0.78–1.68)	
					High	GSTM1	Null/positive	No correlation	–	
					High	GSTM1/T1	Null/positive	No correlation	–	
Literature review	30 studies	–	Dietary intake	High	GSTT1/M1	Null	Reduced risk	OR = 0.41 (0.26–0.65)	[47]	
–	Cohort	Chinese (45–74 years age) 111 men, 135 women	Singapore	Dietary intake and urinary ITC analysis	–	GSTM1	Null	No correlation	P = 0.61	[40]
					–	GSTM1	Positive	No correlation		
					–	GSTT1	Null	Low excretion	P = 0.006	
					–	GSTT1	Positive	High excretion		
					–	GSTP1	a/a	No correlation	P = 0.77	
					–	GSTP1	a/b	No correlation		
					–	GSTP1	b/b	No correlation		
	Cohort (114 subjects) 18–50 years	Mixed	USA	Urinary ITC and metabolites	Single intake	GSTM1	Null	High excretion in 62% subjects	ITC (mol/24 h) = 9.9 ± 1.4	[45]
						GSTM1	Positive	High excretion in 39% subjects	ITC (mol/24 h) = 14.0 ± 1.3 (P = 0.48)	
						GSTT1	Null	Marginally significant	ITC (mol/24 h) = 9.9 ± 2.7	
						GSTM1	Positive	Marginally significant	ITC (mol/24 h) = 11.6 ± 0.9 (P = 0.05)	
						GSTP1	A/A	Non significant	ITC (mol/24 h) = 9.9 ± 1.5	
						GSTP1	G/A	Non significant	ITC (mol/24 h) = 14.8 ± 1.4	
	Cross-over intervention study	20 healthy subjects	–	Plasma ITC	–	GSTM1	Null/Positive	No correlation	–	[49]
					–	GSTT1	Null/Positive	No correlation	–	
	48 subjects, 28 GSTT1 and M1 positive and 20 null genotypes	Healthy volunteers	–	Urinary ITC	Watercress juice	GSTT1/M1	Null/Positive	No correlation	–	[51]

Table 3B
Pre-clinical evidence for effect of GST polymorphism on isothiocyanate efficacy.

Model	Agent used	GST polymorph	Outcome	Reference
Male Wistar rats	BITC	Subunits 3 & 2	Increased	[52]
Rat T9 glioma cells	AITC, BITC	GSTP	Increased marginally	[53]
	AITC, BITC + dibutyryl cAMP	GSTP	Increased	
Male ACI/N rats	BITC, BTC	GSTP	Reduced	[54]
Kinetic study	ITC	GSTP1-1 and M1-1	Most efficient catalysis	[55]
		GATM4-4	Least efficient catalysis	
Kinetic study	PEITC, BITC	GSTA1, A2, P1	Catalyze forward and reverse reactions in a concentration dependent manner	[56]
Male Wistar rats	PEITC	GSTA	Increased in liver	[57]
		GSTM	Increased in stomach and liver	
		GSTP	Increased on colon	
Male F344 rats	PEITC	GSTP	Reduced in liver (Pre-initiation period of tumors) with reduced hyperplasia in esophagus and kidney Enhanced in liver (Post-initiation period of tumors) with increased urinary bladder tumors	[58]
RL34 cells (rat liver epithelial)	BITC, PEITC	GSTP1	Increased	[59]
RL34 cells	ITC	GSTA1/2 GSTA3 GSTM1	Nrf2 dependent increase	[60]
PBMC cells	Watercress/PEITC	GSTM1-0	Increased GPX and SOD	[61]
		GSTM1-1	Not increased GPX and SOD	
		GSTT1	No impact	
HL60 cells	ITC	GSTA (Overexpression)	Reduced efficacy	[62]
Male Wistar rats	ITC	GSTM	Increased in liver	[63]
		GSTA	Increased in Liver	
		GSTT	Increased in kidney	

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 10 μmol 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 *in vitro* and *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 development. Further detailed studies are required to elucidate the complete effects of PEITC on immune system.

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 (5 μM) 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 chemopreventive 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 *in vitro* study using prostate cancer cells. However, no studies have yet been performed to confirm these effects *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_2O_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 *in vitro* study can vary significantly from an *in vivo* study with the same agent [173]. Thus, outcomes based on the *in vitro* studies may be debatable. Hence, it is crucial to confirm ROS generation by PEITC and its manifestations in an *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].

Table 4
Molecular targets and major mechanism of action of PEITC in various cancer types.

Cancer type	Mechanism	Molecular Targets	Outcome	Concentration and duration	Model used	Reference
–	Epigenetic	CYP2E1	Reduced activation of carcinogens	25, 50 μM	*Escherichia coli* MV1304 (cDNA CYP2E1)	[89]
–	Thiol modification	Topoisomerase IIα	Apoptosis	2.5, 5, 10 μM (6 h)	*Top2*β$^{+/+}$; *top2*β$^{-/-}$ primary MEFs	[175]
Breast	Protein suppression	BIM, PUMA, BCL2, BCL-xl, BAX, Bak,	Apoptosis	2.5, 5 μM (24 h)	BRI-JM04; MDA-MB-231; MCF-7; HCT-116; HCT-116 (PUMA$^{-/-}$)	[136]
	Synergism with paclitaxel	–	Apoptosis; G2/M cell cycle arrest	1–10 μM (24, 48 h)	MCF7; MDA-MB-231	[190]
	Protein suppression	HSP90, HSF1, Cyclin B1, CDK1, Cdc25C, PLK-1, and p21, caspase 3, 9	Apoptosis; G2/M cell cycle arrest	0.1, 0.5, 2.5, 5 μM (24 h)	MCF7; MDA-MB-231	[192]
	Protein suppression	BCL2, BAX, NRF2, GSH, caspase 3, 8 and 9	Apoptosis; G2/M cell cycle arrest	20 μM (2, 4, 6, 24, 48 h)	MCF7; BT549; MDA-MB-231; ZR-75-1; SKBR3; T47D	[171]
	Protein suppression	p70S6K, Akt, NDRG1, HSP70, ERK, AMPK, 4E-BP1, mTORC1	Proliferation inhibition	0–20 μM (3,4 h)	MCF-7; PTEN deficient MEF; TSC2 deficient MEF	[194]
	Chemoprevention	–	Reduced tumor incidence and increased tumor free survival	50 μmol/kg or 150 μmol/kg every 2 days (pre-tumor initiation); PEITC continued (18 weeks)	*NMU induced breast cancer in Sprague Dalwey rats*	[85]
	Chemoprevention	–	Angiogenesis inhibition and tumor growth inhibtion	3 μmol/g diet (29 weeks)	MMTV-neu mice	[86]
	Epigenetic changes	PARP-1, BCL-2, Bax, Cdk-1, Cyclin B1, α-tubulin, β-tubulin, acetyl-α-tubulin	Increase in Taxol (10, 100 nM) sensitivity	5, 10 μM (24 h)	MCF-7; MDA-MB-231	[191]
	Genetic expression	P57, CYP19, BRCA2, IL2, ATF2, p53, Hsp27	–	3 μM (48 h)	MCF-7	[123]
	ROS	HER2, EGFR, Akt, STAT3, BIM, BAX, BCL-XL, XIAP,	Proliferation inhibition; apoptosis; reduced tumor growth	5, 10, 15 μM (24 h)	MCF-7; MCF-7 high HER2; MDA-MB-231; MDA-MB-231 high HER2; MDA-MB-231 high HER2 xenograft in athymic nude mice	[102]
Breast and prostate	Protein suppression	Vimentin	Migration inhibition; apoptosis; tumor growth inhibition	2.5, 5 μM (6, 12, 24 h); 3 μmol PEITC/g diet (29 weeks)	MDA-MB-231; PC-3; DU145; MMTV-neu mice	[156]
Cervical	Protein suppression	DR4, DR5, HO-1, cytochrome c, clusterin, claspin, cIAP1, cIAP2, catalase, Bcl2, Bcl-xl, BAX, BAD, Bid, ERK, JNK, MEK	Apoptosis	2.5, 5, 10 μM (48 h)	*HEp-2*; *KB*	[141]
Cervical, Breast	Synergism with cisplatin	MAPKs, MEK1/2, NFkB, Bcl2, Bcl-xl, BAX, NOXA, PUMA, DR4, DR5, TRAIL	Apoptosis	5 μM (2, 4, 8, 12, 24 h)	HeLa; C33A; MCF-7	[200]
Cholangiocarcinoma	Increase of free calcium level in cytosol	Cyt c, BCL-XL, BAX, AIF	Apoptosis	1, 3, 10 μM (3, 6 h)	KKU-M214; Chang cells (reference)	[133]
	ROS	Cyt c, BCL2, caspases 3, 8, 9, AIF	Apoptosis	3, 10 μM (3, 6, 24 h)	KKU-100	[135]
Colon	ROS	DDB2	Tumor regression	10 μM (0.5, 1, 2, 4, 6, 12 h)	HCT116; HCT116 expressing control shRNA or DDB2 shRNA xenograft	[139]
Colon and blood	Epigenetic (Histone modification and genetic modulations)	NFkB, STAT1, chemokines, MMPs	Proliferation inhibition	2.5, 5, 10, 15 μM (5, 8, 12, 18 h)	SW480; HT-29; THP-1	[189]
Gastric	ROS; Rhodamine-123; Cisplatin resistance reversal	ERCC1, survivin, and Mad2, Akt, NfkB, Glutathione, PGP, MRP1	Apoptosis; cell cycle arrsest, Cisplatin resistance reversal	1, 5 μM (24 h)	SGC7901/DDP	[201]
Glioma	ROS; Sensitization to TRAIL	DR5, p53, caspase 3, 8 and 9	Apoptosis	2.5 μM (24 h)	U87MG; A172; U343; T98G	[199]
Glioma, prostate, colon, liver, breast	Sensitivity to hypoxia	HIF1α, VEGF, ERK, Akt, MMP2, MMP9, uPA,	Apoptosis	1, 2.5, 5 μM (8, 16 h)	U87; DU145; HCT116; HepG2; SKBR3	[154]

Leukemia	Protein suppression	Akt, JNK, XIAP, MCl1, BCL2, BCL-XL, BAD, BAX	Apoptosis; tumor growth suppression	2, 4, 6, 8 μM (0–24 h); 50 mg/kg, i.p. (20 days)	U937; HL-60; Jurkat cells	[116]
	Immune-modulation	CD3, CD19, Mac-3	Enhanced phagocytic activity of macrophages; Increased NK cell activity; Enhanced T cell proliferation	80 & 160 mg/kg (2 weeks)	*WEHI-3*; *BALB/c mice*	[162]
	ROS, nitric oxide	Mitochondrial complexes, peroxiredoxin III (Prx III), superoxide dismutase-2 (SOD-2), Bcl-2, Glutathione peroxidase 4, NDUSF3	Apoptosis	10 μM (1, 3, 6, 12 h)	HL-60; Raji	[169]
Lung	Epigenetic (Covalent protein binding)	Actin, Tubulin, Tropomyosin, HSP's, Vimentin, ADAM7, thioredoxin etc.	–	20 μM (1 h)	A549	[114]
	Cytoskeletal changes	F-actin, α-tubulin,	Apoptosis; G2/M cell cycle arrest	1–10 μM (24 & 72 h)	A549; H1299	[126]
	Epigenetic	Tubulin	Cell growth inhibition and apoptosis	5, 10, 20 μM (4 h)	A549	[176]
	Genetic expression	AP1, JNK, p38 MAPK, p44/42 MAPK, cyclin B1	Apoptosis; G2/M cell cycle arrest	12.5, 20 μM (24 h)	L9981	[188]
	Post translational modification	p53 (mutant)	Apoptosis	15, 20 μM (0–24 h)	H596; MDA-MB-231;	[195]
Lung, colon, ovarian	ROS; Sensitization of platinum resistant cells	GSH	Reduced cell survival	5, 7.5 μM (6, 12, 24 h); 25 mg/kg (bid, once a week–3 weeks)	*A549*; *A549/CDDP*; THC8307; THC8307/L-OHP; HCT116; A2780; A549/CDDP xenografts in BALB/c nude mice	[155]
Myeloid leukemia	ROS	Fas, Fas ligand, caspase 9 and 3	Apoptosis	5, 10, 15, 20 μM (24, 48, 72 h)	K562	[143]
Melanoma	ROS, ER stress	CDC25C, CDK1, Chk1, Chk2, cyclin A, Wee1 p53 and p21 (a); Bax, Bcl2 and Bid (b); Apaf-1, caspase-9 and -3, PARP, AIF and Endo G (c); GRP 78 and GADD153 (d); caspase-8, Fas and FasL (e); i-NOS, catalase, SOD (Cu/Zn) and SOD (Mn)	Apoptosis; G2/M cell cycle arrest	5–20 μM (24–48 h)	A375.S2	[193]
Multiple Myeloma (MM)	Protein suppression	AIF, XIAP, MCL1, Survivin, Hsp70 Hsp90,	Apoptosis; G2/M cell cycle arrest; tumor growth suppression	1.56–20 μM (12, 24, 48 h); 60 mg/kg (34 days)	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	[122]
Myeloma	ROS; Proteasomal activity inhibition	p53, IkBα	Proliferation inhibition	10, 20, 40 μM (0–24 h)	U266; RPMI-8226; A549	[127]
Oral	Protein suppression	DR5, caspase 8, BID, p38	Proliferation inhibition; apoptosis	7.5, 15, 20 μM (48 h)	HN22; HSC-2; HSC-4; YD-15; Mc-3	[140]
	ROS	p53, p27, p21, CDK2, Cyclin E, p15, Cdc25a, CDK6, Cyclin D, AIF, NFkB, GRP78	Apoptosis; G0/G1 cell cycle arrest	0.5, 1, 2, 2.5, and 5 μM (24, 48 h)	HSC-3	[121]
Oral squamous carcinoma	Protein suppression	EGF receptor signaling, MMP2, 9, Akt, ERK, PDK1, PI3K, IkBα, JNK, p38	Invasion inhibition	1, 2 μM (24 h)	OSCC SAS	[157]
Ovarian	Protein suppression	EGFR, Akt, mTOR, complex,	Apoptosis; tumor growth inhibition	2.5–40 μM (24 h); 12 μmol/mouse	SKOV-3; OVCAR-3; TOV-21G	[117]
	ROS; combination effect with metformin	–	Proliferation inhibition	5 μM (24 h)	OVCAR3; CAOV3; SKOV3; PA-1; A2780; A2780cis	[197]
Pancreatic	Protein suppression	Bcl-2 and Bcl-XL, upregulated the proapoptotic protein Bak, and suppressed Notch 1 and 2 levels	Apoptosis; G2/M cell cycle arrest; tumor growth delayed	2.5, 5, 10 μM (24 h); 12 μmol/day (5 days/wk–1 wk pre-inoculation)	MIAPaca2; PL-45; BxPC3; MIAPaca2 xenograft	[134]
	ROS, miRNA	miR-27a/miR-20a:miR-17-5p, Sp1, Sp3,Sp4, CD1, VEGF, c-Myc	Apoptosis	10, 20 μM (24 h)	Panc28; L3.6PL; Panc1; SW480; RKO;	[184]
Prostate	Protein suppression	ITGB1, ITGA2, ITGA6	Proliferation inhibition; tumor growth inhibition	0.1, 1, 5 μM (24 h); 3 μmol/g diet (2 weeks)	LNCaP; xenograft	[179]
	Protein suppression	P53, Wee1, Cdc25c, caspases 3, 8, 9, FAS, AIF, cytochrome c, Bcl2, BAK, BID, GRP78, GADD153	Apoptosis; G2/M cell cycle arrest	10 μM (6, 12, 24, 48 h)	DU 145	[124]

(continued on next page)

Table 4 *(continued)*

Cancer type	Mechanism	Molecular Targets	Outcome	Concentration and duration	Model used	Reference
	Autophagy	p62, LC3	Apoptosis; Tumor growth suppression	3 μmol PEITC/g diet (19 weeks)	Mouse model	[148]
	DNA Fragmentation	XIAP, Survivin	Proliferation inhibition and apoptosis	0–5 μM (6, 12, 24 h)	PC-3; LNCaP	[144]
	miRNA and transcriptional activity	Androgen receptor, miR-17, PCAF	Proliferation inhibition	10–20 μM (24–48 h)	LNCaP; PC-3; DU145; C4–2B PCa; ALVA31	[183]
	Mitchondrial structure, autophagy	Mitochondria, β-tubulin	Apoptosis	8 μM (4–18 h)	LNCaP	[146]
	Gene expression	Insulin-like growth factor binding protein 3, fibronectin, thyroxine degradation enzyme, and integrin β6	Tumor growth suppression	3 μmol/g diet (7 weeks)	LNCaP	[161]
Prostate, head and neck	Protein suppression	TLR3, IRF3,	Inhibition of anchorage independent growth and colony formation; tumor growth inhibition	2.5, 5, 10 μM; 3 μmol/g diet (19 weeks)	HEK293; HEK293 derived TLR3 expressing stable cells (Wt11); RL24 cells; HT1080 cells; RAW264.7 cells; SV40 T antigen expressing FVB MEF; PCI15A; LNCaP; C57/BL6 mice	[163]
Sarcoma	ROS, DNA damage	Cyclin A, Cyclin B1, Chk1, p53, catalase, iNOS, Mn-SOD, AIF, cytochrome c, caspase 9 and 3	Apoptosis; G2/M cell cycle arrest	5, 7.5, 10, 15 μM (12, 18, 24 h)	U-2 OS	[125]

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 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]. Jutooru 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 Section 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. 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. 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 benzoypyrene (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. 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 200 mg PEITC by human volunteers, 10 μM 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 10 μM. 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. 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.

Acknowledgements

The work was supported in part by R01 grants CA106953 and CA129038 (to S.K.S.) awarded by the National Cancer Institute, NIH and MRC grant 2007-0054931 (to S.H.K.). Dr. Sanjay K. Srivastava is currently an International Scholar at Kyung Hee University, Seoul, South Korea.

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