

Invited review

Predicting the physiological relevance of *in vitro* cancer preventive activities of phytochemicals¹

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Abstract

There is growing interest in the ability of phytochemicals to prevent chronic diseases, such as cancer and heart disease. However, some of these agents have poor bioavailability and many of the in-depth studies into their mechanisms of action have been carried out *in vitro* using doses which are unachievable in humans. In order to optimize the design of chemopreventive treatment, it is important to determine which of the many reported mechanisms of action are clinically relevant. In this review we consider the physiologically achievable doses for a few of the best studied agents (indole-3-carbinol, diindolylmethane, curcumin, epigallocatechin-3-gallate and resveratrol) and summarize the data derived from studies using these low concentrations in cell culture. We then cite examples of *in vitro* effects which have been observed *in vivo*. Finally, the ability of agent combinations to act synergistically or antagonistically is considered. We conclude that each of the compounds shows an encouraging range of activities *in vitro* at concentrations which are likely to be physiologically relevant. There are also many examples of *in vivo* studies which validate *in vitro* observations. An important consideration is that combinations of agents can result in significant activity at concentrations where any single agent is inactive. Thus, for each of the compounds reviewed here, *in vitro* studies have provided useful insights into their mechanisms of action in humans. However, data are lacking on the full range of activities at low doses *in vitro* and the benefits or otherwise of combinations *in vivo*.

Introduction

In recent years there has been increasing interest in the use of biologically active phytochemicals in cancer prevention. In particular, many *in vitro* studies using a wide range of natural products have demonstrated a preferential induction of cell cycle arrest or apoptosis in tumor cell lines compared to lines derived from non-tumor tissue. On further investigation, phytochemicals have been found to modulate the expression or activity of a large number of cellular proteins which are key for cell survival and the transformed phenotype. However, there is also much concern that many of these effects^[1–3] are irrelevant *in vivo*, since the concentrations used are often orders of magnitude greater than

appear to be achievable in the human body.

In this review, we have attempted to address this concern for a few of the most studied diet-derived compounds. For each agent, we have assimilated reported *in vivo* concentrations, based where possible on human data. We have then surveyed the *in vitro* biological effects at these or lower doses. For the indoles only, we have included data on phase I drug metabolizing activity relating to altered estrogen metabolism. Encouragingly, a significant amount of published data validates some of these changes *in vivo*.

For a number of reasons, some guesswork was involved in deciding on the most relevant *in vitro* doses for consideration. First, adding a compound directly to a cell culture may deliver a much higher local dose than occurs following

ingestion in the body. Second, some if not all of the compounds may undergo metabolism *in vivo* to other more or less active derivatives and such metabolism may not be possible in culture. Third, higher *in vivo* doses than those so far reported may be achievable by administration of a pure compound rather than a dietary source, or an optimized formulation of a pure compound. Fourth, some target tissues may receive a higher (or more prolonged) dose than the reported peak levels in plasma. Fifth, some target tissues, such as skin, oral cavity, gastrointestinal tract, and bladder, may receive higher doses because they are not dependent on circulating levels. The colon, for example, can be exposed to significant amounts of (unabsorbed) excreted material and the lining of the bladder may be exposed for substantial periods of time to any compounds concentrated in urine. Finally, where diet is concerned, any one compound may be poorly bioavailable, but with dozens, even hundreds, of active molecules being ingested together, the cumulative dose of similar acting compounds may be significantly higher.

It should not be forgotten that some natural products have exhibited toxicity *in vivo* when given in high doses, so there is also an argument for the use of higher concentrations *in vitro* to indicate the full range of activity of these molecules.

Indole-3-carbinol and diindolylmethane

Indole-3-carbinol (I3C) is derived from glucobrassicin, found in cruciferous vegetables. Diindolylmethane (DIM) is an acid condensation product formed from 2 molecules of I3C. *In vivo*, this is thought to occur in the acid conditions of the stomach. Data for mammals only are considered here.

Bioavailability of I3C and DIM in humans Information on the bioavailability and tissue distribution of I3C or DIM in humans is very limited^[4-6]. However, there are a number of studies in which oral administration of I3C resulted in a biochemically or clinically measurable outcome, indicating that the absorption of I3C and/or its acid-condensation products does occur. Administered doses of I3C have ranged

from 200 to 500 mg/d (~ 6–7 mg/kg), typically for periods of 1–6 months, although treatments up to 82 months have also been reported^[7-14]. A dose-dependent effect (placebo, 200 and 400 mg/d for 3 months) was observed in the treatment of cervical intraepithelial neoplasia^[7], and 200 or 400 mg/d were similarly effective against vulval intraepithelial neoplasia^[14]. In dose-escalation studies for breast cancer prevention, 300 mg/d (minimum) increased the urinary estrogen metabolite ratio of 2-hydroxyestrone to 16 alpha-hydroxyestrone; 800 mg/d did not provide additional benefits over 400 mg/d in adult women^[15,16]. Elevated cytochrome P450 activity was responsible for an increase in 2-hydroxylation of estrogen, increasing the ratio of 2-OH:16-OH estrone^[16], which is regarded as favorable for the prevention of breast cancer and human papilloma virus (HPV)-related neoplasias^[7-14].

Two studies detected DIM in plasma (2.5 µmol/L maximum at 2 h, gradually decreasing by 12 h) or in urine following oral administration of I3C (Table 1). I3C was not detected in plasma or serum following oral doses of 400–1200 mg^[4,5]. DIM was also detected in the urine of a patient receiving DIM^[4]. In a pilot study using a formulation of enhanced absorption DIM (BioResponse DIM, 108 mg/d for 30 d), increased 2-hydroxylation of estrogen was also reported^[6].

Bioavailability of I3C and DIM in animals Radio-labeled I3C was used to follow distribution and tissue content in several studies, although this method did not differentiate between I3C and related products. In rats receiving 50 mg ¹⁴C-I3C by gavage, I3C equivalents peaked at 28 µmol/L in the blood and 121 µmol/L in the liver after 30 min. The labeled product was detectable in the 100 µmol/L range from 10 min to 2 h following dosing^[17]. High maximal concentrations of the I3C equivalent, but not I3C itself, were detected in the liver (1154 µmol/L), kidney, lung (436 µmol/L), blood (320 µmol/L), and tongue of the rats given ³H-I3C in the diet for 1 week (0.88±0.074 mmol/kg/d)^[18]. Once a steady state had been reached, excretion in feces and urine accounted for 75% dose/d, the majority of this being present in the feces by 110 h, indicating that either the dose was not absorbed or that a major excretory route was via bile. When ¹⁴C-I3C was

Table 1. Bioavailability of DIM in humans following oral administration of I3C.

I3C dose mg/day	Peak time point (h)	DIM plasma µg/mL (µmol/L)	DIM urine µg/mg creatinine	Reference
200			12.1	4
400			15.6	
400–1200	2 (1 g)–3 (400 mg)	0.3 (400 mg)–2.5 (1 g)		5
400 chronic	1.3	0.3		

given to pregnant mice, it was detected in the fetal liver, stomach, kidney, intestine, and lung (100–300 $\mu\text{mol/L}$) after 8 h of maternal exposure^[19].

Anderton *et al* detected I3C and DIM in tissues following the dosing of mice with 250 mg/kg I3C (Table 2) using an HPLC method allowing the simultaneous identification and quantification of I3C and its derivatives^[20]. The maximum level of 28 $\mu\text{mol/L}$ I3C was observed at 15 min, falling below the level of detection by 1 h after dosing. I3C was detected in the liver (170 $\mu\text{mol/L}$)>kidney (116 $\mu\text{mol/L}$)>lung and heart>plasma>brain. The levels of DIM peaked at around 2 h in the liver (16 $\mu\text{mol/L}$)>lung and kidney>heart>brain>plasma (4 $\mu\text{mol/L}$), and by 24 h, were still detectable at approximately 0.5 $\mu\text{g/g}$ in the brain and liver. The presence of linear trimer, 1-(3-hydroxymethyl)-indolyl-3-indolylmethane and indolo(3, 2b)carbazole, together with oxidative metabolites of I3C, was also documented. In a further study, Anderton *et al* compared concentrations of DIM in tissues of mice dosed with either pure DIM (250 mg/kg; Table 2) or an equivalent dose of the enhanced absorption BioResponse DIM^[21]. The tissue distribution of DIM was similar to that reported previously following the administration of I3C with maximal concentrations around 160 $\mu\text{mol/L}$ in the liver^[20]. The BioResponse DIM resulted in levels approximately 50% higher than those obtained with unformulated DIM.

Thus, following the oral administration of I3C, both I3C and DIM were detectable at $\mu\text{mol/L}$ concentrations in the blood and multiple organs. I3C was rapidly absorbed and cleared from the blood and tissues within 1 h, while DIM peaked slightly later and was more persistent. The observation of I3C in the blood and tissues at these very early time points belies previous assumptions that I3C is not absorbed, but undergoes complete acid condensation in the stomach. Several studies have revealed distinct responses to I3C and DIM in animal models^[22–26]. Therefore, the *in vivo* activity of dietary I3C cannot be attributed completely to the production of DIM, although response due partially to DIM conversion is probable.

Physiologically relevant concentrations of I3C and DIM

As no data are available for achievable levels of I3C in

humans, we extrapolated from animal studies^[20,21]. The maximum plasma and tissue concentrations attained in mice for I3C were 28 and 170 $\mu\text{mol/L}$ (15 min) and for DIM, 4 and 16 $\mu\text{mol/L}$ (2 h), respectively (Table 2). By allometric scaling, the I3C dose given to mice would equate to a 20 mg/kg dose in humans (1200 mg/d), which yielded the maximal serum concentration 2.5 $\mu\text{mol/L}$ DIM (2 h), with no I3C detectable after 1 h^[5]. Therefore, maximal detectable DIM concentrations following I3C administration are similar in mice and humans, and the discrepancy in I3C detection is likely to be caused by the sensitivity of methods and selection of time points.

The maximum levels of DIM achieved in animals following a dose of a pure compound range from 24–200 $\mu\text{mol/L}$ (Table 2), with BioResponse DIM resulting in 50% higher bioavailability. The dose of BioResponse DIM used in humans was 108 mg (~1.3–1.9 mg/kg)^[6], which might be expected to give levels in the range 3–30 $\mu\text{mol/L}$.

For the purposes of this review, the effects of the physiological concentrations up to 150 $\mu\text{mol/L}$ I3C and 50 $\mu\text{mol/L}$ DIM *in vitro* have been considered.

***In vitro* mechanistic studies using low doses of I3C or DIM** Several mechanisms are responsible for the chemopreventive activities of I3C and DIM, as summarized in Tables 3 and 4. Both agents induce activity of phase I and II enzymes involved in the biotransformation and elimination of carcinogens and steroid hormones. While detailed molecular interactions involved have not been completely elucidated, DIM interacts with the aryl hydrocarbon receptor (AhR), resulting in its nuclear translocation and induction of the genes encoding phase I and II enzymes^[27]. Several lines of evidence suggest that DIM exerts agonist and/or modulator activity on the AhR^[28,29]. I3C can activate the NF-E2-related factor-2 (Nrf2) transcription factor which interacts with the antioxidant response element in the promoter of many cytoprotective enzymes, as described later. The induction of cytochrome P450 (CYP450) by physiological concentrations of I3C and DIM was observed in cancer cells^[30] and confirmed by an analysis of mRNA expression profiles^[31]. Increased CYP450 activity led to increased

Table 2. Bioavailability of I3C and DIM in animals following oral administration.

Dose	Time point	C _{max} plasma $\mu\text{g/mL}$ ($\mu\text{mol/L}$)	Tissue concentration $\mu\text{g/g}$ ($\mu\text{mol/L}$)	Reference
I3C (250 mg/kg)	15 min	I3C 4 (28)	27–25 (20–170)	20
	2 h	DIM 1 (4)	1–4 (4–16)	
DIM (250 mg/kg)	0.5–1 h	DIM 6 (24)	8–50 (32–200)	21

Table 3. Bioactivity of I3C *in vitro*.

Model	Dose (μmol/L)	Biomarkers affected	Outcome	Reference
Breast cancer MDA-MB468	100 25–100 125	↓ p-Akt ↓ MUC1 protein and mRNA ↓ mitochondrial potential, ↑ caspase-9, ↑ Src ⇒ EGFR signaling, ↓ EGFR	Growth IC ₅₀ 30 μmol/L, apoptosis	40, 41, 180, 181
MDA-MB231	50	↑ 2-hydroxylation of E	Apoptosis	182
MDA-MB435	30–60	↑ Bax and induced translocation to mitochondria	Apoptosis	183
MCF7	Sub μmol/L -50 10–50 50–60 25–50 100	↑ 2-hydroxylation of E ↓ E2-induced ER–DNA binding and ERE reporter activity; ↓ E responsive genes; ↓ E2-stimulated ER phosphorylation; ↑ BRCA1 and BRCA2 mRNA and protein ↑ CYP1A ↓ MUC1 protein, mRNA, and promoter activity ↓ cdk6; ↓ p-Rb; ↑ p27; ↑ p21; ↓ cdk2 activity; altered cdk2/cyclin E complex and localization; ↓ ERα mRNA and protein	Growth IC ₅₀ 50 μmol/L Apoptosis, G ₀ /G ₁ arrest	32, 33, 37, 43, 181, 184–190
MCF10CA1a	60–100	↓ p-Akt; ↓ Akt activity; ↓ BclX _L ; ↓ NF-κB–DNA-binding	Apoptosis	49, 191
T47D	50 60	↑ 2-hydroxylation of E; ↓ E2-induced ER-DNA binding; ↓ cell migration ↑ BRCA1 and BRCA2 mRNA and protein	Growth inhibition	32, 186, 192 43
Prostate cancer PC-3	30–60 60	↓ p-Akt; ↓ Bad; ↓ BclX _L ; ↓ EGFR ↓ gene expression of EGFR, PI3K, TGF-β ₂ , FGF, cyclin E2, ATF, Bcl2	Apoptosis	31, 193
LNCaP	30–90	↑ DR4 and DR5, ↑ BRCA1 and BRCA2 mRNA and protein	Growth inhibition, ↑ TRAIL-induced apoptosis	43, 194
DU145	60	↑ BRCA1 and BRCA2 mRNA and protein		43
Myeloid and leukemia cells (Jurkat, KBM-5, U266, MM.1), epithelial cancers H1299, SCC-4, A293	25–50 1–25	↓ TNFα-induced activation of NF-κB, ↓ activity of IKK and phosphorylation of IκBα, ↓ NF-κB DNA binding, ↓ NF-κB-regulated expression of a reporter gene and endogenous genes	↑ cytotoxicity, induced by TNFα, cisplatin, and doxorubicin	52
Colon cancer HCT-116	50–100	↑ NAG-1 expression	Growth inhibition	179
HCT-116 clones	10	PARP cleavage, caspase-9 activation, ↓ Bcl-X _L	IC ₅₀ ≅ 5–10 μmol/L	195

estrogen metabolism and the degradation of estradiol (E2)^[27], which is required for the growth of estrogen receptor-alpha (ERα)-positive cancer cells. I3C (50 μmol/L) inhibited the estradiol-stimulated growth of estrogen-responsive

MCF7, T47D and ZR75.1 breast and cervical cells. It inhibited receptor phosphorylation and DNA binding as well as estrogen-dependent reporter gene activity in breast tumor cells and cervical cancer cell lines^[32,33]. DIM (10 μmol/L) also

Table 4. Bioactivity of DIM *in vitro*.

Model	Dose ($\mu\text{mol/L}$)	Biomarkers affected	Outcome	Reference
Breast cancer MCF7	10–50	\uparrow CYP1A1; \uparrow formation of Ah-receptor nuclear complex	Apoptosis, G_1 cell cycle arrest, growth inhibition	27, 34, 37, 45, 48, 69, 196–198
	1–10	\uparrow ER-DNA binding, \uparrow ER-regulated pS2 mRNA and reporter gene expression, \downarrow ER α mRNA		
	50	\uparrow GADD proteins; \uparrow IFN γ expression; \uparrow p-JNK; \uparrow p-p38; \uparrow p-Jun; \uparrow p-ATF-2, \uparrow p21Cip1 mRNA and protein, \downarrow cdk2 activity, \uparrow Sp1 DNA binding, \downarrow Bcl-2; inhibits mitochondrial H ⁺ -ATP synthase, \uparrow ROS		
MCF10CA1a	15–50		Growth inhibition	50
	30–50	\downarrow NF- κ B-DNA-binding		
	50	\downarrow p-Akt		
MDA-MB231	40–50	\uparrow mRNA levels for p21Cip1, p57Kip2, \downarrow mRNA levels for genes involved in cell proliferation and survival as well as angiogenesis and metastasis, \downarrow Bcl-2	Apoptosis, G_1 cell cycle arrest, growth inhibition, IC ₅₀ 60 $\mu\text{mol/L}$	196, 198, 199
Endometrial cancer Ishikawa	1–30	\uparrow p-ERK1/2; \uparrow p-CREB; \uparrow ERE-reporter gene activity; \uparrow ER-responsive genes (TGF- α , alkaline phosphatase, PR)	Growth inhibition	35, 36
HEC-1B (ER transfected)	30	\uparrow ERE-reporter gene activity		36
Prostate cancer LNCaP	10	\downarrow PSA protein and mRNA levels; binds to AR and blocks nuclear translocation, \downarrow expression of AR-regulated reporter gene expression, \downarrow AR function.	Growth IC ₅₀ 40 $\mu\text{mol/L}$	38, 53, 200
	10–50 B-DIM	\downarrow DHT-induced NF- κ B DNA binding		
	50	\downarrow expression of androgen receptor		
PC-3	40	\downarrow gene expression of EGFR, PI3K, TGF- β 2, FGF, cyclin E2, ATF, Bcl2	Growth IC ₅₀ 40 $\mu\text{mol/L}$	31, 51, 200
	15–60	\downarrow p-Akt, \downarrow PI3K activity; \downarrow NF- κ B-DNA-binding, \downarrow EFGR	Apoptosis	
DU 145	25–50	\downarrow p-Akt, \downarrow cdk4, \downarrow cdk6, \uparrow Ca ²⁺ mobilization	Apoptosis, growth inhibition, G_1 cell cycle arrest, IC ₅₀ 20 $\mu\text{mol/L}$	47, 200, 201
Colon cancer HCT-116	12–25	\uparrow NAG-1 expression, \uparrow NAG-1-promoter regulated reporter gene expression		179
	25–50	\uparrow ATF3		
HCT-116 clones	20	PARP cleavage, caspase-9 activation, \downarrow Bcl-X _L	IC ₅₀ \cong 11 $\mu\text{mol/L}$	195
HT29		Blocked exit from G_1	Growth IC ₅₀ 10 $\mu\text{mol/L}$	202
HepG2 (hepatoma)	30–50	Inhibits topoisomerase II α	Growth inhibition, G_1 cell cycle arrest	44
Adrenocortical carcinoma H295R	<10	\uparrow EROD activity		203
Pancreatic cancer Panc-1 and Panc-28	20	\uparrow endoplasmic reticulum stress, \uparrow GRP78, CHOP, \uparrow DR5, \uparrow caspase-8, \uparrow PARP cleavage, \uparrow caspase3	Decreased survival, apoptosis	46

inhibited the estradiol-stimulated growth of MCF7, but in the absence of 17 β -estradiol it appeared to stimulate growth^[34].

DIM also exerted other estrogenic effects in these and endometrial cells^[34–36]. Conversely, earlier data indicated an inhibitory effect of DIM on E2-regulated reporter activity and ER DNA binding^[27]. Both I3C (75–100 μ mol/L) and DIM (1–25 μ mol/L) reduced the expression of ER α mRNA in MCF7 cells^[33,37]. DIM also interfered with androgen receptor expression, DNA binding, and signaling^[38,39].

Both compounds are growth inhibitory to a wide range of tumor cell lines, including ER-negative cancer cells. The increased loss of viability of several breast cancer cell lines when grown in a 3-D environment in the presence of I3C implied greater susceptibility *in vivo* than in a monolayer cell culture^[40]. Recent studies have shown that I3C decreases proliferation and induces apoptosis by reducing the expression and signaling of the genes essential for tumor cell viability, such as ER and epidermal growth factor receptor (EGFR) in breast cells of luminal A and basal-like subtypes^[33,41]. I3C (50 μ mol/L) can also inhibit phosphoinositide-3-kinase (PI3K)^[42], resulting in the inhibition of protein kinase B (Akt) phosphorylation and decreased survival in cancer cells dependent on this pathway. A mechanism dependent on breast cancer-related protein (BRCA)1/2 upregulation has also been proposed^[43].

DIM, which is considerably more potent than I3C, also inhibits the growth of a range of cells (Table 4). Topoisomerase II- α and mitochondrial H⁺-ATP synthase were identified as direct targets of DIM. The inhibition of the latter enzyme results in increased mitochondrial reactive oxygen species (ROS) production and signaling via the p38 stress activation pathway^[44,45]. Apoptosis via the activation of the endoplasmic reticulum stress pathway has also been reported^[46,47].

Both agents have a significant effect on several other signaling pathways, such as those involving p38^[45,48] and NF- κ B signaling^[49–53]. They modulate a variety of growth-, cell cycle-, and apoptotic-regulatory proteins at the mRNA or protein level, including EGFR, PI3K, transforming growth factor (TGF)- β 2, fibroblast growth factor (FGF), cyclin E2, activating transcription factor (ATF), B cell lymphoma Bcl2, BclX_L, Bad, and Bax.

In vitro* effects of I3C or DIM observed *in vivo Both I3C and DIM clearly inhibit tumor cell growth *in vivo* in a range of animal models^[54]. The reduced incidence and multiplicity of mammary tumors was concurrent with increased phase I and II drug metabolizing enzymes in I3C-treated animals^[55–57]. An analysis of transgenic Nrf2^{-/-} mice indicated that the I3C-induced upregulation of phase II enzymes required the Nrf2 transcription factor^[58,59]. The upregulation of CYP450 activ-

ity by DIM *in vivo* has been proposed to occur via a mechanism involving the AhR^[30], as reported in MCF7 cells *in vitro*^[27]. The induction of phase I and II enzymes has been reported in liver, small intestine, and lungs of rodents receiving I3C (in the diet or by gavage)^[30,54,57,60–63]. DIM also induced P450 activity and flavin-containing monooxygenase 1 in the rat liver^[30,60].

Apoptosis in response to I3C was observed *in vivo* in initiated mammary glands with activation of caspases-8, -9, and -3^[25] and in cervical epithelium of transgenic mice (HPV16), developing cervical cancer in response to estrogen^[56]. Few studies have investigated the effect of either agent on signal transduction intermediates *in vivo*, but in one study, dietary I3C (0.5%) caused a significant decrease in total tyrosine phosphorylation and ornithine decarboxylase activity in the rat liver^[62]. Many of the signaling events modulated by I3C *in vitro* involve tyrosine phosphorylation^[41], but interestingly, changes in ornithine decarboxylase activity in breast and colon cells *in vitro* were only observed at relatively high concentrations (>100 μ mol/L)^[62,64]. The downregulation of NF- κ B-regulated genes by I3C occurring in a variety of cancer cells *in vitro*, was also observed in mouse xenografts of MDA-MB231 cells^[52,65].

Evidence for I3C or DIM acting synergistically/ antagonistically In rats, I3C (5 mg/kg) reversed vinblastine- or vincristine-induced P-glycoprotein levels^[66]. This group also showed that a very high dose of I3C (10 mmol/L) decreased P-glycoprotein levels *in vitro* in the multidrug resistant cell line K562/R 10, sensitizing it to vinblastine, but had no growth-inhibitory effect on the parent K562 cell line^[67]. I3C (333 or 500 mg/kg per day) also reversed the MDR phenotype of the B16/hMDR1 (drug-resistant MDR1-expressing murine melanoma) tumor *in vivo*, and in combination with vinblastine, actually reduced the tumor mass^[68]. In the same study, an I3C acid-condensation mixture (12.5 μ mol/L) sensitized the B16/hMDR1 cell line *in vitro* to vinblastine, while DIM (45 μ mol/L) increased the drug content of cells by 50%^[68]. Combined treatments using I3C (50–125 μ mol/L) in combination with Src or/and EGFR inhibitors reduced the viability of breast cancer cells MDA-MB468 and MCF7^[41].

DIM (25 μ mol/L) plus genistein (5 μ mol/L) synergistically induced growth arrest and DNA damage-inducible (GADD)34 protein levels and apoptosis, and at higher concentrations induced estrogen receptor response element (ERE)-driven reporter gene activity^[69]. I3C (50 or 100 μ mol/L) has been shown to cooperate with tamoxifen (1 μ mol/L) *in vitro* to increase cell growth inhibition and G₀/G₁ cell cycle arrest of MCF7 cells^[70]; *in vivo*, it reduces tumor mass and increases latency of mammary cancers^[71]. An

I3C acid condensation mixture also enhanced the efficacy of vinblastine in mouse melanoma cells, while I3C itself had no effect^[68].

In vivo, dietary I3C, together with crambene (1-cyano 2-hydroxy 3-butene), another glucosinolate from vegetables, showed a greater than additive induction of glutathione-S-transferase (GST) and quinone reductase activity^[72].

Curcumin

Curcumin (diferuloylmethane) is a major constituent of the spice turmeric, derived from the roots of *Curcuma longa*. The major dietary source is curry, but it is also used as a food coloring and in some medicines.

Bioavailability of curcumin in humans Curcumin exhibits poor gastrointestinal absorption, with much of an oral dose passing unchanged through the gastrointestinal tract, and a further proportion undergoing conjugation, without

absorption, prior to fecal loss. Absorbed curcumin undergoes sequential reduction and conjugation (glucuronidation and/or sulfation) within the gastrointestinal tract and liver, with the resultant formation of metabolites and low systemic levels of the parent compound^[73,74].

Studies in humans have demonstrated that the oral administration of curcumin furnishes very low systemic levels, mostly in the low nanomolar range (Table 5). An exception is the study by Cheng *et al*, which reported serum levels in the low micromolar range using the maximum tenable dose (8 g/d)^[75]. Other groups have failed to replicate this finding, with Sharma *et al*, for example, administering up to 3.6 g/d of curcumin to patients for up to 4 months, yet only achieving levels in the 10 nmol/L range^[76]. The discrepancy between these studies remains to be explained, but may have resulted from the use of different formulations of curcumin.

Due to its poor bioavailability, curcumin levels in tissues beyond the gastrointestinal tract are also in the low nanomolar

Table 5. Bioavailability of curcumin in humans following oral administration.

Source	Maximum dose (mg/d)	C _{max} plasma/serum (T _{max})	Urinary/ fecal excretion	Tissue levels (nmol/g)	Metabolites	Reference	
Curcumin	8000 (3 months)	1.77 μmol/L ¹	60–120 min Urine	ND		75	
Curcuma extract (8.2% curcumin)	2200 (up to 4 months) (=180 mg/d curcumin)	ND	Urine Faeces (dried)	ND 64–1054 nmol/g	Plasma/urine Feces	ND CS detected in 1 case	76
Curcumin	3600 (7 d)	low nmol/L (systemic/portal) (Sampled 60 min post-dose)		Liver ND Bile ND	Blood Liver	CS/CG (low nmol/L) Hexahydrocurcumin and hexahydrocurcuminol (trace)	204
Curcuminoid capsules (90% curcumin)	3600 curcumin (up to 4 months)	11.1 nmol/L (60 min)	Urine Faeces (dried)	1.3 μmol/L 25–116 nmol/g	Plasma Urine Faeces	CS (8.9 nmol/L); CG (15.8 nmol/L); CS (45 nmol/L); CG (510 nmol/L) CS (trace)	76
Curcuminoid capsules (90% curcumin)	3600 curcumin (7 d)	<3 nmol/L (Sampled 60 min post-dose)		Colorectal mucosa CR cancer	7.7 12.7	Plasma Colon ND CS/CG detected	77
Curcumin extract (75% curcumin)	12000 (1 dose)	150 nmol/L ² (60 min)					205

¹ Serum level declined within 12 h; figures given are maximum levels achieved unless otherwise indicated. ² Curcumin detectable in only 1 of 3 patients receiving this dose; no curcumin was detectable at doses ≤8000 mg/d. ND, not detected; CS, curcumin sulfate; CG, curcumin glucuronide.

range or below. Garcea *et al* were unable to detect curcumin in normal liver or colorectal liver metastases in patients who had received 3.6 g/d for 1 week. In the only human study to examine colorectal tissue to date, this oral dose resulted in levels in the 10 $\mu\text{mol/L}$ range^[77].

Bioavailability of curcumin in animals A number of groups have examined the bioavailability of curcumin in animals, following oral, intragastric (ig) or intraperitoneal (ip) dosing (Table 6). Studies suggest that oral dosing may give rise to significant levels of curcumin within the gastrointestinal tract. In a rat model, approximately 1.8 μmol curcumin/g of tissue was demonstrated in colonic mucosa following the dietary administration of 1200 mg/kg daily^[78]. Perkins *et al* reported 750 mg/kg of curcumin/d to result in ~ 100 nmol/g in mouse small intestine mucosa and 500 nmol/g within colonic mucosa^[79]. Following oral dosing of 400 mg per rat, liver and kidney levels were less than 20 μg per tissue^[80]. Significant levels of curcumin may also be achieved locally when administered topically to the skin or within the oral cavity, but the exact dose achieved in these scenarios remains to be confirmed.

It is neither practicable nor desirable to increase the oral dose of curcumin above that already investigated. Recent animal studies, however, have demonstrated that the reformulation of curcumin may enable further improvements in bioavailability. It has previously been shown that the formulation of drugs with phosphatidylcholine increases their plasma bioavailability. Such a formulation led to significantly higher levels of curcumin within plasma and the liver compared with the parent compound, although lower levels within the intestinal mucosa^[81]. Several other animal studies have also found curcumin bioavailability to be significantly increased by its administration as a phospholipid complex^[82,83]. These increases in bioavailability now require confirmation in human studies. Although the bioavailability data are lacking, *in vitro* and animal studies have also shown promising anticancer potential for a liposomal preparation of curcumin^[84,85]. In addition, nanoparticle-encapsulated curcumin may provide an alternative means to increase the bioavailability of this agent^[86].

Physiologically relevant concentrations of curcumin The bioavailability data suggest that *in vitro* studies with curcumin in the 10 $\mu\text{mol/L}$ range or below might have human physiological relevance, but that its role as a chemopreventive agent may lie primarily within the gastrointestinal tract.

***In vitro* mechanistic studies using low doses of curcumin**

The anticancer effects of curcumin have been demonstrated in multiple cell types, at concentrations between 5 and 50 $\mu\text{mol/L}$ ^[87]. Selected studies demonstrating the anticancer

activity of curcumin at or below the 10 $\mu\text{mol/L}$ level achievable in the human colon *in vivo* are summarized in Table 7. Where available, data are presented from studies using colorectal cell lines; results from other cell types using a maximal dose of 10 $\mu\text{mol/L}$ are also included. In addition to these studies, curcumin also inhibited the proliferation of squamous carcinoma SCC-25 cells^[88] and the proliferation and invasion of HBL100 breast cells^[89].

***In vitro* effects of curcumin observed in vivo** In a rat model, dietary curcumin significantly increased the apoptotic index in azoxymethane-induced colonic tumors^[90]. Rao *et al* demonstrated the effect of a curcumin-containing diet on azoxymethane-induced rat carcinogenesis^[91]. Curcumin significantly reduced tumor volume, as well as colonic mucosa and tumor prostaglandin (PGE)₂ expression by over 38%. Similarly, it enhanced 2-amino-1-methyl-6-phenylimidazol(4,5-b)pyridine-induced apoptosis in Min/+ mice and inhibited tumorigenesis in the proximal small intestine. Also in mice, Mahmoud *et al* found dietary curcumin to normalize enterocyte proliferation and restore the level of enterocyte apoptosis to that of wild-type animals^[92]. In rats, a gavage administration of curcumin (200 or 600 mg/kg) inhibited diethylnitrosamine (DEN)-induced hepatic hyperplasia and inflammation. Specifically, the increased expression of p21ras and p53 in the liver was prevented. The decreased expression of proliferating cell nuclear antigen, cyclin E, and cdc2 was also observed, along with the inhibition of DEN-induced NF- κ B activation^[93].

While there are no *in vitro* studies for comparison, there is evidence from both animal and human studies showing that curcumin suppresses malondialdehyde-deoxyguanosine adduct (M₁dG) adduct formation in DNA^[77,78]. However, Garcea *et al*, while noting decreased M₁dG adduct formation in the colorectum following curcumin treatment, found no alteration in cyclooxygenase 2 (COX2) protein levels^[77].

Despite the low bioavailability of curcumin, there are examples in animal studies of its biological activity at sites distant from the locus of absorption, where levels are expected to be inefficacious based upon the results of *in vitro* studies. Sharma *et al*, for example, demonstrated increased hepatic GST expression and the attenuation of hepatotoxin-induced adduct formation following curcumin treatment^[78]. Oral curcumin also led to the complete suppression of tumor NF- κ B activation in an orthotopic mouse model of pancreatic cancer^[94]. Anticancer activity has also been reported at a number of other sites distant from the gastrointestinal tract, including the breast^[95], prostate^[96], lung^[97], and liver^[98].

Table 6. Bioavailability of curcumin in animals.

Source	Model	Maximum dose ¹	C _{max} plasma/serum (T _{max})	Urinary/fecal excretion (% of dose)		Tissue levels	Metabolites	Reference
Curcumin	Rat	1000 mg/kg (po)	Trace	Urine	trace	Bile	trace	206
Curcumin	Rat	600 mg (po) 600 mg (ip)		Urine	6%			
				Feces	89%	Bile	11% excreted	207
				Feces	73%		Major biliary metabolites- glucuronides	
Curcumin	Rat	400 mg (po)	Systemic ND Portal <5 µg/mL (13.5 µmol/L)*	Urine	ND	Liver <20 µg Kidney <20 µg	Urine-glucuronides and sulfates	80
				Feces	38%			
					remaining in large intestine (24 h)			
Curcumin	Rat	400 mg (po)	Detectable in blood	Urine	Minimal	Detectable in liver		208
				Feces	Major route	and kidney		
Curcumin	Mouse	1000 mg/kg (ig) 100 mg/kg (ip)	0.22 µg/mL (60 min) ² (0.6 µmol/L) 2.25 µg/mL (15 min) ³ (6.1 µmol/L)			Following IP: Kidney 7.5 µg/g (20 µmol/L) Spleen 26.1 µg/g (71 µmol/L) Liver 26.9 µg/g (73 µmol/L) Brain 0.4 µg/g (1 µmol/L) Intestine 117.0 µg/g (316 µmol/L)	99% plasma curcumin conjugated with glucuronide	209
Curcumin (91% curcumin)	Rat	1200 mg·kg ⁻¹ ·d ⁻¹ (14 d in diet)	Systemic/portal ND	Faeces (dried)	8.6 µmol/g	Colon mucosa 1.8 µmol/g (mmol/L) Liver 0.8 nmol/g (µmol/L)	CS/CG ND in plasma/ tissues/ feces	78
		200–400 mg/kg (diet)	<10 nmol/L (30 min)			Colon mucosa 279 nmol/g (µmol/L) Liver 0.1 nmol/g (µmol/L)	Plasma conjugates ND	
		500 mg/kg (ig)	30 nmol/L (30 min)			Colon mucosa 1.7 nmol/g (µmol/L) Liver 0.1 nmol/g (µmol/L)	Plasma conjugates detected	
Curcumin	Rat	500 mg/kg (ig)	<20 nmol/L				Plasma CG (1.7 µmol/L), CS (0.35 µmol/L), CG and others detectable	73
Curcumin (97% curcumin)	Mouse	300–750 mg·kg ⁻¹ ·d ⁻¹ (7 d in diet)	~5 pmol/L	Urine	ND	Small intestine ⁴ 39–240 nmol/g (µmol/L) Colon ⁴ 15–715 nmol/g (µmol/L) Liver ⁴ 119 pmol/g (nmol/L)	Colon and feces-CS (trace)	79
				Feces	3.2–3.8 µmol/g			

(Continue)

Source	Model	Maximum dose ¹	C _{max} plasma/serum (T _{max})	Urinary/fecal excretion (% of dose)	Tissue levels	Metabolites	Reference
Curcumin Meriva (curcumin+ phosphatidylcholine)	Rat	340 mg/kg (ig)	6.5 nmol/L 33.4 nmol/L	30 min 15 min	Liver <10 ng/mL Intestinal 2.8 mg/g mucosa Liver 80 ng/mL Intestinal 2 mg/g mucosa	Plasma metabolites detected. Levels of metabolites in liver and gastrointestinal mucosa higher following administration of Meriva	81
Curcumin (>99% purity)	Rat	100 mg (po)	266.7 ng/mL (0.72 μmol/L)	97 min			82
Curcumin-phospholipid complex		300 mg (po)	600.98 ng/mL (1.63 μmol/L)	140 min			
Curcumin	Rat	500 mg/kg (po)	0.06 μg/mL (0.16 μmol/L)	42 min			210
Curcumin	Rat	g/kg (po)	0.5 μg/mL (1.36 μmol/L)	45 min			83
Curcumin-phospholipid complex		1.0 g/kg of curcumin (po)	1.2 μg/mL (3.26 μmol/L)	90 min			

¹ Single dose unless otherwise stated; ² Levels ND at 6 h; ³ Levels decline rapidly within 1 h; ⁴ Tissue levels decline to ND by 3–6 h; *Figures in brackets are derived from the published data. po, per os; ND, not detected; CS, curcumin sulfate; CG, curcumin glucuronide; CGS, curcumin glucuronide sulfate.

Evidence for curcumin acting synergistically/antagonistically The treatment of MCF7 breast cells with curcumin (10 μmol/L) and genistein (25 μmol/L) demonstrated a synergistic effect, leading to the total inhibition of proliferation induced by an endosulfane/chlordane/DDT mixture^[99]. Curcumin also synergistically potentiated the inhibitory effect of celecoxib on pancreatic carcinoma cells^[100] and additively inhibited the growth of colorectal cancer with celecoxib in the 1,2-dimethylhydrazine rat model^[101].

In an *in vitro* model of oral cancer, EGCG blocked cells in the G₀/G₁ phase, while curcumin blocked in the G₂/M phase of the cell cycle. The combination showed synergistic interactions in growth inhibition^[102]. While tea or curcumin individually decreased the number and volume of dimethylbenzanthracene (DMBA)-induced oral tumors in hamsters, only the combination decreased the proliferation index of squamous cell carcinoma^[103].

LNCAp prostate cancer cells are relatively insensitive to tumor necrosis factor related, apoptosis-inducing ligand (TRAIL). At low concentrations, neither TRAIL (20 ng/mL) nor curcumin (10 μmol/L) produced significant cytotoxicity,

whereas cell death was markedly enhanced by the combination. Both agents together induced the cleavage of procaspases-8, -9, and -3, the truncation of Bid, the release of cytochrome c, and apoptosis^[104].

Recent studies have also demonstrated promising interactions between curcumin and established chemotherapeutic agents. In colorectal carcinoma lines, the antiproliferative and pro-apoptotic effects of curcumin and oxaliplatin increased markedly when cells were treated with both agents^[84,105]. Similarly, curcumin potentiated the pro-apoptotic effects of gemcitabine and paclitaxel in bladder cancer cell lines^[106] and the antitumor activity of gemcitabine in an orthotopic model of pancreatic cancer. Antagonistic interactions have also been demonstrated, however, with curcumin shown to inhibit chemotherapy-induced apoptosis in breast tumor lines. Camptothecin-, mechlorethamine-, and doxorubicin-induced apoptosis in MCF7, MDA-MB231 and BT474 cells was inhibited by as much as 70%, following 3 h exposure to as little as 1 μmol/L curcumin. The inhibition of both c-jun N-terminal kinase (JNK) activation and cytochrome c release occurred^[107]. The same authors, using an *in vivo*

Table 7. Bioactivity of curcumin *in vitro*.

Model	Minimum effective dose ($\mu\text{mol/L}$)	Biomarkers affected	Outcome	Reference
Colo320	12.5		Growth inhibition; apoptosis	211
HCT-116	5 10	\uparrow GADD153 mRNA and protein expression; \uparrow DNA damage	Apoptosis	212, 213
Moser	15 10	\uparrow PPAR γ ; \downarrow cyclin D1; \downarrow EGF signaling \downarrow EGFR gene expression	Growth inhibition Growth inhibition	214, 215
HCA-7	5	\downarrow CD/PMA-induced COX2 and PGE $_2$ production		216
HCEC	10	\downarrow TNF α -induced COX2 mRNA		217
HT29	5 10	\downarrow COX2 mRNA and protein \downarrow Arachidonic acid metabolism	Growth inhibition	218, 219
SK-Hep-1	10	\downarrow MMP-9 secretion;	\downarrow invasion (matrigel)	220
MDA MB468	1–5 10	\downarrow JNK activation by anisomycin	Growth inhibition, \downarrow invasion (matrigel)	89
MCF7	10		Apoptosis (p53-dependent); growth inhibition; G $_2$ /M arrest	99, 221, 222
KB-V1	1 5	\downarrow MDR1 mRNA \downarrow P-glycoprotein expression		223
B cell lymphoma	10	\downarrow egr-1; \downarrow c-myc; \downarrow Bcl-X $_L$; \downarrow p53; \downarrow NF- κ B	Growth inhibition; apoptosis	224
Jurkat	5–10	\downarrow JNK activation by PMA and ionomycin	Growth inhibition	225
HL-60	3.5	\downarrow Bcl-2 expression	Apoptosis	226
U266; RPMI 8226; MM.1S	10	\downarrow constitutive STAT3 phosphorylation	\downarrow IL-6 induced proliferation	227

xenograft model, found dietary curcumin (25 g/kg) decreased the level of cyclophosphamide-induced tumor regression, again with decreased JNK activation and less apoptosis.

Epigallocatechin-3-gallate (EGCG)

Green tea and its constituent molecules, including EGCG, have been found to prevent tumor formation in a wide range of tissues in animal models. However, the possible influ-

ence of green tea on cancer in humans has been difficult to interpret due to confounding factors, such as diversity in types of tea used, preparation methods, including temperature of infusion, and frequency of tea drinking.

The relevance of *in vitro* studies with EGCG has been reviewed by Lambert and Yang^[108], who concluded that the effectiveness of tea consumption in cancer prevention remained unclear and required a better understanding of bioavailability and fundamental mechanisms.

Bioavailability of EGCG in humans A number of studies have reported the bioavailability of EGCG in various human body fluids (Table 8) following the administration of green tea or EGCG. Levels in plasma up to a maximum of 7.3 µmol/L (±3.6) have been reported, but more often are in the submicromolar range. Bioavailability in two early studies found plasma

levels at 0.2%–2% of the ingested amount of EGCG (up to 4 µmol/L), but higher plasma concentrations have since been reported in fasting patients compared to those who consumed catechins with food^[109–111]. The oral administration in human patients resulted in high plasma clearance levels and volume distribution, suggesting that the bioavailability

Table 8. Bioavailability of EGCG in humans following oral administration.

Source	Dose	C _{max} plasma	T _{max} plasma	Elimination half-life	Reference
Decaffeinated tea solids >98%purity	1.5 g (in 500 mL)	119 ng/mL (0.26 µmol/L)*	1.4–2.4 h	5.0–5.5 h	228
	3.0	326 ng/mL (0.71 µmol/L)			
	4.5	321 ng/mL (0.70 µmol/L)			
EGCG	2.78 mg/kg	0.15 µmol/L	2 h	2.7 h	229
Green tea	20 mg/kg	77.9 ng/mL (0.17 µmol/L)	1.6 h	3.4 h	230
Decaffeinated green tea	20	24.4 ng/mL (0.05 µmol/L)	1.2 h	2.3 h	
EGCG	2	34.7 ng/mL (0.08 µmol/L)	1.6 h	3.7 h	
Green tea	2–3 cups/d	4.8–22 µg/mL (10–40 µmol/L) in saliva		10–20 min	115
EGCG	200 mg	74 ng/mL (0.16 µmol/L)	127.1±76.6 min	118.0±77.0 min	109
	400	119 ng/mL (0.26 µmol/L)	108.7±26.4	162.3±84.3	
	600	169 ng/mL (0.37 µmol/L)	180.0±84.8	183.7±67.6	
	800	439 ng/mL (0.96 µmol/L)	240.6±84.6	114.0±33.3	
EGCG	50–1600 mg	130–3392 ng/mL (0.28–7 µmol/L)	1.3–2.2 h	1.9–4.6 h	231
EGCG	688 mg	1.3 µmol/L	2.9 h	3.9 h	232
EGCG	225 mg	0.66 µmol/L	1.5 h	6.9 h	110
	375	4.3			
	525	4.4			
Black tea	16.74 mg EGCG 4×/6 h	0.02 µmol/L	5 h		233
Polyphenon E containing EGCG	(mg of EGCG)	(free EGCG, ng/mL)	(free EGCG, min)	(free EGCG, min)	112,
	400 mg fed	141.8±89.1 (0.30 µmol/L)	122.9±83.7	145.2±129.1	234
	400 mg fasting	798.7±573.1 (1.74 µmol/L)	93.9±59.0	170.5±104.6	
	800 mg fed	294.0±113.5 (0.64 µmol/L)	154.9±78.3	220.9±209.3	
	800 mg fasting	1522.4±1357.8 (3.32 µmol/L)	83.3±31.0	156.5±77.5	
	1200 mg fed	923.6±755.3 (2.01 µmol/L)	175.1±74.6	254.9±59.9	
1200 mg fasting	3371.6±1651.2 (7.34 µmol/L)	90.6±28.4	228.4±75.3		
Polyphenon E containing EGCG	618 mg	0.7±0.4 µmol/L	2.5 h±1.1		235
Purified EGCG	580 mg	0.5±0.2 µmol/L	2.6 h±1.2		

*Figures in brackets are derived from published data.

of EGCG in the blood may be low, similar to the situation found in rodents^[109,112]. Dvorakova *et al* suggested that topical application to skin of an ointment containing 10% EGCG was likely to result in substantial intradermal uptake, but very poor systemic absorption^[113].

It has been found that holding a green tea solution (1.2 g/200 mL water) in the mouth for 1 min resulted in salivary EGCG concentrations (mean) of 27 $\mu\text{mol/L}$, with values up to 48 $\mu\text{mol/L}$ recorded, several fold higher than that achieved through normal drinking, and many more times greater than plasma concentrations^[114,115]. However, holding the tea in the mouth for 5 min resulted in salivary concentrations 4–5 times higher, whilst taking tea solids in capsules resulted in no detectable salivary catechin levels. Thus, drinking tea slowly may be an effective way of delivering relatively high concentrations to the oral cavity and esophagus.

EGCG can undergo metabolism through glucuronidation, sulfation, methylation, or ring fission^[108], processes which are subject to interindividual variation.

Bioavailability of EGCG in animals Surprisingly, few studies have documented the bioavailability of EGCG in animals. Most have shown a maximum plasma concentration in the nmol/L to low $\mu\text{mol/L}$ range, similar to the human situation, although 1 study using a large dose of EGCG in rats reported plasma concentrations up to 20 $\mu\text{mol/L}$ (Table 9). No animal studies have examined the effect of fasting on

bioavailability. Fang *et al*, using liposomes for the local (injection) delivery of EGCG, found that a liposomal cocktail containing deoxycholic acid and ethanol greatly increased the tumor uptake of EGCG in both melanoma and colon murine tumor models^[116,117]. However, liposomal delivery was not superior following topical application.

Lambert and colleagues^[118] reported that piperine from black pepper enhanced the bioavailability of EGCG in mice. Small intestinal levels following EGCG administration alone resulted in a C_{max} of 37.5 \pm 2.5 nmol/g at 60 min, decreasing to 5.1 \pm 1.7 nmol/g at 90 min. Following cotreatment with piperine, the C_{max} was 31.6 \pm 15.1 nmol/g at 90 min, with levels still above 20 nmol/g at 180 min. The appearance of EGCG in the colon and feces was slower in the cotreated mice.

In rats and mice 24 h after the intragastric administration of radiolabeled EGCG, 10% of the dose was present in the blood, with around 1% in tissues, such as liver, kidney, heart, lung, and prostate^[119]. Major elimination occurred in the feces. In line with these findings, another study suggested that the transporter-mediated intestinal efflux of catechins may play a role in the systemic elimination of these compounds^[120]. Following an intravenous (iv) dose in rats, >70% was eliminated in bile and 2% in urine^[119,121]. A study in rats, in which different green tea catechins were administered by iv or ip, suggested that first-pass hepatic elimination did not play a major role in the metabolism of orally-

Table 9. Bioavailability of EGCG in animals.

Source	Model	Dose	C_{max} plasma	T_{max} plasma	Tissue	Excretion	Reference	
EGCG	Mouse	0.3% (<i>po</i>) 0.1% (<i>po</i>) in drinking water	90.6 nmol/L	22.4	Liver	42.9 ng/g (94.6 nmol/L)*	Urine 26.9–98.6 $\mu\text{mol/L}$	229
			13.4 (29.3 nmol/L)		Kidney	14.3 (31.5 nmol/L)	Feces 18–89 $\mu\text{g/g}$	
			4.8 (10.6 nmol/L)		Intestine	14.4 (31.7 nmol/L)	24–53	
			14.8 (32.6 nmol/L)					
EGCG	Mouse	21.8 $\mu\text{mol/kg}$ (iv) 163.8 (ig)	2.70 $\mu\text{mol/L}$	0.28	Lung, Prostate	0.31 nmol/g ($\mu\text{mol/L}$)	236	
			7.9		Colon			
			45.2		Intestine			
EGCG	Rat	10 mg/kg (iv) 75 mg/kg (ig)	85.5 min	Intestine, Kidney, Lung, Liver	High	Bile	237	
					Low			
EGCG	Rat	6000 μg (iv)	≥ 10 $\mu\text{g/mL}$	Approx 30 min			122	
		6041 μg (ip)	(21.8 $\mu\text{mol/L}$)					

*Figures in brackets are derived from the published data.

administered epigallocatechin, epicatechin, and EGCG^[122].

Physiologically relevant concentrations of EGCG The plasma bioavailability of EGCG, whether administered as tea or a pure compound, is in the range of 0.1–7 µmol/L in humans, with concentrations over 100 µmol/L observed in saliva. No significant excretion occurred in urine (generally <0.1% of dose). Rodent data indicate levels up to 20 µmol/L may be achievable. Based on these data, we chose 20 µmol/L as the maximum concentration at which to consider *in vitro* findings.

***In vitro* mechanistic studies using low doses of EGCG**

Many *in vitro* studies show that EGCG, at concentrations ≤20 µmol/L, inhibits growth and induces cell cycle arrest or apoptosis in a variety of cell types (Table 10). A wide range of signaling molecules is affected, including growth factor receptors [EGFR, platelet derived (PDGFR), fibroblast (FGFR), vascular endothelial (VEGFR)], survival signaling pathway components [extracellular signal regulated kinase (ERK), p38, activating protein-1 (AP-1), signal transducer and activator of transcription (STAT), PI3K, Akt, and NF-κB], cell cycle regulators [cyclin D1, p21, p27, phosphorylated retinoblastoma (pRb), cyclin-dependent kinases (cdk)2/4/6], and regulators of apoptosis [Bcl2, Bax, Bad, caspases-3/7/8/9, and poly (ADP ribose) polymerase (PARP)]. One interesting effect of EGCG at the lowest doses (0.01–0.1 µmol/L) is the inhibition of VEGF-dependent phosphorylation of the VEGFR (Table 10), an anti-angiogenesis effect which also occurs *in vivo*, as discussed later.

In vitro* effects of EGCG observed *in vivo There is substantial evidence that the effects of EGCG or green tea recorded *in vitro* have also been observed in animal models or humans. Green tea polyphenols inhibited the growth of 4T1 breast cancer cells and their metastasis to lungs in BALB/c mice. A reduction in tumor weight, increased survival time, and later tumor appearance were observed. The ratio of Bax/Bcl2 was altered in favor of apoptosis, along with a decrease in proliferating cell nuclear antigen and the activation of caspase-3^[123]. The topical application of EGCG to SKH-1 hairless mice that had been pretreated twice weekly with UVB light decreased the multiplicity of skin tumors by 44%–72% and increased the apoptotic index by 56%–92%, again measured by increased caspase-3 activity^[124]. Fang and colleagues, who demonstrated that the liposomal delivery of EGCG resulted in increased tumor uptake, also found that this delivery system led to increased antiproliferative activity in basal carcinoma cells *in vitro*, where the EGCG concentration in the liposomes was 21.3 µmol/L^[117].

A study investigating the effect of EGCG in murine colon 26-L5 cells found that, using *in vitro* assays, 1,1-diphenyl-2-picryl-hydrazyl free radicals were reduced with an ED₅₀ of

2.9 µmol/L, and cell growth was inhibited with an IC₅₀ of 41.8 µmol/L. Following the injection of these colon cells into female BALB/c mice to analyze the effect on lung metastases, they found that EGCG, administered ip, reduced the number of tumor nodules in a dose-dependent manner^[125].

When green tea extract (400–500 mg/cup, 5 cups/d) was administered for 4 weeks to 3 heavy smokers^[126], smoking-induced DNA damage was decreased, cell growth (keratinocytes) was inhibited, and the percentage of cells in S phase was reduced, with accumulation in the G₀/G₁ phase. DNA content became less aneuploid and p53 and caspase-3 were increased. Li and colleagues found that in hamsters, 0.6% green tea inhibited DMBA-induced oral tumor number and volume, with increased apoptosis and a decreased proliferation index and microvessel density^[103]. *In vitro* EGCG inhibits AP-1 transcriptional activation, and this was also observed *in vivo* in UVB-treated transgenic mice carrying a luciferase reporter gene with an AP-1 binding sequence^[127].

EGCG (10 or 50 µg/mL) significantly decreased the proliferation of bovine capillary endothelial cells, and at 1–100 µg per disc, it also inhibited neovascularization in the chick chorioallantoic membrane assay^[128]. These authors also showed that green tea in drinking water (1.25% containing 708 µg/mL EGCG, giving plasma levels of 0.1–0.3 µmol/L) could significantly suppress VEGF-induced corneal neovascularization. Such results suggest that EGCG may be a useful *in vivo* inhibitor of angiogenesis.

Green tea consumption in 2 study groups, 1 in China and 1 in the USA, decreased oxidative DNA damage (8-hydroxydeoxyguanosine in white blood cells and urine), lipid peroxidation (malondialdehyde in urine), and free radical generation (2,3-dihydroxy benzoic acid in urine) in smokers. Non-smokers (USA group) also exhibited a decrease in overall oxidative stress, which was correlated to decreased levels of free radicals^[129].

A recent clinical trial involving 60 volunteers with (pre-malignant) prostate intraepithelial neoplasia, conducted by Bettuzzi *et al*, showed that after 1 year, only 1 man (3%) in the group receiving 600 mg/d green tea compounds in (oral) capsule form, presented with cancer compared to 9 (30%) from the placebo group^[130]. No significant side-effects were reported. Therefore, despite the apparent poor bioavailability of green tea catechins in many studies, they appear to have great promise as chemopreventive agents.

Evidence for EGCG acting synergistically/antagonistically There are a number of reports documenting an enhanced chemopreventive effect when EGCG or green tea is used in combination with another chemopreventive agent or a therapeutic drug.

Table 10. Bioactivity of EGCG *in vitro*.

Model	Dose	Biomarkers affected	Outcome	Reference
HT29	10 $\mu\text{mol/L}$ 10–20	↓ p-ERK1/2; ↓ VEGF (serum starved) ↓ VEGF reporter gene activity ; ↑ AP-1 reporter activity; ↑ cyclin D1 ↑ NF- κB reporter gene activity		238, 239
HT29 spheroids	10–20		Inhibition of spheroid formation	240
LNCaP	1–10 $\mu\text{mol/L}$ 10 $\mu\text{mol/L}$ 20 $\mu\text{mol/L}$ – 10 $\mu\text{g/mL}$ (22 $\mu\text{mol/L}$)	↑ p27; ↑ I κB - α ↓ Arachidonic acid-stimulated PGE ₂ levels ↓ COX2 mRNA; ↓ PI3K (p85); ↓ p-Akt; ↑ p-ERK1/2 ↓ Chymotrypsin-like activity of 20S proteasome	↓ arachidonic acid-stimulated cell growth; G ₀ /G ₁ arrest; Apoptosis, Growth inhibition	241–243
DUI45	From 10 $\mu\text{g/mL}$ (22 $\mu\text{mol/L}$)	↓ PI3K (p85); ↓ p-Akt; ↑ p-ERK1/2	Growth inhibition	243, 244
Jurkat T	From 1 $\mu\text{mol/L}$ 10 5–20	↑ p27 ↓ chymotrypsin-like activity of 20S proteasome (IC ₅₀ 18 $\mu\text{mol/L}$)	G ₀ /G ₁ arrest	241
CLL B	5–10 $\mu\text{g/mL}$ (10–22 $\mu\text{mol/L}$) 3 $\mu\text{g/mL}$ (6.5 $\mu\text{mol/L}$)	↑ caspase 3 and PARP cleavage; ↓ Bcl-2; ↓ XIAP; ↓ Mcl-1 ↓ VEGF-R1; ↓ VEGF-R2 phosphorylation	Apoptosis	245
Foreskin keratinocytes	10 $\mu\text{g/mL}$ (22 $\mu\text{mol/L}$)	↑ involucrin promoter activity; ↑ cornification	Growth inhibition	246
HCL14	From 5.45 nmol/L	↓ UVB-induced AP-1 activity	No effect	247
HaCaT	7.5–10 $\mu\text{mol/L}$	↓ UVB-induced c-fos; ↓ UVB-induced p38 activation		248
A-375	1–5 $\mu\text{g/mL}$ (0.5–10 $\mu\text{mol/L}$) 2.5–10 $\mu\text{g/mL}$ (1.5–22 $\mu\text{mol/L}$) 10 $\mu\text{g/mL}$ (22 $\mu\text{mol/L}$)	↓ colony formation ↑ Bax; ↓ Bcl2; ↑ Bax:Bcl2 ratio; ↑ caspase 3, 7, 9; ↑ PARP cleavage; ↑ p21; ↑ p16; ↓ cdk2 ↓ cyclin D1; ↑ p27	Growth inhibition; G ₀ /G ₁ arrest; apoptosis	249
Hs-294T	1–5 $\mu\text{g/mL}$ (0.5–10 $\mu\text{mol/L}$) 2.5–10 $\mu\text{g/mL}$ (1.5–22 $\mu\text{mol/L}$)	↓ colony formation ↑ Bax; ↑ Bax:Bcl2 ratio; ↑ caspase 7; ↑ PARP cleavage ↓ cyclin D1; ↓ cdk2; ↑ p16; ↑ p27; ↑ p21	Growth inhibition; ↑S phase; apoptosis	249
JB6 (C1 41)	1 $\mu\text{mol/L}$ 5–20	↓ UVB-induced phospho-p70S6K (possible direct effect) ↓ p-Akt and PI3K activity and expression ↓ TPA-induced cell transformation; ↓ TPA-induced NF- κB activity; ↓ NF- κB DNA-binding; ↓ TPA-induced p-I κB α ; ↓ AP-1–DNA binding and reporter activity		250–252

(Continue)

Model	Dose	Biomarkers affected	Outcome	Reference
A431	0.5–1 µg/mL (0.5–2 µmol/L) 10 µmol/L– 10 µg/mL (22 µmol/L)	↓ tyrosine kinase activity of EGFR, PDGFR, FGFR ↓ NF-κB–DNA binding; ↓ nuclear NF-κB (p65); ↑ caspases-3,-8,-9 activation	Apoptosis	253-255
Immortalized ECE16-1	10 µmol/L 20	↓ p-ERK1/2; ↓ERK1/2 and Akt activity (direct effect); ↓ p-Bad ↑ p21; ↑ p27; ↑ p53	Growth inhibition	256
HeLa cells	1–25 µmol/L	↓ oxidative stress (↓ 8-OHdG and HMdU)		257
NBT-II	10 µmol/L 20 10–40	↓ p-Rb ↓ cyclin D1 ↓ cdk 4/6	Growth inhibition G ₀ /G ₁ arrest Apoptosis	258
YCU-N861 YCU-H891	10 µg/mL (22 µmol/L) 0.1–1.0 µg/mL (0.2–2 µmol/L)	↑ p21; ↑ p27; ↓ cyclin D1; ↓ pRb; ↓ p-EGFR; ↓ p-ERK1/2; ↓ p-STAT3; ↓ Bcl2; ↓ BclX _L ; ↓ Bax; ↓ p-HER2	Growth IC ₇₀ 10 µg/mL; G ₀ /G ₁ arrest; apoptosis ↑ growth inhibition by 5-FU and Taxol	133, 134
NOE (normal) MSK-Leuk1 MSK-Leuk1S 1483	5.5 µmol/L 8 µmol/L 11.5 µmol/L 18 µmol/L	↓ pRb; ↑ hypo-pRb	ED ₅₀ 5.5 µmol/L; G ₀ /G ₁ arrest ED ₅₀ 8 µmol/L; G ₀ /G ₁ arrest ED ₅₀ 11.5 µmol/L; G ₀ /G ₁ arrest ED ₅₀ 18 µmol/L; G ₀ /G ₁ arrest	259
KYSE	<10 µmol/L 20 µmol/L	↓ DNA methyltransferase activity (<i>in vitro</i> assay) ↓Hypermethylation; ↑mRNA expression <i>p16^{INK}</i> , <i>RARβ</i> , <i>MGMT</i> , <i>hMLH1</i>	Growth inhibition	260
Bovine capillary cells	From 10 µg/mL (22 µmol/L)		Growth inhibition ↓ <i>in vitro</i> angiogenesis	128
BAEC	0.01–1 µmol/L	↓ VEGF-dependent phosphorylation of VEGFR-2;	↓ <i>in vitro</i> angiogenesis	261
HMVEC	2–20 µmol/L 20	↓ VEGF-stimulated VE-cadherin; ↓ <i>in vitro</i> VEGF-induced angiogenesis ↓ VEGF-stimulated p-VE-cadherin and p-Akt		262
Vascular smooth muscle cells	3 and 10 µmol/L 1,3, and 10 µmol/L	↓ Thrombin-induced activation of MMP-2 at 10 µmol/L ↓ Thrombin-induced activation of membrane- type MMP-1 at 1, 3, and 10 µmol/L	Prevent smooth muscle cell invasion (at 30 µmol/L, lower concentration not tested)	263
HEK293 cells	5 and 10 µmol/L	↓ nuclear and membrane-associated β-catenin and membrane-associated E-cadherin		264
A549 cells	10 µmol/L	↑ PGE ₂		265
PC-3 cells	10 µmol/L		↓ viability	266
SH-SY5Y cells	1 µmol/L		↓ Bad	267

Suganuma *et al* found that epicatechin significantly enhanced the uptake of labeled EGCG into human lung PC-9 cells and suggested that whole green tea was a better preventive agent than EGCG alone^[131]. In this study, the proapoptotic effects of EGCG were also increased by tamoxifen or sulindac. Another study using the prostate cancer cell lines PC-3, LNCaP, and CWR22Rv1 showed that while 10 $\mu\text{mol/L}$ EGCG only resulted in a 12%–21% inhibition in cell viability, the addition of 10 $\mu\text{mol/L}$ NS-398 (a COX2 inhibitor), resulted in a 44%–49% inhibition, greater than the additive effect of either agent alone. These results corresponded to decreases in Bcl-2, procaspases-6 and -9, phospho-p65 and peroxisome proliferator-activated receptor (PPAR) γ , and increases in Bax and PARP^[132].

EGCG at 0.1 $\mu\text{g/mL}$ (equivalent to serum concentrations) markedly enhanced the growth inhibitory effects of 5-fluorouracil in head and neck squamous carcinoma cells^[133]. The IC_{50} values for 2 different cell lines were reduced by ~4-fold (sensitive line) and 45-fold (resistant line). EGCG on its own at this concentration had no effect. The same group also found that EGCG enhanced the sensitivity of HNSCC (0.1 $\mu\text{g/mL}$) and breast (1.0 $\mu\text{g/mL}$) cells to Taxol^[134].

Min/+ mice treated with a combination of white tea (1.5%) and sulindac (80 ppm) had significantly fewer intestinal tumors than mice treated with either agent alone. While β -catenin and β -catenin/T cell factor-4-regulated genes, cyclin D1, and c-jun were detected in polyps, the expression of these proteins was markedly reduced in the normal intestine^[135]. A combination of EGCG and sulindac was also found to be efficacious in preventing azoxymethane-induced colon cancer in rat, where the combination synergistically enhanced apoptosis^[136].

Resveratrol

The phytoalexin resveratrol is found largely in grape products and peanuts, with red wine a major source of human consumption. Its potential role in disease prevention is well documented, as it exhibits vasorelaxing, anti-inflammatory, antilipidemic, anti-estrogenic, antioxidant, antifungal, and antibacterial properties^[137–139].

Whilst resveratrol appears to have great potential *in vitro*, the relevance to *in vivo* effects in both humans and animals is less clear, as its chemopreventive effect *in vivo* depends greatly on its absorption, metabolism, and tissue distribution.

Bioavailability of resveratrol in humans Several studies have looked at the rate of uptake in healthy human volunteers via the oral administration of either resveratrol in its pure form or when present in foodstuffs (Table 11). Estimates of the amount of resveratrol in red wine (mainly trans), vary from 0.3 to 10.6 mg/L (1.3–46 $\mu\text{mol/L}$)^[140–142]. Recent studies by Boocock *et al*^[143,144] found peak resveratrol plasma concentrations of 0.3–2.4 $\mu\text{mol/L}$ following single oral doses of 0.5–5 g in healthy human volunteers. Observed peak plasma concentrations for resveratrol metabolites ranged between 0.92 and 4.3 $\mu\text{mol/L}$ (mono-glucuronides), and 3.7–14 $\mu\text{mol/L}$ (resveratrol 3-sulfate). Plasma half-lives for the parent compound and major conjugates were of a similar order (2.9–10.6 h).

Subjects receiving a lower dose of 10–25 mg pure resveratrol/70 kg body mass were similarly found to have serum resveratrol levels between 1.83 and 2.06 $\mu\text{mol/L}$ at 30 min post-dose, returning to baseline by 4 h^[145]. Wang *et al*^[146] found both resveratrol and resveratrol glucuronide present

Table 11. Bioavailability of resveratrol in humans following oral administration.

Source	Dose	C_{max} plasma	Clearance	Urinary excretion (% of intake)	Reference
Resveratrol	25 mg/70 kg body weight	491 ng/mL (2 $\mu\text{mol/L}$)*	~9 h		268
White wine	25 mg/70 kg body weight resveratrol	416 $\mu\text{g/L}$ (1.82 $\mu\text{mol/L}$)		16.8	
Grape juice		416 $\mu\text{g/L}$ (1.82 $\mu\text{mol/L}$)	~4 h	16.0	145
Vegetable juice		471 $\mu\text{g/L}$ (2.06 $\mu\text{mol/L}$)		17.0	
Resveratrol	0.5–5 g	73–539 ng/mL (0.3–2.4 $\mu\text{mol/L}$)			143, 144
Red wine	7.5 $\mu\text{g/kg}$ body weight				269

*Figures in brackets are derived from the published data.

at up to 6 h following a dose of 1 g. It is likely that conjugated resveratrol is the main component in the circulation^[147], and plasma concentrations around 2 $\mu\text{mol/L}$ appear typical (Table 11).

Several major metabolites of resveratrol are found in human subjects, including the sulfate-glucuronide, mono-glucuronides, and mono- and di-sulfates^[143,144], with sulfation thought to occur primarily via the sulfotransferase 1A1^[148] and glucuronidation via the UDP-glucuronosyltransferases^[149]. The rate of glucuronidation in the human liver ranges between 0.23 and 1.2 nmol/min/mg and the rate of sulfation is 80 pmol/min per mg^[141,150]. The presence of sulfated products *in vivo* may vary depending upon whether pure resveratrol is administered, as quercetin (also present in red wine) is known to inhibit its sulfation. Resveratrol glucuronide has been assumed to be pharmacologically inactive, although it has been suggested that glycosylation of polyphenols is an important step in protecting them from enzymatic oxidation, so extending their half-life and biological properties^[151]. However, it is possible that the glucuronide may be converted back to resveratrol *in vivo* by the action of beta-glucuronidases^[152]. The aqueous solubility of resveratrol is low, and it is thought that albumin is the main carrier in plasma, with little free resveratrol^[153]. The presence of fatty acids increases binding to serum albumin, which may have important consequences for the delivery of resveratrol to cell membranes and thus signaling events.

Bioavailability of resveratrol in animals Marier *et al*^[154] observed that resveratrol was absorbed within minutes following an oral dose of 50 mg/kg to rats. Resveratrol aglycone (parent compound) plasma concentrations dropped from 10 $\mu\text{mol/L}$ to levels at the limit of detection by 12 h. The glucuronide, however, was present at $\sim 100 \mu\text{mol/L}$, falling to 3 $\mu\text{mol/L}$ after 12 h, again suggesting that resveratrol is absorbed from the intestine mainly in this form. A lower dose of 20 mg/kg gave a maximal resveratrol plasma content of 3 $\mu\text{mol/L}$, falling to $<0.1 \mu\text{mol/L}$ after 1 h^[140], with a 5 mg/kg dose showing maximal plasma levels of 1.5 $\mu\text{mol/L}$ ^[155].

The disposition of resveratrol revealed far higher levels in tissues involved in absorption and excretion, such as the intestine, liver, and kidney, than in plasma. In mice, the highest accumulation was in intestinal mucosa^[156], brain^[157], kidneys, and liver, reaching 25–30 $\mu\text{mol/L}$ following a dose of 5 mg/kg^[155]. The major tissue metabolites were resveratrol-3,4'-disulfate, -3,4',5-trisulfate and β -D-glucuronide^[158]. Following a 20 mg/kg dose, the rat lung contained 0.8 nmol/g resveratrol, the mouse liver 1.03 nmol/g, and the mouse kidney 0.17 nmol/g^[140].

Physiologically relevant concentrations of resveratrol

Animal and human studies consistently indicate resveratrol levels of 1–2 $\mu\text{mol/L}$ in plasma (Tables 11, 12). Concentrations 10–20 times higher than this have been achieved through ig or iv dosing in animal studies. An examination of tissue distribution revealed that concentrations in some tissues can be significantly higher than those in plasma. Therefore, in considering the relevance of *in vitro* studies, we have focused on those which have reported effects at concentrations of 10 $\mu\text{mol/L}$ or less.

***In vitro* mechanistic studies using low doses of resveratrol** Cell culture studies with resveratrol indicate anticancer potential over a range of doses and in a wide variety of tissues, including breast, colon, pancreas, stomach, prostate, head and neck, ovary, liver, lung, and cervix^[137] (Table 13). At physiological concentrations of 10 $\mu\text{mol/L}$ or less, resveratrol exhibits a range of activities which modulate signal transduction. These include the downregulation of growth factors (EGF and VEGF), alterations to survival signaling (ERK, JNK, AP-1, and NF- κ B), cell cycle regulators (cyclinD1, cdk4/6, p21, and p53) and apoptosis regulators (PARP, ceramide, and caspases). In U937 monocytes, concentrations as low as 0.1 $\mu\text{mol/L}$ effectively inhibited the production of ROS, with the inhibition of Akt phosphorylation at 10 $\mu\text{mol/L}$ ^[159].

***In vitro* effects of resveratrol observed in vivo** Whether or not the IC_{50} values *in vitro* are achievable *in vivo* depends to some extent on the target tissue. It is likely that the highest concentration of resveratrol and its metabolites following ingestion would be achieved in colorectal mucosal tissue and in the liver. Several studies administering resveratrol via ingestion of red wine have used the mean serum antioxidant capacity as a marker of efficacy. In healthy volunteers consuming 300 mL red wine over a 30 min period, blood taken up to 2 h post-dose showed significantly raised serum antioxidant capacity^[160].

MDA-MB231 xenografts in nude mice exhibited an increase in the apoptotic index and decreased angiogenesis when treated daily with 25 mg/kg resveratrol for 3 weeks, while the same cell line in the culture did not undergo apoptosis at concentrations less than 100 $\mu\text{mol/L}$ ^[161]. Conversely, when B16M tumor cells were inoculated into mice, 20 mg/kg resveratrol did not affect tumor growth (tumor concentration of 0.04 nmol/g), even though in the culture the cells underwent 60% apoptosis following a 5 $\mu\text{mol/L}$ treatment for 24 h^[140]. The rats inoculated with the Yoshida ascites hepatoma receiving daily ip injections (1 mg/kg) of resveratrol exhibited decreased tumor growth due to the induction of apoptosis and a G_2/M cell cycle arrest. This effect was not seen *in vitro* using resveratrol in the range of

Table 12. Bioavailability of resveratrol in animals.

Source	Model	Dose	C _{max} plasma (μmol/L)	T _{max} plasma	Clearance	Reference	
Grape juice	Rat	2 mg/kg resveratrol (ig)	1.2			270	
Resveratrol	Rat	20 mg/kg (po)	1.2	5 min	2 h	140	
	Mouse		2.6				
	Rabbit		1.1				
	Mouse	2.6 mg·kg ⁻¹ ·d ⁻¹ (po)	0.05–0.1				
	Rabbit	20 mg/kg (iv)	38.4–47.2				
Resveratrol	Mouse	1 mmol/kg (ig)	32	10 min	1 h	156	
Resveratrol	Rat	15 mg/kg (iv)	~20	0.08 h	8 h	154	
		50 mg/kg (po)	~7		12 h		
Resveratrol (¹⁴ C)	Mouse	5 mg/kg (po)	1.5	1.5 h (earliest measured)	High urinary content by 6 h	155	
Resveratrol	Rat	2 mg/kg (po)	0.175 mg/L (0.7 μmol/L)*			271	
Resveratrol	Mouse	20 mg/kg (ip)	Resveratrol	<0.5	15 min	Metabolites cleared by 2 h	147
			Resveratrol sulfate	13 (ip); 5 (ig)			
		20 mg/kg (ig)	Resveratrol glucuronide	3 (ip); 1 (ig)			
		60 mg/kg (ig)	Resveratrol	<0.5			
	Resveratrol sulfate	300					
	Resveratrol glucuronide	175					

*Figures in brackets are derived from the published data.

15–30 μmol/L over 24 h^[162]. Daily ip injections of 40 mg/kg resveratrol (estimated serum level of 25 μmol/L) reduced neuroblastoma growth in rats and increased survival by 70%. In culture, resveratrol was also cytotoxic to neuroblastoma cells in a range from 10 to 100 μmol/L^[163]. Resveratrol at 100 mg/kg per day prolonged the survival time for rats with intracerebral tumors generated from RT-2 glioma cell xenografts. The IC₅₀ for RT-2 cells in the culture equated to 12.8 μmol/L following a 48 h treatment, with 39% cells undergoing apoptosis at the higher concentration of 25 μmol/L^[164].

A number of reports have shown that resveratrol can inhibit NF-κB activation *in vitro*. Banerjee *et al* found that in rats, 10 ppm produced striking reductions in DMBA-induced breast tumor incidence and multiplicity, while extending tumor latency^[165]. They reported that resveratrol suppressed DMBA-induced COX2 and matrix metalloproteinase (MMP)-9 expressions through the downregulation of NF-κB activation.

Resveratrol treatment may also inhibit preneoplastic conditions. In both an experimentally induced model of colitis^[166] and the Min/+ mouse^[167], resveratrol was able to reduce damage/adenoma load and COX2 protein expression. The spontaneous development of mammary tumors in HER2/neu mice was delayed with the reduction in both size and number of tumors following resveratrol treatment^[168]. In rats, azoxymethane treatment caused the formation of aberrant crypt foci, the number of which was significantly reduced in the presence of resveratrol (200 μg/kg per day for 100 d), with decreased bax and increased p21 expression in the crypts^[169]. The treatment of dimethylhydrazine-induced aberrant crypt foci with resveratrol (8 mg/kg per day) resulted in a marked reduction in tumor incidence and degree of histological lesions^[170]. Similarly, rats fed a diet containing 15% grape extract showed a decrease in the number and area of GST-P⁺ foci^[171].

Table 13. Bioactivity of resveratrol *in vitro*.

Model	Dose (µmol/L)	Biomarkers affected	Outcome	Reference
LNCaP	2.5	↓ pAkt; ↓ Bcl2:Bax ratio	Growth inhibition; apoptosis; G ₀ /G ₁ arrest.	272
	1–5		Growth inhibition; apoptosis	273
A2780/CP70	12.5	↓ HIF-1α; ↓ VEGF		274
Ishikawa	10	↓ EGF	Growth inhibition	275
MCF7	0.1–10	↓ CYP1A1 mRNA and protein	Growth inhibition	276–278
	1–10			
	1–15	↓ progesterone receptor and pS2	Increased growth	279
	10			
T47D	0.1–10	↓ CYP1A1 mRNA and protein	Growth inhibition	276, 278
	1–15	↓ Progesterone receptor; ↓ pS2		
MDA-MB231	16	PARP cleavage; ↑ ceramide	Apoptosis	280
184B5/HER	2.5–20	↓ COX2 mRNA and activity		281
BHP2–7, 18-21	1, 10	↑ activation of ERK1/2, ↑ p53	Apoptosis	282
JB6	5-20	↑ p53 activation	Growth inhibition; Apoptosis; G ₀ /G ₁ arrest	283, 284
	10–20	↑ JNK activation; ↑ p53 phosphorylation		
HCT-116	10–20	Bax-independent	Apoptosis	163
CaSki	10	↓ MMP-9 transcription; ↓ AP-1 activation		285
BAEC	6–20		↓ migration, tube formation, ↓ angiogenesis	286
A431	5–10	↑ p21; ↓ cyclin D1; ↓ cdk4, 6	Growth inhibition, Apoptosis; G ₀ /G ₁ arrest	287
HL60	4-8	↓ caspase activation, ↑ DNA fragmentation	Apoptosis	288
MEF (p53+,p53-)	10	↑ p53 activation	Apoptosis	289
U937	0.1–20	↓ ROS production		159, 290
	5–10	↓ AP-1 activation; ↓ NF-κB activation		
	10	↓ p-Akt		
HeLa; glioma (H4); Jurkat	5–10	↓ AP-1 activation; ↓ NF-κB activation		290
BEPD2; BEAS-2B	10	↓ B[a]P-induced adducts; ↓ CYP1A1/CYP1B1 mRNA		291
Melanoma	5	↑ p53; ↑ quinone reductase 2	Growth inhibition; S- and G ₂ /M arrest	292
SCC-9; HEPG2	5–10	↓ hypoxia-induced HIF-1α; ↓ VEGF; ↓ pERK; ↓ pAkt		293

Evidence for resveratrol acting synergistically/antagonistically Resveratrol sensitized colon cancer cells to CD95 and the TRAIL-mediated induction of apoptosis, and at 10 $\mu\text{mol/L}$, sensitized HT29 cells to cisplatin-induced apoptosis^[172]. Fulda and Debatin found that pretreatment with resveratrol cooperatively enhanced doxorubicin, cytarabine, actinomycin D, Taxol, and methotrexate-induced apoptosis and cell cycle arrest in neuroblastoma cells^[173], and enhanced TRAIL-mediated apoptosis in neuroblastoma and Jurkat T cells^[174]. Resveratrol (10 $\mu\text{mol/L}$) also enhanced the apoptotic effects of paclitaxel in A549, EBC-1, and Lu65 lung cancer cell lines^[175], and of cisplatin and doxorubicin in OVCAR-3 and Ishikawa cells, respectively^[176].

Resveratrol has been used in combination with other phytochemicals, such as beta-sitosterol, resulting in enhanced growth inhibition due to an arrest at both the G₁ and G₂/M phases of the cell cycle in PC-3 cells^[177]. The combination of quercetin/ellagic acid with resveratrol resulted in a synergistic effect on caspase-3 activation leading to apoptosis^[178]. Lee *et al*^[179] found individual concentrations of resveratrol (0.5 $\mu\text{mol/L}$) or I3C (50 $\mu\text{mol/L}$) to induce the non-steroidal, anti-inflammatory, drug-activated gene (NAG)-1, a TGF- β superfamily gene associated with pro-apoptotic and anti-tumorigenic activity. However, when used in combination, the doses could be reduced to 0.025 and 1 $\mu\text{mol/L}$, respectively.

Conclusion

Plasma concentrations in humans, following normal dietary intake or administration of supplements or formulations, have been measured or can be estimated from animal studies for each of the agents reviewed. In general, achievable plasma concentrations were in the low micromolar range, although animal studies revealed the possibility of considerably higher concentrations in some tissues. In summarizing data from many cell culture studies, which have been carried out using the low concentrations achievable *in vivo*, it is apparent that all the compounds still exhibit biological activity. However, the range of activities is more limited, compared to that using a much wider dose range. While this may reflect genuine lack of activity at low doses, it is partly due to the fact that many studies have chosen to use only higher doses and shorter time points. There may therefore be many more useful preventive possibilities to be identified using lower doses.

Two very encouraging themes emerge from the data reviewed here. First, while not all *in vitro* findings are matched *in vivo*, many observations have been validated in animals or humans, giving credibility to the value of cell

cultures for screening and more detailed mechanistic studies. However, some caution is required in extrapolation, as in many cases it is not known whether exactly the same signaling mechanisms are operational *in vivo*. There can also be significant discrepancies in the effective doses, even to the extent that where low levels are active *in vivo*, much higher concentrations are required to achieve the same effect *in vitro*.

Second, there is a growing body of evidence to suggest that even if single agents are inactive at low concentrations, combinations of 2 or more compounds might be much more efficacious. Combinations with chemotherapeutic drugs also offer the possibility of lowering the dose of the latter, with a consequent reduction of unwanted side-effects.

Thus, studies in cell culture have provided valuable insights into chemopreventive mechanisms of action, and there is now a need to pursue these at physiological doses and in novel combinations. One further aspect, which has not been tackled in any detail here, is the need to address more rigorously the question of tissue specificity and cancer subtype.

References

- 1 Manson MM. Inhibition of survival signalling by dietary polyphenols and indole-3-carbinol. *Eur J Cancer* 2005; 41: 1842–53.
- 2 Aggarwal BB, Shishodia S. Molecular targets of dietary agents for prevention and therapy of cancer. *Biochem Pharmacol* 2006; 71: 1397–421.
- 3 Surh YJ. Cancer chemoprevention with dietary phytochemicals. *Nat Rev Cancer* 2003; 3: 768–80.
- 4 Sepkovic DW, Bradlow HL, Bell MC. Quantitative determination of 3,3'-diindolylmethane in urine of individuals receiving indole-3-carbinol. *Nutr Cancer* 2001; 42: 57–63.
- 5 Reed GA, Arneson DW, Putnam WC, Smith HJ, Gray JC, Sullivan DK, *et al*. Single-dose and multiple-dose administration of indole-3-carbinol to women: pharmacokinetics based on 3,3'-diindolylmethane. *Cancer Epidemiol Biomarkers Prev* 2006; 15: 2477–81.
- 6 Dalessandri KM, Firestone GL, Fitch MD, Bradlow HL, Bjeldanes LF. Pilot study: Effect of 3,3'-diindolylmethane supplements on urinary hormone metabolites in postmenopausal women with a history of early-stage breast cancer. *Nutr Cancer* 2004; 50: 161–7.
- 7 Bell MC, Crowley-Nowick P, Bradlow HL, Sepkovic DW, Schmidt-Grimminger D, Howell P, *et al*. Placebo-controlled trial of indole-3-carbinol in the treatment of CIN. *Gynaecol Oncol* 2000; 78: 123–9.
- 8 Bradlow HL, Michnovicz JJ, Halper M, Miller DG, Wong GYC, Osborne MP. Long-term responses of women to indole-3-carbinol or a high fiber diet. *Cancer Epidemiol Biomarkers Prev* 1994; 3: 591–5.

- 9 McAlindon TE, Gulin J, Chen T, Klug T, Lahita R, Nuite M. Indole-3-carbinol in women with SLE: effect on estrogen metabolism and disease activity. *Lupus* 2001; 10: 779–83.
- 10 Michnovicz JJ. Increased estrogen 2-hydroxylation in obese women using oral indole-3-carbinol. *Int J Obes* 1998; 22: 227–9.
- 11 Michnovicz JJ, Adlercreutz H, Bradlow HL. Changes in levels of urinary estrogen metabolites after oral indole-3-carbinol treatment in humans. *J Natl Cancer Inst* 1997; 89: 718–23.
- 12 Michnovicz JJ, Bradlow HL. Altered estrogen metabolism and excretion in humans following consumption of indole-3-carbinol. *Nutr Cancer* 1991; 16: 59–66.
- 13 Rosen CA, Bryson PC. Indole-3-carbinol for recurrent respiratory papillomatosis: long-term results. *J Voice* 2004; 18: 248–53.
- 14 Naik R, Nixon S, Lopes A, Godfrey K, Hatem MH, Monaghan JM. A randomized phase II trial of indole-3-carbinol in the treatment of vulvar intraepithelial neoplasia. *Int J Gynecol Cancer* 2006; 16: 786–90.
- 15 Wong GYC, Bradlow L, Sepkovic D, Mehl S, Mailman J, Osborne MP. Dose-ranging study of indole-3-carbinol for breast cancer prevention. *J Cell Biochem Suppl* 1997; 28/29: 111–6.
- 16 Reed GA, Peterson KS, Smith HJ, Gray JC, Sullivan DK, Mayo MS, *et al*. A phase I study of indole-3-carbinol in women: tolerability and effects. *Cancer Epidemiol Biomarkers Prev* 2005; 14: 1953–60.
- 17 Shertzer HG, Berger ML, Tabor MW. Intervention in free-radical mediated hepatotoxicity and lipid-peroxidation by indole-3-carbinol. *Biochem Pharmacol* 1988; 37: 333–8.
- 18 Stresser DM, Williams DE, Griffin DA, Bailey GS. Mechanisms of tumor modulation by indole-3-carbinol. Disposition and excretion in male Fischer 344 rats. *Drug Metab Disp* 1995; 23: 965–75.
- 19 Yu Z, Mahadevan B, Lohr CV, Fischer KA, Louderback MA, Krueger SK, *et al*. Indole-3-carbinol in the maternal diet provides chemoprotection for the fetus against transplacental carcinogenesis by the polycyclic aromatic hydrocarbon, dibenzo[a, l]pyrene. *Carcinogenesis* 2006; 27: 2116–23.
- 20 Anderton MJ, Manson MM, Verschoyle RD, Gescher A, Lamb JH, Farmer PB, *et al*. Pharmacokinetics and tissue disposition of indole-3-carbinol and its acid condensation products after oral administration to mice. *Clin Cancer Res* 2004; 10: 5233–41.
- 21 Anderton MJ, Manson MM, Verschoyle R, Gescher A, Steward WP, Williams ML, *et al*. Physiological modeling of formulated and crystalline 3,3'-diindolylmethane pharmacokinetics following oral administration in mice. *Drug Metab Disp* 2004; 32: 632–8.
- 22 Crowell JA, Page JG, Levine BS, Tomlinson MJ, Hebert CD. Indole-3-carbinol, but not its major digestive product 3,3'-diindolylmethane, induces reversible hepatocyte hypertrophy and cytochromes P450. *Toxicol Appl Pharmacol* 2006; 211: 115–23.
- 23 Donald S, Verschoyle RD, Greaves P, Colombo T, Zuchetti M, Falcioni C, *et al*. Dietary agent indole-3-carbinol protects female rats against the hepatotoxicity of the antitumor drug ET-743 (trabectedin) without compromising efficacy in a rat mammary carcinoma. *Int J Cancer* 2004; 111: 961–7.
- 24 Leibelt DA, Hedstrom OR, Fischer KA, Pereira CB, Williams DE. Evaluation of chronic dietary exposure to indole-3-carbinol and absorption-enhanced 3,3'-diindolylmethane in sprague-dawley rats. *Toxicol Sci* 2003; 74: 10–21.
- 25 Zhang X, Malejka-Giganti D. Effects of treatment of rats with indole-3-carbinol on apoptosis in the mammary gland and mammary adenocarcinomas. *Anticancer Res* 2003; 23: 2473–79.
- 26 Parkin DR, Malejka-Giganti D. Differences in the hepatic P450-dependent metabolism of estrogen and tamoxifen in response to treatment of rats with 3,3'-diindolylmethane and its parent compound indole-3-carbinol. *Cancer Detect Prev* 2004; 28: 72–9.
- 27 Chen I, McDougal A, Wang F, Safe S. Aryl hydrocarbon receptor-mediated antiestrogenic and antitumorigenic activity of diindolylmethane. *Carcinogenesis* 1998; 19: 1631–39.
- 28 Hestermann EV, Brown M. Agonist and chemopreventative ligands induce differential transcriptional cofactor recruitment by aryl hydrocarbon receptor. *Mol Cell Biol* 2003; 23: 7920–25.
- 29 Liu S, Abdelrahim M, Khan S, Ariazi E, Jordan VC, Safe S. Aryl hydrocarbon receptor agonists directly activate estrogen receptor alpha in MCF-7 breast cancer cells. *Biol Chem* 2006; 387: 1209–13.
- 30 Jellinek PH, Forkert P-G, Riddick DS, Okey AB, Michnovicz JJ, Bradlow HL. Ah receptor binding properties of indole carbinols and induction of hepatic estradiol hydroxylation. *Biochem Pharmacol* 1993; 45: 1129–36.
- 31 Li Y, Li X, Sarkar FH. Gene expression profiles of I3C- and DIM-treated PC3 human prostate cancer cells determined by cDNA microarray analysis. *J Nutr* 2003; 133: 1011–19.
- 32 Meng Q, Yuan F, Goldberg ID, Rosen EM, Auburn K, Fan S. Indole-3-carbinol is a negative regulator of estrogen receptor- α signalling in human tumor cells. *J Nutr* 2000; 130: 2927–31.
- 33 Sundar SN, Kerekatte V, Equinzio CN, Doan VB, Bjeldanes LF, Firestone GL. Indole-3-carbinol selectively uncouples expression and activity of estrogen receptor subtypes in human breast cancer cells. *Mol Endocrinol* 2006; 20: 3070–82.
- 34 Riby JE, Chang GHF, Firestone GL, Bjeldanes LF. Ligand-independent activation of estrogen receptor function by 3,3'-diindolylmethane in human breast cancer cells. *Biochem Pharmacol* 2000; 60: 167–77.
- 35 Leong H, Firestone GL, Bjeldanes LF. Cytostatic effects of 3,3'-diindolylmethane in human endometrial cancer cells result from an estrogen receptor-mediated increase in transforming growth factor- α expression. *Carcinogenesis* 2001; 22: 1809–17.
- 36 Leong H, Riby JE, Firestone GL, Bjeldanes LF. Potent ligand-independent estrogen receptor activation by 3,3'-diindolylmethane is mediated by cross talk between the protein kinase A and mitogen-activated protein kinase signaling pathways. *Mol Endocrinol* 2004; 18: 291–302.
- 37 Wang TT, Milner MJ, Milner JA, Kim YS. Estrogen receptor alpha as a target for indole-3-carbinol. *J Nutr Biochem* 2006; 17: 659–64.
- 38 Le HT, Schaldach CM, Firestone GL, Bjeldanes LF. Plant-derived 3,3'-diindolylmethane is a strong androgen antagonist in human prostate cancer cells. *J Biol Chem* 2003; 278: 21136–45.
- 39 Hsu JC, Zhang J, Dev A, Wing A, Bjeldanes LF, Firestone GL.

- Indole-3-carbinol inhibition of androgen receptor expression and down-regulation of androgen responsiveness in human prostate cancer cells. *Carcinogenesis* 2005; 26: 1896–904.
- 40 Moiseeva EP, Fox LH, Howells LM, Temple LA, Manson MM. Indole-3-carbinol-induced death in cancer cells involves EGFR downregulation and is exacerbated in a 3D environment. *Apoptosis* 2006; 11: 799–812.
- 41 Moiseeva EP, Heukers R, Manson MM. EGFR and Src are involved in indole-3-carbinol-induced death and cell cycle arrest of human breast cancer cells. *Carcinogenesis* 2007; 28: 435–45.
- 42 Howells LM, Hudson EA, Manson MM. Inhibition of phosphatidylinositol 3-kinase/protein kinase B signaling is not sufficient to account for indole-3-carbinol-induced apoptosis in some breast and prostate tumor cells. *Clin Cancer Res* 2005; 11: 8521–27.
- 43 Fan S, Meng Q, Auburn K, Carter T, Rosen EM. BRCA1 and BRCA2 as molecular targets for phytochemicals indole-3-carbinol and genistein in breast and prostate cancer cells. *Br J Cancer* 2006; 94: 407–26.
- 44 Gong Y, Firestone GL, Bjeldanes LF. 3,3'-diindolylmethane is a novel topoisomerase II α catalytic inhibitor that induces S-phase retardation and mitotic delay in human hepatoma HepG2 cells. *Mol Pharmacol* 2006; 69: 1320–27.
- 45 Gong Y, Sohn H, Xue L, Firestone GL, Bjeldanes LF. 3,3'-Diindolylmethane is a novel mitochondrial H(+)-ATP synthase inhibitor that can induce p21(Cip1/Waf1) expression by induction of oxidative stress in human breast cancer cells. *Cancer Res* 2006; 66: 4880–87.
- 46 Abdelrahim M, Newman K, Vanderlaag K, Samudio I, Safe S. 3,3'-diindolylmethane (DIM) and its derivatives induce apoptosis in pancreatic cancer cells through endoplasmic reticulum stress-dependent upregulation of DR5. *Carcinogenesis* 2006; 27: 717–28.
- 47 Savino JA, 3rd, Evans JF, Rabinowitz D, Auburn KJ, Carter TH. Multiple, disparate roles for calcium signaling in apoptosis of human prostate and cervical cancer cells exposed to diindolylmethane. *Mol Cancer Ther* 2006; 5: 556–63.
- 48 Xue L, Firestone GL, Bjeldanes LF. DIM stimulates IFN γ gene expression in human breast cancer cells via the specific activation of JNK and p38 pathways. *Oncogene* 2005; 24: 2343–53.
- 49 Rahman KMW, Li Y, Sarkar FH. Inactivation of Akt and NF- κ B play important roles during indole-3-carbinol-induced apoptosis in breast cancer cells. *Nutr Cancer* 2004; 48: 84–94.
- 50 Rahman KMW, Sarkar FH. Inhibition of nuclear translocation of nuclear factor- κ B contributes to 3,3'-diindolylmethane-induced apoptosis in breast cancer cells. *Cancer Res* 2005; 65: 364–71.
- 51 Li Y, Chinni SR, Sarkar FH. Selective growth regulatory and pro-apoptotic effects of DIM is mediated by AKT and NF- κ B pathways in prostate cancer cells. *Front Biosci* 2005; 10: 236–43.
- 52 Takada Y, Andreeff M, Aggarwal BB. Indole-3-carbinol suppresses NF- κ B and I κ B α kinase activation causing inhibition of expression of NF- κ B-regulated anti-apoptotic and metastatic gene products and enhancement of apoptosis in myeloid and leukemia cells. *Blood* 2005; 106: 641–9.
- 53 Bhuiyan MM, Li Y, Banerjee S, Ahmed F, Wang Z, Ali S, *et al*. Down-regulation of androgen receptor by 3,3'-diindolylmethane contributes to inhibition of cell proliferation and induction of apoptosis in both hormone-sensitive LNCaP and insensitive C4-2B prostate cancer cells. *Cancer Res* 2006; 66: 10 064–72.
- 54 IARC. Handbooks of cancer prevention; No 9. Cruciferous vegetables, isothiocyanates and indoles. Lyon: IARC Press; 2004.
- 55 Grubbs CJ, Steele VE, Casebolt T, Juliana MM, Eto I, Whitaker LM, *et al*. Chemoprevention of chemically-induced mammary carcinogenesis by indole-3-carbinol. *Anticancer Res* 1995; 15: 709–16.
- 56 Chen DZ, Qi M, Auburn KJ, Carter TH. Indole-3-carbinol and diindolylmethane induce apoptosis of human cervical cancer cells and in murine HPV-16 transgenic preneoplastic cervical epithelium. *J Nutr* 2001; 131: 3294–302.
- 57 Bradlow HL, Michnovicz JJ, Telang NT, Osborne MP. Effects of dietary indole-3-carbinol on estradiol metabolism and spontaneous mammary tumors in mice. *Carcinogenesis* 1991; 12: 1571–74.
- 58 McMahan M, Itoh K, Yamamoto M, Chanas SA, Henderson CJ, McLellan LI, *et al*. The Cap'n'Collar basic leucine zipper transcription factor Nrf2 (NF-E2 p45-related factor 2) controls both constitutive and inducible expression of intestinal detoxification and glutathione biosynthetic enzymes. *Cancer Res* 2001; 61: 3299–307.
- 59 Chanas SA, Jiang Q, McMahan M, McWalter GK, McLellan LI, Elcombe CR, *et al*. Loss of the Nrf2 transcription factor causes a marked reduction in constitutive and inducible expression of the glutathione S-transferase Gsta1, Gsta2, Gstm1, Gstm2, Gstm3 and Gstm4 genes in the livers of male and female mice. *Biochem J* 2002; 365: 405–16.
- 60 Katchamart S, Stresser DM, Dehal SS, Kupfer D, Williams DE. Concurrent flavin-containing monooxygenase down-regulation and cytochrome P-450 induction by dietary indoles in rat: implications for drug-drug interaction. *Drug Metab Disp* 2000; 28: 930–6.
- 61 Katchamart S, Williams DE. Indole-3-carbinol modulation of hepatic monooxygenases CYP1A1, CYP1A2 and FMO1 in guinea pig, mouse and rabbit. *Comp Biochem Physiol Part C* 2001; 129: 377–84.
- 62 Manson MM, Hudson EA, Ball HWL, Barrett MC, Clark HL, Judah DJ, *et al*. Chemoprevention of aflatoxin B $_1$ -induced carcinogenesis by indole-3-carbinol in rat liver - predicting the outcome using early biomarkers. *Carcinogenesis* 1998; 19: 1829–36.
- 63 Park J-Y, Bjeldanes LF. Organ-selective induction of cytochrome P-450-dependent activities by indole-3-carbinol-derived products: influence on covalent binding of benzo[a]pyrene to hepatic and pulmonary DNA in the rat. *Chem Biol Interact* 1992; 83: 235–47.
- 64 Hudson EA, Howells LM, Gallacher-Horley B, Fox LH, Gescher A, Manson MM. Growth-inhibitory effects of the chemopreventive agent indole-3-carbinol are increased in combination with the polyamine putrescine in the SW480 colon tumour cell line. *BMC Cancer* 2003; 3: 2.
- 65 Rahman KM, Sarkar FH, Banerjee S, Wang Z, Liao DJ, Hong X, *et al*. Therapeutic intervention of experimental breast cancer bone metastasis by indole-3-carbinol in SCID-human mouse model. *Mol Cancer Ther* 2006; 5: 2747–56.

- 66 Arora A, Shukla Y. Modulation of vinca-alkaloid induced P-glycoprotein expression by indole-3-carbinol. *Cancer Lett* 2003; 189: 167–73.
- 67 Arora A, Seth K, Kalra N, Shukla Y. Modulation of P-glycoprotein-mediated multidrug-resistance in K562 leukemic cells by indole-3-carbinol. *Toxicol Appl Pharmacol* 2005; 202: 237–43.
- 68 Christensen JG, LeBlanc GA. Reversal of multidrug resistance *in vivo* by dietary administration of the phytochemical indole-3-carbinol. *Cancer Res* 1996; 56: 574–81.
- 69 Auburn KJ, Fan S, Rosen EM, Goodwin L, Chandraskaren A, Williams DE, *et al*. Indole-3-carbinol is a negative regulator of estrogen. *J Nutr* 2003; 133: S2470–5.
- 70 Cover CM, Hsieh SJ, Cram EJ, Hong C, Riby JE, Bjeldanes LF, *et al*. Indole-3-carbinol and tamoxifen cooperate to arrest the cell cycle of MCF-7 human breast cancer cells. *Cancer Res* 1999; 59: 1244–51.
- 71 Malejka-Giganti D, Parkin DR, Bennett KK, Lu Y, Decker RW, Niehans GA, *et al*. Suppression of mammary gland carcinogenesis by post-initiation treatment of rats with tamoxifen or indole-3-carbinol or their combination. *Eur J Cancer Prev* 2007; 16: 130–41.
- 72 Nho CW, Jeffery E. The synergistic upregulation of phase II detoxification enzymes by glucosinolate breakdown products in cruciferous vegetables. *Toxicol Appl Pharmacol* 2001; 174: 146–52.
- 73 Ireson C, Orr S, Jones DJ, Verschoyle R, Lim CK, Luo JL, *et al*. Characterization of metabolites of the chemopreventive agent curcumin in human and rat hepatocytes and in the rat *in vivo*, and evaluation of their ability to inhibit phorbol ester-induced prostaglandin E2 production. *Cancer Res* 2001; 61: 1058–64.
- 74 Ireson CR, Jones DJ, Orr S, Coughtrie MW, Boocock DJ, Williams ML, *et al*. Metabolism of the cancer chemopreventive agent curcumin in human and rat intestine. *Cancer Epidemiol Biomarkers Prev* 2002; 11: 105–11.
- 75 Cheng AL, Hsu CH, Lin JK, Hsu MM, Ho YF, Shen TS, *et al*. Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions. *Anticancer Res* 2001; 21: 2895–900.
- 76 Sharma RA, Euden SA, Platton SL, Cooke DN, Shafayat A, Hewitt HR, *et al*. Phase I clinical trial of oral curcumin: biomarkers of systemic activity and compliance. *Clin Cancer Res* 2004; 10: 6847–54.
- 77 Garcea G, Berry DP, Jones DJ, Singh R, Dennison AR, Farmer PB, *et al*. Consumption of the putative chemopreventive agent curcumin by cancer patients: assessment of curcumin levels in the colorectum and their pharmacodynamic consequences. *Cancer Epidemiol Biomarkers Prev* 2005; 14: 120–5.
- 78 Sharma RA, Ireson CR, Verschoyle RD, Hill KA, Williams ML, Leuratti C, *et al*. Effects of dietary curcumin on glutathione S-transferase and malondialdehyde-DNA adducts in rat liver and colon mucosa: relationship with drug levels. *Clin Cancer Res* 2001; 7: 1452–8.
- 79 Perkins S, Verschoyle RD, Hill K, Parveen I, Threadgill MD, Sharma RA, *et al*. Chemopreventive efficacy and pharmacokinetics of curcumin in the min/+ mouse, a model of familial adenomatous polyposis. *Cancer Epidemiol Biomarkers Prev* 2002; 11: 535–40.
- 80 Ravindranath V, Chandrasekhara N. Absorption and tissue distribution of curcumin in rats. *Toxicol* 1980; 16: 259–65.
- 81 Marczylo TH, Verschoyle RD, Cooke DN, Morazzoni P, Steward WP, Gescher AJ. Comparison of systemic availability of curcumin with that of curcumin formulated with phosphatidylcholine. *Cancer Chemother Pharmacol* 2006; 60: 171–7.
- 82 Liu A, Lou H, Zhao L, Fan P. Validated LC/MS/MS assay for curcumin and tetrahydrocurcumin in rat plasma and application to pharmacokinetic study of phospholipid complex of curcumin. *J Pharm Biomed Anal* 2006; 40: 720–7.
- 83 Maiti K, Mukherjee K, Gantait A, Saha BP, Mukherjee PK. Curcumin-phospholipid complex: Preparation, therapeutic evaluation and pharmacokinetic study in rats. *Int J Pharm* 2007; 330: 155–63.
- 84 Li L, Ahmed B, Mehta K, Kurzrock R. Liposomal curcumin with and without oxaliplatin: effects on cell growth, apoptosis, and angiogenesis in colorectal cancer. *Mol Cancer Ther* 2007; 6: 1276–82.
- 85 Li L, Braith FS, Kurzrock R. Liposome-encapsulated curcumin: *in vitro* and *in vivo* effects on proliferation, apoptosis, signaling, and angiogenesis. *Cancer* 2005; 104: 1322–31.
- 86 Bisht S, Feldmann G, Soni S, Ravi R, Karikari C, Maitra A, *et al*. Polymeric nanoparticle-encapsulated curcumin (nanocurcumin): a novel strategy for human cancer therapy. *J Nanobiotechnol* 2007; 5: 3.
- 87 Aggarwal BB, Kumar A, Bharti AC. Anticancer potential of curcumin: preclinical and clinical studies. *Anticancer Res* 2003; 23: 363–98.
- 88 Elattar TM, Virji AS. The inhibitory effect of curcumin, genistein, quercetin and cisplatin on the growth of oral cancer cells *in vitro*. *Anticancer Res* 2000; 20: 1733–8.
- 89 Squires MS, Hudson EA, Howells L, Sale S, Houghton CE, Jones JL, *et al*. Relevance of mitogen activated protein kinase (MAPK) and phosphatidylinositol-3-kinase/protein kinase B (PI3K/PKB) pathways to induction of apoptosis by curcumin in breast cells. *Biochem Pharmacol* 2003; 65: 361–76.
- 90 Samaha HS, Kelloff GJ, Steele V, Rao CV, Reddy BS. Modulation of apoptosis by sulindac, curcumin, phenylethyl-3-methylcaffeate, and 6-phenylhexyl isothiocyanate: apoptotic index as a biomarker in colon cancer chemoprevention and promotion. *Cancer Res* 1997; 57: 1301–5.
- 91 Rao CV, Rivenson A, Simi B, Reddy BS. Chemoprevention of colon carcinogenesis by dietary curcumin, a naturally occurring plant phenolic compound. *Cancer Res* 1995; 55: 259–66.
- 92 Mahmoud NN, Carothers AM, Grunberger D, Bilinski RT, Churchill MR, Martucci C, *et al*. Plant phenolics decrease intestinal tumors in an animal model of familial adenomatous polyposis. *Carcinogenesis* 2000; 21: 921–7.
- 93 Chuang SE, Cheng AL, Lin JK, Kuo ML. Inhibition by curcumin of diethylnitrosamine-induced hepatic hyperplasia, inflammation, cellular gene products and cell-cycle-related proteins in rats. *Food Chem Toxicol* 2000; 38: 991–5.
- 94 Kunnumakkara AB, Guha S, Krishnan S, Diagaradjane P, Gelovani J, Aggarwal BB. Curcumin potentiates antitumor activity of gemcitabine in an orthotopic model of pancreatic cancer through suppression of proliferation, angiogenesis, and inhibition of nuclear factor- κ B-regulated gene products. *Cancer Res* 2007; 67: 3853–61.

- 95 Inano H, Onoda M. Prevention of radiation-induced mammary tumors. *Int J Radiat Oncol Biol Phys* 2002; 52: 212–23.
- 96 Frank N, Knauff J, Amelung F, Nair J, Wesch H, Bartsch H. No prevention of liver and kidney tumors in Long-Evans Cinnamon rats by dietary curcumin, but inhibition at other sites and of metastases. *Mutat Res* 2003; 523-524: 127–35.
- 97 Menon LG, Kuttan R, Kuttan G. Inhibition of lung metastasis in mice induced by B16F10 melanoma cells by polyphenolic compounds. *Cancer Lett* 1995; 95: 221–5.
- 98 Busquets S, Carbo N, Almendro V, Quiles MT, Lopez-Soriano FJ, Argiles JM. Curcumin, a natural product present in turmeric, decreases tumor growth but does not behave as an anticachectic compound in a rat model. *Cancer Lett* 2001; 167: 33–8.
- 99 Verma SP, Salamone E, Goldin B. Curcumin and genistein, plant natural products, show synergistic inhibitory effects on the growth of human breast cancer MCF-7 cells induced by estrogenic pesticides. *Biochem Biophys Res Commun* 1997; 233: 692–6.
- 100 Lev-Ari S, Zinger H, Kazanov D, Yona D, Ben-Yosef R, Starr A, *et al*. Curcumin synergistically potentiates the growth inhibitory and pro-apoptotic effects of celecoxib in pancreatic adenocarcinoma cells. *Biomed Pharmacother* 2005; 59 (Suppl 2): S276–80.
- 101 Lev-Ari S, Strier L, Kazanov D, Madar-Shapiro L, Dvory-Sobol H, Pinchuk I, *et al*. Celecoxib and curcumin synergistically inhibit the growth of colorectal cancer cells. *Clin Cancer Res* 2005; 11: 6738–44.
- 102 Khafif A, Schantz SP, Chou TC, Edelstein D, Sacks PG. Quantitation of chemopreventive synergism between (-)-epigallocatechin-3-gallate and curcumin in normal, premalignant and malignant human oral epithelial cells. *Carcinogenesis* 1998; 19: 419–24.
- 103 Li N, Chen X, Liao J, Yang G, Wang S, Josephson Y, *et al*. Inhibition of 7,12-dimethylbenz[a]anthracene (DMBA)-induced oral carcinogenesis in hamsters by tea and curcumin. *Carcinogenesis* 2002; 23: 1307–13.
- 104 Deeb D, Xu YX, Jiang H, Gao X, Janakiraman N, Chapman RA, *et al*. Curcumin (diferuloyl-methane) enhances tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis in LNCaP prostate cancer cells. *Mol Cancer Ther* 2003; 2: 95–103.
- 105 Howells LM, Mitra A, Manson MM. Comparison of oxaliplatin- and curcumin-mediated antiproliferative effects in colorectal cell lines. *Int J Cancer* 2007; 121: 175–83.
- 106 Kamat AM, Sethi G, Aggarwal BB. Curcumin potentiates the apoptotic effects of chemotherapeutic agents and cytokines through down-regulation of nuclear factor-kappaB and nuclear factor-kappaB-regulated gene products in IFN-alpha-sensitive and IFN-alpha-resistant human bladder cancer cells. *Mol Cancer Ther* 2007; 6: 1022–30.
- 107 Somasundaram S, Edmund NA, Moore DT, Small GW, Shi YY, Orłowski RZ. Dietary curcumin inhibits chemotherapy-induced apoptosis in models of human breast cancer. *Cancer Res* 2002; 62: 3868–75.
- 108 Lambert JD, Yang CS. Mechanisms of cancer prevention by tea constituents. *J Nutr* 2003; 133: S3262–7.
- 109 Chow HH, Cai Y, Alberts DS, Hakim I, Dorr R, Shahi F, *et al*. Phase I pharmacokinetic study of tea polyphenols following single-dose administration of epigallocatechin gallate and polyphenon E. *Cancer Epidemiol Biomarkers Prev* 2001; 10: 53–8.
- 110 Nakagawa K, Okuda S, Miyazawa T. Dose-dependent incorporation of tea catechins, (-)-epigallocatechin-3-gallate and (-)-epigallocatechin, into human plasma. *Biosci Biotechnol Biochem* 1997; 61: 1981–5.
- 111 Unno T, Kondo K, Itakura H, Takeo T. Analysis of (-)-epigallocatechin gallate in human serum obtained after ingesting green tea. *Biosci Biotechnol Biochem* 1996; 60: 2066–68.
- 112 Chow HH, Cai Y, Hakim IA, Crowell JA, Shahi F, Brooks CA, *et al*. Pharmacokinetics and safety of green tea polyphenols after multiple-dose administration of epigallocatechin gallate and polyphenon E in healthy individuals. *Clin Cancer Res* 2003; 9: 3312–9.
- 113 Dvorakova K, Dorr RT, Valcic S, Timmermann B, Alberts DS. Pharmacokinetics of the green tea derivative, EGCG, by the topical route of administration in mouse and human skin. *Cancer Chemother Pharmacol* 1999; 43: 331–5.
- 114 Yang CS, Lee MJ, Chen L. Human salivary tea catechin levels and catechin esterase activities: implication in human cancer prevention studies. *Cancer Epidemiol Biomarkers Prev* 1999; 8: 83–9.
- 115 Yang CS, Kim S, Yang GY, Lee MJ, Liao J, Chung JY, *et al*. Inhibition of carcinogenesis by tea: bioavailability of tea polyphenols and mechanisms of actions. *Proc Soc Exp Biol Med* 1999; 220: 213–7.
- 116 Fang JY, Hung CF, Hwang TL, Huang YL. Physicochemical characteristics and *in vivo* deposition of liposome-encapsulated tea catechins by topical and intratumor administrations. *J Drug Target* 2005; 13: 19–27.
- 117 Fang JY, Lee WR, Shen SC, Huang YL. Effect of liposome encapsulation of tea catechins on their accumulation in basal cell carcinomas. *J Dermatol Sci* 2006; 42: 101–9.
- 118 Lambert JD, Hong J, Kim DH, Mishin VM, Yang CS. Piperine enhances the bioavailability of the tea polyphenol (-)-epigallocatechin-3-gallate in mice. *J Nutr* 2004; 134: 1948–52.
- 119 Kohri T, Nanjo F, Suzuki M, Seto R, Matsumoto N, Yamakawa M, *et al*. Synthesis of (-)-[4-³H]epigallocatechin gallate and its metabolic fate in rats after intravenous administration. *J Agric Food Chem* 2001; 49: 1042–8.
- 120 Vaidyanathan JB, Walle T. Transport and metabolism of the tea flavonoid (-)-epicatechin by the human intestinal cell line Caco-2. *Pharm Res* 2001; 18: 1420–5.
- 121 Sukanuma M, Okabe S, Oniyama M, Tada Y, Ito H, Fujiki H. Wide distribution of [³H](-)-epigallocatechin gallate, a cancer preventive tea polyphenol, in mouse tissue. *Carcinogenesis* 1998; 19: 1771–6.
- 122 Cai Y, Anavy ND, Chow HH. Contribution of presystemic hepatic extraction to the low oral bioavailability of green tea catechins in rats. *Drug Metab Dispos* 2002; 30: 1246–9.
- 123 Baliga MS, Meleth S, Katiyar SK. Growth inhibitory and antimetastatic effect of green tea polyphenols on metastasis-specific mouse mammary carcinoma 4T1 cells *in vitro* and *in vivo* systems. *Clin Cancer Res* 2005; 11: 1918–27.
- 124 Lu YP, Lou YR, Xie JG, Peng QY, Liao J, Yang CS, *et al*. Topical applications of caffeine or (-)-epigallocatechin gallate (EGCG) inhibit carcinogenesis and selectively increase apoptosis

- in UVB-induced skin tumors in mice. *Proc Natl Acad Sci USA* 2002; 99: 12 455–60.
- 125 Ogasawara M, Matsunaga T, Suzuki H. Differential effects of antioxidants on the *in vitro* invasion, growth and lung metastasis of murine colon cancer cells. *Biol Pharm Bull* 2007; 30: 200–4.
- 126 Schwartz JL, Baker V, Larios E, Chung FL. Molecular and cellular effects of green tea on oral cells of smokers: a pilot study. *Mol Nutr Food Res* 2005; 49: 43–51.
- 127 Dong Z. Effects of food factors on signal transduction pathways. *Biofactors* 2000; 12: 17–28.
- 128 Cao Y, Cao R. Angiogenesis inhibited by drinking tea. *Nature* 1999; 398: 381.
- 129 Klaunig JE, Xu Y, Han C, Kamendulis LM, Chen J, Heiser C, *et al*. The effect of tea consumption on oxidative stress in smokers and nonsmokers. *Proc Soc Exp Biol Med* 1999; 220: 249–54.
- 130 Bettuzzi S, Brausi M, Rizzi F, Castagnetti G, Peracchia G, Corti A. Chemoprevention of human prostate cancer by oral administration of green tea catechins in volunteers with high-grade prostate intraepithelial neoplasia: a preliminary report from a one-year proof-of-principle study. *Cancer Res* 2006; 66: 1234–40.
- 131 Suganuma M, Okabe S, Kai Y, Sueoka N, Sueoka E, Fujiki H. Synergistic effects of (–)-epigallocatechin gallate with (–)-epicatechin, sulindac, or tamoxifen on cancer-preventive activity in the human lung cancer cell line PC-9. *Cancer Res* 1999; 59: 44–7.
- 132 Adhami VM, Malik A, Zaman N, Sarfaraz S, Siddiqui IA, Syed DN, *et al*. Combined inhibitory effects of green tea polyphenols and selective cyclooxygenase-2 inhibitors on the growth of human prostate cancer cells both *in vitro* and *in vivo*. *Clin Cancer Res* 2007; 13: 1611–9.
- 133 Masuda M, Suzui M, Weinstein IB. Effects of epigallocatechin-3-gallate on growth, epidermal growth factor receptor signaling pathways, gene expression, and chemosensitivity in human head and neck squamous cell carcinoma cell lines. *Clin Cancer Res* 2001; 7: 4220–9.
- 134 Masuda M, Suzui M, Lim JT, Weinstein IB. Epigallocatechin-3-gallate inhibits activation of HER-2/neu and downstream signaling pathways in human head and neck and breast carcinoma cells. *Clin Cancer Res* 2003; 9: 3486–91.
- 135 Orner GA, Dashwood WM, Blum CA, Diaz GD, Li Q, Dashwood RH. Suppression of tumorigenesis in the Apc(min) mouse: down-regulation of beta-catenin signaling by a combination of tea plus sulindac. *Carcinogenesis* 2003; 24: 263–7.
- 136 Ohishi T, Kishimoto Y, Miura N, Shiota G, Kohri T, Hara Y, *et al*. Synergistic effects of (–)-epigallocatechin gallate with sulindac against colon carcinogenesis of rats treated with azoxymethane. *Cancer Lett* 2002; 177: 49–56.
- 137 Aggarwal BB, Bhardwaj A, Aggarwal RS, Seeram NP, Shishodia S, Takada Y. Role of resveratrol in prevention and therapy of cancer: preclinical and clinical studies. *Anticancer Res* 2004; 24: 2783–840.
- 138 Baur JA, Sinclair DA. Therapeutic potential of resveratrol: the *in vivo* evidence. *Nat Rev Drug Discov* 2006; 5: 493–506.
- 139 Bhat KP, Pezzuto JM. Cancer chemopreventive activity of resveratrol. *Ann N Y Acad Sci* 2002; 957: 210–29.
- 140 Asensi M, Medina I, Ortega A, Carretero J, Bano MC, Obrador E, *et al*. Inhibition of cancer growth by resveratrol is related to its low bioavailability. *Free Radic Biol Med* 2002; 33: 387–98.
- 141 de Santi C, Pietrabissa A, Mosca F, Pacifici GM. Glucuronidation of resveratrol, a natural product present in grape and wine, in the human liver. *Xenobiotica* 2000; 30: 1047–54.
- 142 Fremont L. Biological effects of resveratrol. *Life Sci* 2000; 66: 663–73.
- 143 Boocock DJ, Faust GES, Patel KR, Schinas AM, Brown VA, Ducharme MP, *et al*. Phase I dose escalation pharmacokinetic study in healthy volunteers of resveratrol, a potential cancer chemopreventive agent. *Cancer Epidemiol Biomarkers Prev* 2007; 16:1246–52.
- 144 Boocock DJ, Patel KR, Faust GE, Normolle DP, Marczylo TH, Crowell JA, *et al*. Quantitation of trans-resveratrol and detection of its metabolites in human plasma and urine by high performance liquid chromatography. *J Chromatogr B Analyt Technol Biomed Life Sci* 2007; 848: 182–7.
- 145 Goldberg DM, Yan J, Soleas GJ. Absorption of three wine-related polyphenols in three different matrices by healthy subjects. *Clin Biochem* 2003; 36: 79–87.
- 146 Wang LX, Heredia A, Song H, Zhang Z, Yu B, Davis C, *et al*. Resveratrol glucuronides as the metabolites of resveratrol in humans: characterization, synthesis, and anti-HIV activity. *J Pharm Sci* 2004; 93: 2448–57.
- 147 Yu C, Shin YG, Chow A, Li Y, Kosmeder JW, Lee YS, *et al*. Human, rat, and mouse metabolism of resveratrol. *Pharm Res* 2002; 19: 1907–14.
- 148 Miksits M, Maier-Salamon A, Aust S, Thalhammer T, Reznicek G, Kunert O, *et al*. Sulfation of resveratrol in human liver: evidence of a major role for the sulfotransferases SULT1A1 and SULT1E1. *Xenobiotica* 2005; 35: 1101–19.
- 149 Sabolovic N, Humbert AC, Radomska-Pandya A, Magdalou J. Resveratrol is efficiently glucuronidated by UDP-glucuronosyltransferases in the human gastrointestinal tract and in Caco-2 cells. *Biopharm Drug Dispos* 2006; 27: 181–9.
- 150 De Santi C, Pietrabissa A, Spisni R, Mosca F, Pacifici GM. Sulphation of resveratrol, a natural compound present in wine, and its inhibition by natural flavonoids. *Xenobiotica* 2000; 30: 857–66.
- 151 Regev-Shoshani G, Shoseyov O, Bilkis I, Kerem Z. Glycosylation of resveratrol protects it from enzymic oxidation. *Biochem J* 2003; 374: 157–63.
- 152 Kuhnle G, Spencer JP, Chowrimootoo G, Schroeter H, Debnam ES, Srai SK, *et al*. Resveratrol is absorbed in the small intestine as resveratrol glucuronide. *Biochem Biophys Res Commun* 2000; 272: 212–7.
- 153 Jannin B, Menzel M, Berlot JP, Delmas D, Lancon A, Latruffe N. Transport of resveratrol, a cancer chemopreventive agent, to cellular targets: plasmatic protein binding and cell uptake. *Biochem Pharmacol* 2004; 68: 1113–8.
- 154 Marier JF, Vachon P, Gritsas A, Zhang J, Moreau JP, Ducharme MP. Metabolism and disposition of resveratrol in rats: extent of absorption, glucuronidation, and enterohepatic recirculation evidenced by a linked-rat model. *J Pharmacol Exp Ther* 2002; 302: 369–73.
- 155 Vitrac X, Desmouliere A, Brouillaud B, Krisa S, Deffieux G, Barthe N, *et al*. Distribution of [¹⁴C]-trans-resveratrol, a cancer chemo-

- preventive polyphenol, in mouse tissues after oral administration. *Life Sci* 2003; 72: 2219–33.
- 156 Sale S, Verschoyle RD, Boocock D, Jones DJ, Wilsher N, Ruparelia KC, *et al*. Pharmacokinetics in mice and growth-inhibitory properties of the putative cancer chemopreventive agent resveratrol and the synthetic analogue trans 3,4,5,4'-tetramethoxystilbene. *Br J Cancer* 2004; 90: 736–44.
- 157 Abd El-Mohsen M, Bayele H, Kuhnle G, Gibson G, Debnam E, Kaila Srai S, *et al*. Distribution of [3H]trans-resveratrol in rat tissues following oral administration. *Brit J Nutr* 2006; 96: 62–70.
- 158 Wenzel E, Soldo T, Erbersdobler H, Somoza V. Bioactivity and metabolism of trans-resveratrol orally administered to Wistar rats. *Mol Nutr Food Res* 2005; 49: 482–94.
- 159 Poolman TM, Ng LL, Farmer PB, Manson MM. Inhibition of the respiratory burst by resveratrol in human monocytes: correlation with inhibition of PI3K signaling. *Free Radic Biol Med* 2005; 39: 118–32.
- 160 Whitehead TP, Robinson D, Allaway S, Syms J, Hale A. Effect of red wine ingestion on the antioxidant capacity of serum. *Clin Chem* 1995; 41: 32–5.
- 161 Garvin S, Ollinger K, Dabrosin C. Resveratrol induces apoptosis and inhibits angiogenesis in human breast cancer xenografts *in vivo*. *Cancer Lett* 2006; 231: 113–22.
- 162 Carbo N, Costelli P, Baccino FM, Lopez-Soriano FJ, Argiles JM. Resveratrol, a natural product present in wine, decreases tumour growth in a rat tumour model. *Biochem Biophys Res Commun* 1999; 254: 739–43.
- 163 Chen Y, Tseng SH, Lai HS, Chen WJ. Resveratrol-induced cellular apoptosis and cell cycle arrest in neuroblastoma cells and antitumor effects on neuroblastoma in mice. *Surgery* 2004; 136: 57–66.
- 164 Tseng SH, Lin SM, Chen JC, Su YH, Huang HY, Chen CK, *et al*. Resveratrol suppresses the angiogenesis and tumor growth of gliomas in rats. *Clin Cancer Res* 2004; 10: 2190–202.
- 165 Banerjee S, Bueso-Ramos C, Aggarwal BB. Suppression of 7,12-dimethylbenz(a)anthracene-induced mammary carcinogenesis in rats by resveratrol: role of nuclear factor-kappaB, cyclooxygenase 2, and matrix metalloproteinase 9. *Cancer Res* 2002; 62: 4945–54.
- 166 Martin AR, Villegas I, Sanchez-Hidalgo M, de la Lastra CA. The effects of resveratrol, a phytoalexin derived from red wines, on chronic inflammation induced in an experimentally induced colitis model. *Br J Pharmacol* 2006; 147: 873–85.
- 167 Sale S, Tunstall RG, Ruparelia KC, Potter GA, Steward WP, Gescher AJ. Comparison of the effects of the chemopreventive agent resveratrol and its synthetic analog trans 3,4,5,4'-tetramethoxystilbene (DMU-212) on adenoma development in the Apc(Min+) mouse and cyclooxygenase-2 in human-derived colon cancer cells. *Int J Cancer* 2005; 115: 194–201.
- 168 Provinciali M, Re F, Donnini A, Orlando F, Bartozzi B, Di Stasio G, *et al*. Effect of resveratrol on the development of spontaneous mammary tumors in HER-2/neu transgenic mice. *Int J Cancer* 2005; 115: 36–45.
- 169 Tessitore L, Davit A, Sarotto I, Caderni G. Resveratrol depresses the growth of colorectal aberrant crypt foci by affecting bax and p21(CIP) expression. *Carcinogenesis* 2000; 21: 1619–22.
- 170 Sengottuvelan M, Viswanathan P, Nalini N. Chemopreventive effect of trans-resveratrol — a phytoalexin against colonic aberrant crypt foci and cell proliferation in 1,2-dimethylhydrazine induced colon carcinogenesis. *Carcinogenesis* 2006; 27: 1038–46.
- 171 Kweon S, Kim Y, Choi H. Grape extracts suppress the formation of preneoplastic foci and activity of fatty acid synthase in rat liver. *Exp Mol Med* 2003; 35: 371–8.
- 172 Delmas D, Rebe C, Micheau O, Athias A, Gambert P, Grazide S, *et al*. Redistribution of CD95, DR4 and DR5 in rafts accounts for the synergistic toxicity of resveratrol and death receptor ligands in colon carcinoma cells. *Oncogene* 2004; 23: 8979–86.
- 173 Fulda S, Debatin KM. Sensitization for anticancer drug-induced apoptosis by the chemopreventive agent resveratrol. *Oncogene* 2004; 23: 6702–11.
- 174 Fulda S, Debatin KM. Resveratrol-mediated sensitisation to TRAIL-induced apoptosis depends on death receptor and mitochondrial signalling. *Eur J Cancer* 2005; 41: 786–98.
- 175 Kubota T, Uemura Y, Kobayashi M, Taguchi H. Combined effects of resveratrol and paclitaxel on lung cancer cells. *Anticancer Res* 2003; 23: 4039–46.
- 176 Rezk YA, Balulad SS, Keller RS, Bennett JA. Use of resveratrol to improve the effectiveness of cisplatin and doxorubicin: study in human gynecologic cancer cell lines and in rodent heart. *Am J Obstet Gynecol* 2006; 194: e23–6.
- 177 Awad AB, Burr AT, Fink CS. Effect of resveratrol and beta-sitosterol in combination on reactive oxygen species and prostaglandin release by PC-3 cells. *Prostag Leukotr Ess* 2005; 72: 219–26.
- 178 Mertens-Talcott SU, Percival SS. Ellagic acid and quercetin interact synergistically with resveratrol in the induction of apoptosis and cause transient cell cycle arrest in human leukemia cells. *Cancer Lett* 2005; 218: 141–51.
- 179 Lee SH, Kim JS, Yamaguchi K, Eling TE, Baek SJ. Indole-3-carbinol and 3,3'-diindolylmethane induce expression of NAG-1 in a p53-independent manner. *Biochem Biophys Res Commun* 2005; 328: 63–9.
- 180 Howells LM, Gallacher-Horley B, Houghton CE, Manson MM, Hudson EA. Indole-3-carbinol inhibits protein kinase B/Akt and induces apoptosis in the human breast tumor cell line MDA MB468 but not in the nontumorigenic HBL100 line. *Mol Cancer Ther* 2002; 1: 1161–72.
- 181 Lee IJ, Han F, Baek J, Hisatsune A, Kim KC. Inhibition of MUC1 expression by indole-3-carbinol. *Int J Cancer* 2004; 109: 810–16.
- 182 Telang NT, Katdare M, Bradlow HL, Osborne MP, Fishman J. Inhibition of proliferation and modulation of estradiol metabolism: novel mechanisms for breast cancer prevention by the phytochemical indole-3-carbinol. *Proc Soc Exp Biol Med* 1997; 216: 246–52.
- 183 Rahman KMW, Aranha O, Glazyrin A, Chinni SR, Sarkar FH. Translocation of Bax to mitochondria induces apoptotic cell death in Indole-3-carbinol (I3C) treated breast cancer cells. *Oncogene* 2000; 19: 5764–71.
- 184 Ge X, Fares FA, Yannai S. Induction of apoptosis in MCF-7 cells by indole-3-carbinol is independent of p53 and Bax. *Anticancer Res* 1999; 19: 3199–204.

- 185 Cover CM, Hsieh SJ, Tran SH, Hallden G, Kim GS, Bjeldanes LF, *et al*. Indole-3-carbinol inhibits the expression of cyclin-dependent kinase-6 and induces a G1 cell cycle arrest of human breast cancer cells independent of estrogen receptor signalling. *J Biol Chem* 1998; 273: 3838–47.
- 186 Ashok BT, Chen Y, Liu X, Bradlow HL, Mittelman A, Tiwari RK. Abrogation of estrogen-mediated cellular and biochemical effects by indole-3-carbinol. *Nutr Cancer* 2001; 41: 180–7.
- 187 Niwa T, Swaneck G, Bradlow HL. Alterations in estradiol metabolism in MCF-7 cells induced by treatment with indole-3-carbinol and related compounds. *Steroids* 1994; 59: 523–7.
- 188 Tiwari RK, Guo L, Bradlow HL, Telang NT, Osborne MP. Selective responsiveness of human breast cancer cells to indole-3-carbinol, a chemopreventive agent. *J Natl Cancer Inst* 1994; 86: 126–31.
- 189 Ashok BT, Chen YG, Liu X, Garikapaty VPS, Sepowitz R, Tschorn J, *et al*. Multiple molecular targets of indole-3-carbinol, a chemopreventive anti-estrogen in breast cancer. *Eur J Cancer Prev* 2002; 11: S86–93.
- 190 Garcia HH, Brar GA, Nguyen DHH, Bjeldanes LF, Firestone GL. Indole-3-carbinol (I3C) inhibits cyclin-dependent kinase-2 function in human breast cancer cells by regulating the size distribution, associated cyclin E forms, and subcellular localization of the CDK2 protein complex. *J Biol Chem* 2005; 280: 8756–64.
- 191 Rahman KMW, Aranha OP, Sarkar FH. Indole-3-carbinol (I3C) induces apoptosis in tumorigenic but not in nontumorigenic breast epithelial cells. *Nutr Cancer* 2003; 45: 101–12.
- 192 Meng QH, Goldberg ID, Rosen EM, Fan SJ. Inhibitory effects of indole-3-carbinol on invasion and migration in human breast cancer cells. *Breast Cancer Res Treat* 2000; 63: 147–52.
- 193 Chinni SR, Sarkar FH. Akt inactivation is a key event in indole-3-carbinol-induced apoptosis in PC-3 cells. *Clin Cancer Res* 2002; 8: 1228–36.
- 194 Jeon KI, Rih JK, Kim HJ, Lee YJ, Cho CH, Goldberg ID, *et al*. Pretreatment of indole-3-carbinol augments TRAIL-induced apoptosis in a prostate cancer cell line, LNCaP. *FEBS Lett* 2003; 544: 246–51.
- 195 Pappa G, Lichtenberg M, Iori R, Barillari J, Bartsch H, Gerhauser C. Comparison of growth inhibition profiles and mechanisms of apoptosis induction in human colon cancer cell lines by isothiocyanates and indoles from Brassicaceae. *Mutat Res* 2006; 599: 76–87.
- 196 Hong C, Firestone GL, Bjeldanes LF. Bcl-2 family-mediated apoptotic effects of 3,3'-diindolylmethane (DIM) in human breast cancer cells. *Biochem Pharmacol* 2002; 63: 1085–97.
- 197 Ge X, Yannai S, Rennert G, Gruener N, Fares FA. 3,3'-Diindolylmethane induces apoptosis in human cancer cells. *Biochem Biophys Res Commun* 1996; 228: 153–8.
- 198 Hong C, Kim HA, Firestone GL, Bjeldanes LF. 3,3'-Diindolylmethane (DIM) induces a G(1) cell cycle arrest in human breast cancer cells that is accompanied by Sp1-mediated activation of p21(WAF1/CIP1) expression. *Carcinogenesis* 2002; 23: 1297–305.
- 199 Rahman KW, Li Y, Wang Z, Sarkar SH, Sarkar FH. Gene expression profiling revealed survivin as a target of 3,3'-diindolylmethane-induced cell growth inhibition and apoptosis in breast cancer cells. *Cancer Res* 2006; 66: 4952–60.
- 200 Nachshon-Kedmi M, Yannai S, Haj A, Fares FA. Indole-3-carbinol and 3,3'-diindolylmethane induce apoptosis in human prostate cancer cells. *Food Chem Toxicol* 2003; 41: 745–52.
- 201 Garikapaty VP, Ashok BT, Tadi K, Mittelman A, Tiwari RK. 3, 3'-Diindolylmethane downregulates pro-survival pathway in hormone independent prostate cancer. *Biochem Biophys Res Commun* 2006; 340: 718–25.
- 202 Gamet-Payraastre L, Lumeau S, Gasc N, Cassar G, Rollin P, Tulliez J. Selective cytostatic and cytotoxic effects of glucosinolates hydrolysis products on human colon cancer cells in vitro. *Anti-cancer Drugs* 1998; 9: 141–8.
- 203 Sanderson JT, Slobbe L, Lansbergen GWA, Safe S, van den Berg M. 2,3,7,8-tetrachlorodibenzo-p-dioxin and diindolylmethanes differentially induce cytochrome P450 1A1, 1B1, and 19 in H295R human adrenocortical carcinoma cells. *Toxicol Sci* 2001; 61: 40–8.
- 204 Garcea G, Jones DJ, Singh R, Dennison AR, Farmer PB, Sharma RA, *et al*. Detection of curcumin and its metabolites in hepatic tissue and portal blood of patients following oral administration. *Br J Cancer* 2004; 90: 1011–5.
- 205 Lao CD, Ruffin MT, Normolle D, Heath DD, Murray SI, Bailey JM, *et al*. Dose escalation of a curcuminoid formulation. *BMC Complement Altern Med* 2006; 6: 10.
- 206 Wahlstrom B, Blennow G. A study on the fate of curcumin in the rat. *Acta Pharmacol Toxicol (Copenh)* 1978; 43: 86–92.
- 207 Holder GM, Plummer JL, Ryan AJ. The metabolism and excretion of curcumin (1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) in the rat. *Xenobiotica* 1978; 8: 761–8.
- 208 Ravindranath V, Chandrasekhara N. Metabolism of curcumin—studies with [³H]curcumin. *Toxicol* 1981; 22: 337–44.
- 209 Pan MH, Huang TM, Lin JK. Biotransformation of curcumin through reduction and glucuronidation in mice. *Drug Metab Dispos* 1999; 27: 486–94.
- 210 Yang KY, Lin LC, Tseng TY, Wang SC, Tsai TH. Oral bioavailability of curcumin in rat and the herbal analysis from *Curcuma longa* by LC-MS/MS. *J Chromatogr B Analyt Technol Biomed Life Sci* 2007; 853: 183–9.
- 211 Mori H, Niwa K, Zheng Q, Yamada Y, Sakata K, Yoshimi N. Cell proliferation in cancer prevention; effects of preventive agents on estrogen-related endometrial carcinogenesis model and on an in vitro model in human colorectal cells. *Mutat Res* 2001; 480–481: 201–7.
- 212 Collett GP, Campbell FC. Curcumin induces c-jun N-terminal kinase-dependent apoptosis in HCT116 human colon cancer cells. *Carcinogenesis* 2004; 25: 2183–9.
- 213 Scott DW, Loo G. Curcumin-induced GADD153 gene up-regulation in human colon cancer cells. *Carcinogenesis* 2004; 25: 2155–64.
- 214 Chen A, Xu J. Activation of PPAR{gamma} by curcumin inhibits Moser cell growth and mediates suppression of gene expression of cyclin D1 and EGFR. *Am J Physiol Gastrointest Liver Physiol* 2005; 288: G447–56.
- 215 Chen A, Xu J, Johnson AC. Curcumin inhibits human colon cancer cell growth by suppressing gene expression of epidermal growth factor receptor through reducing the activity of the transcription factor Egr-1. *Oncogene* 2006; 25: 278–87.
- 216 Zhang F, Altorki NK, Mestre JR, Subbaramaiah K, Dannenberg AJ. Curcumin inhibits cyclooxygenase-2 transcription in bile acid- and phorbol ester-treated human gastrointestinal epithelial

- lial cells. *Carcinogenesis* 1999; 20: 445–51.
- 217 Plummer SM, Holloway KA, Manson MM, Munks RJ, Kaptein A, Farrow S, *et al*. Inhibition of cyclo-oxygenase 2 expression in colon cells by the chemopreventive agent curcumin involves inhibition of NF-kappaB activation via the NIK/IKK signalling complex. *Oncogene* 1999; 18: 6013–20.
- 218 Goel A, Boland CR, Chauhan DP. Specific inhibition of cyclooxygenase-2 (COX-2) expression by dietary curcumin in HT-29 human colon cancer cells. *Cancer Lett* 2001; 172: 111–8.
- 219 Hong J, Bose M, Ju J, Ryu JH, Chen X, Sang S, *et al*. Modulation of arachidonic acid metabolism by curcumin and related beta-diketone derivatives: effects on cytosolic phospholipase A(2), cyclooxygenases and 5-lipoxygenase. *Carcinogenesis* 2004; 25: 1671–9.
- 220 Lin LI, Ke YF, Ko YC, Lin JK. Curcumin inhibits SK-Hep-1 hepatocellular carcinoma cell invasion *in vitro* and suppresses matrix metalloproteinase-9 secretion. *Oncol* 1998; 55: 349–53.
- 221 Choudhuri T, Pal S, Agwarwal ML, Das T, Sa G. Curcumin induces apoptosis in human breast cancer cells through p53-dependent Bax induction. *FEBS Lett* 2002; 512: 334–40.
- 222 Simon A, Allais DP, Duroux JL, Basly JP, Durand-Fontanier S, Delage C. Inhibitory effect of curcuminoids on MCF-7 cell proliferation and structure-activity relationships. *Cancer Lett* 1998; 129: 111–6.
- 223 Anuchapreeda S, Leechanachai P, Smith MM, Ambudkar SV, Limtrakul PN. Modulation of P-glycoprotein expression and function by curcumin in multidrug-resistant human KB cells. *Biochem Pharmacol* 2002; 64: 573–82.
- 224 Han SS, Chung ST, Robertson DA, Ranjan D, Bondada S. Curcumin causes the growth arrest and apoptosis of B cell lymphoma by downregulation of *egr-1*, *c-myc*, *bcl-XL*, *NF-kappa B*, and *p53*. *Clin Immunol* 1999; 93: 152–61.
- 225 Chen YR, Tan TH. Inhibition of the *c-Jun N-terminal kinase* (JNK) signaling pathway by curcumin. *Oncogene* 1998; 17: 173–8.
- 226 Kuo ML, Huang TS, Lin JK. Curcumin, an antioxidant and anti-tumor promoter, induces apoptosis in human leukemia cells. *Biochim Biophys Acta* 1996; 1317: 95–100.
- 227 Bharti AC, Donato N, Aggarwal BB. Curcumin (diferuloylmethane) inhibits constitutive and IL-6-inducible STAT3 phosphorylation in human multiple myeloma cells. *J Immunol* 2003; 171: 3863–71.
- 228 Yang CS, Chen L, Lee MJ, Balentine D, Kuo MC, Schantz SP. Blood and urine levels of tea catechins after ingestion of different amounts of green tea by human volunteers. *Cancer Epidemiol Biomarkers Prev* 1998; 7: 351–4.
- 229 Meng X, Sang S, Zhu N, Lu H, Sheng S, Lee MJ, *et al*. Identification and characterization of methylated and ring-fission metabolites of tea catechins formed in humans, mice, and rats. *Chem Res Toxicol* 2002; 15: 1042–50.
- 230 Lee MJ, Maliakal P, Chen L, Meng X, Bondoc FY, Prabhu S, *et al*. Pharmacokinetics of tea catechins after ingestion of green tea and (-)-epigallocatechin-3-gallate by humans: formation of different metabolites and individual variability. *Cancer Epidemiol Biomarkers Prev* 2002; 11: 1025–32.
- 231 Ullmann U, Haller J, Decourt JP, Girault N, Girault J, Richard-Caudron AS, *et al*. A single ascending dose study of epigallocatechin gallate in healthy volunteers. *J Int Med Res* 2003; 31: 88–101.
- 232 Van Amelsvoort JM, Van Hof KH, Mathot JN, Mulder TP, Wiersma A, Tijburg LB. Plasma concentrations of individual tea catechins after a single oral dose in humans. *Xenobiotica* 2001; 31: 891–901.
- 233 Warden BA, Smith LS, Beecher GR, Balentine DA, Clevidence BA. Catechins are bioavailable in men and women drinking black tea throughout the day. *J Nutr* 2001; 131: 1731–7.
- 234 Chow HH, Hakim IA, Vining DR, Crowell JA, Ranger-Moore J, Chew WM, *et al*. Effects of dosing condition on the oral bioavailability of green tea catechins after single-dose administration of Polyphenon E in healthy individuals. *Clin Cancer Res* 2005; 11: 4627–33.
- 235 Henning SM, Niu Y, Liu Y, Lee NH, Hara Y, Thames GD, *et al*. Bioavailability and antioxidant effect of epigallocatechin gallate administered in purified form versus as green tea extract in healthy individuals. *J Nutr Biochem* 2005; 16: 610–6.
- 236 Lambert JD, Lee MJ, Lu H, Meng X, Hong JJ, Seril DN, *et al*. Epigallocatechin-3-gallate is absorbed but extensively glucuronidated following oral administration to mice. *J Nutr* 2003; 133: 4172–7.
- 237 Chen L, Lee MJ, Li H, Yang CS. Absorption, distribution, elimination of tea polyphenols in rats. *Drug Metab Dispos* 1997; 25: 1045–50.
- 238 Jeong WS, Kim IW, Hu R, Kong AN. Modulation of AP-1 by natural chemopreventive compounds in human colon HT-29 cancer cell line. *Pharm Res* 2004; 21: 649–60.
- 239 Jung YD, Kim MS, Shin BA, Chay KO, Ahn BW, Liu W, *et al*. EGCG, a major component of green tea, inhibits tumour growth by inhibiting VEGF induction in human colon carcinoma cells. *Br J Cancer* 2001; 84: 844–50.
- 240 McLoughlin P, Roengvoraphoj M, Gissel C, Hescheler J, Certa U, Sachinidis A. Transcriptional responses to epigallocatechin-3-gallate in HT 29 colon carcinoma spheroids. *Genes Cells* 2004; 9: 661–9.
- 241 Nam S, Smith DM, Dou QP. Ester bond-containing tea polyphenols potently inhibit proteasome activity *in vitro* and *in vivo*. *J Biol Chem* 2001; 276: 13322–30.
- 242 Hussain T, Gupta S, Adhami VM, Mukhtar H. Green tea constituent epigallocatechin-3-gallate selectively inhibits COX-2 without affecting COX-1 expression in human prostate carcinoma cells. *Int J Cancer* 2005; 113: 660–9.
- 243 Siddiqui IA, Adhami VM, Afaq F, Ahmad N, Mukhtar H. Modulation of phosphatidylinositol-3-kinase/protein kinase B- and mitogen-activated protein kinase-pathways by tea polyphenols in human prostate cancer cells. *J Cell Biochem* 2004; 91: 232–42.
- 244 Gupta S, Ahmad N, Nieminen AL, Mukhtar H. Growth inhibition, cell-cycle dysregulation, and induction of apoptosis by green tea constituent (-)-epigallocatechin-3-gallate in androgen-sensitive and androgen-insensitive human prostate carcinoma cells. *Toxicol Appl Pharmacol* 2000; 164: 82–90.
- 245 Lee YK, Bone ND, Strege AK, Shanafelt TD, Jelinek DF, Kay NE. VEGF receptor phosphorylation status and apoptosis is modulated by a green tea component, epigallocatechin-3-gallate (EGCG), in B-cell chronic lymphocytic leukemia. *Blood* 2004; 104: 788–94.

- 246 Balasubramanian S, Efimova T, Eckert RL. Green tea polyphenol stimulates a Ras, MEKK1, MEK3, and p38 cascade to increase activator protein 1 factor-dependent involucrin gene expression in normal human keratinocytes. *J Biol Chem* 2002; 277: 1828–36.
- 247 Barthelman M, Bair WB, 3rd, Stickland KK, Chen W, Timmermann BN, Valcic S, *et al.* (-)-Epigallocatechin-3-gallate inhibition of ultraviolet B-induced AP-1 activity. *Carcinogenesis* 1998; 19: 2201–4.
- 248 Chen W, Dong Z, Valcic S, Timmermann BN, Bowden GT. Inhibition of ultraviolet B — induced c-fos gene expression and p38 mitogen-activated protein kinase activation by (-)-epigallocatechin gallate in a human keratinocyte cell line. *Mol Carcinog* 1999; 24: 79–84.
- 249 Nihal M, Ahmad N, Mukhtar H, Wood GS. Anti-proliferative and proapoptotic effects of (-)-epigallocatechin-3-gallate on human melanoma: possible implications for the chemoprevention of melanoma. *Int J Cancer* 2005; 114: 513–21.
- 250 Nomura M, Kaji A, He Z, Ma WY, Miyamoto K, Yang CS, *et al.* Inhibitory mechanisms of tea polyphenols on the ultraviolet B-activated phosphatidylinositol 3-kinase-dependent pathway. *J Biol Chem* 2001; 276: 46624–31.
- 251 Nomura M, Ma W, Chen N, Bode AM, Dong Z. Inhibition of 12-O-tetradecanoylphorbol-13-acetate-induced NF-kappaB activation by tea polyphenols, (-)-epigallocatechin gallate and theaflavins. *Carcinogenesis* 2000; 21: 1885–90.
- 252 Dong Z, Ma W, Huang C, Yang CS. Inhibition of tumor promoter-induced activator protein 1 activation and cell transformation by tea polyphenols, (-)-epigallocatechin gallate, and theaflavins. *Cancer Res* 1997; 57: 4414–9.
- 253 Liang YC, Lin-shiau SY, Chen CF, Lin JK. Suppression of extracellular signals and cell proliferation through EGF receptor binding by (-)-epigallocatechin gallate in human A431 epidermoid carcinoma cells. *J Cell Biochem* 1997; 67: 55–65.
- 254 Gupta S, Hastak K, Afaq F, Ahmad N, Mukhtar H. Essential role of caspases in epigallocatechin-3-gallate-mediated inhibition of nuclear factor kappa B and induction of apoptosis. *Oncogene* 2004; 23: 2507–22.
- 255 Ahmad N, Gupta S, Mukhtar H. Green tea polyphenol epigallocatechin-3-gallate differentially modulates nuclear factor kappaB in cancer cells versus normal cells. *Arch Biochem Biophys* 2000; 376: 338–46.
- 256 Sah JF, Balasubramanian S, Eckert RL, Rorke EA. Epigallocatechin-3-gallate inhibits epidermal growth factor receptor signaling pathway. Evidence for direct inhibition of ERK1/2 and AKT kinases. *J Biol Chem* 2004; 279: 12755–62.
- 257 Bhimani RS, Troll W, Grunberger D, Frenkel K. Inhibition of oxidative stress in HeLa cells by chemopreventive agents. *Cancer Res* 1993; 53: 4528–33.
- 258 Chen JJ, Ye ZQ, Koo MW. Growth inhibition and cell cycle arrest effects of epigallocatechin gallate in the NBT-II bladder tumour cell line. *BJU Int* 2004; 93: 1082–6.
- 259 Khafif A, Schantz SP, al-Rawi M, Edelstein D, Sacks PG. Green tea regulates cell cycle progression in oral leukoplakia. *Head Neck* 1998; 20: 528–34.
- 260 Fang MZ, Wang Y, Ai N, Hou Z, Sun Y, Lu H, *et al.* Tea polyphenol (-)-epigallocatechin-3-gallate inhibits DNA methyltransferase and reactivates methylation-silenced genes in cancer cell lines. *Cancer Res* 2003; 63: 7563–70.
- 261 Lamy S, Gingras D, Beliveau R. Green tea catechins inhibit vascular endothelial growth factor receptor phosphorylation. *Cancer Res* 2002; 62: 381–5.
- 262 Tang FY, Nguyen N, Meydani M. Green tea catechins inhibit VEGF-induced angiogenesis *in vitro* through suppression of VE-cadherin phosphorylation and inactivation of Akt molecule. *Int J Cancer* 2003; 106: 871–8.
- 263 El Bedoui J, Oak MH, Anglard P, Schini-Kerth VB. Catechins prevent vascular smooth muscle cell invasion by inhibiting MT1-MMP activity and MMP-2 expression. *Cardiovasc Res* 2005; 67: 317–25.
- 264 Dashwood WM, Carter O, Al-Fageeh M, Li Q, Dashwood RH. Lysosomal trafficking of beta-catenin induced by the tea polyphenol epigallocatechin-3-gallate. *Mutat Res* 2005; 591: 161–72.
- 265 Moon Y, Lee M, Yang H. Involvement of early growth response gene 1 in the modulation of microsomal prostaglandin E synthase 1 by epigallocatechin gallate in A549 human pulmonary epithelial cells. *Biochem Pharmacol* 2007; 73: 125–35.
- 266 Yu HN, Shen SR, Yin JJ. Effects of interactions of EGCG and Cd (2+) on the growth of PC-3 cells and their mechanisms. *Food Chem Toxicol* 2007; 45: 244–9.
- 267 Kalfon L, Youdim MB, Mandel SA. Green tea polyphenol (-)-epigallocatechin-3-gallate promotes the rapid protein kinase C- and proteasome-mediated degradation of Bad: implications for neuroprotection. *J Neurochem* 2007; 100: 992–1002.
- 268 Walle T, Hsieh F, DeLegge MH, Oatis JE Jr, Walle UK. High absorption but very low bioavailability of oral resveratrol in humans. *Drug Metab Dispos* 2004; 32: 1377–82.
- 269 Vitaglione P, Sforza S, Galaverna G, Ghidini C, Caporaso N, Vescovi PP, *et al.* Bioavailability of trans-resveratrol from red wine in humans. *Mol Nutr Food Res* 2005; 49: 495–504.
- 270 Meng X, Maliakal P, Lu H, Lee MJ, Yang CS. Urinary and plasma levels of resveratrol and quercetin in humans, mice, and rats after ingestion of pure compounds and grape juice. *J Agric Food Chem* 2004; 52: 935–42.
- 271 Juan ME, Lamuela-Raventos RM, de la Torre-Boronat MC, Planas JM. Determination of trans-resveratrol in plasma by HPLC. *Anal Chem* 1999; 71: 747–50.
- 272 Hsieh TC, Wu JM. Differential effects on growth, cell cycle arrest, and induction of apoptosis by resveratrol in human prostate cancer cell lines. *Exp Cell Res* 1999; 249: 109–15.
- 273 Aziz MH, Kumar R, Ahmad N. Cancer chemoprevention by resveratrol: *In vitro* and *in vivo* studies and the underlying mechanisms. *Int J Oncol* 2003; 23: 17–28.
- 274 Cao Z, Fang J, Xia C, Shi X, Jiang BH. trans-3,4,5'-Trihydroxystibene inhibits hypoxia-inducible factor 1alpha and vascular endothelial growth factor expression in human ovarian cancer cells. *Clin Cancer Res* 2004; 10: 5253–63.
- 275 Kaneuchi M, Sasaki M, Tanaka Y, Yamamoto R, Sakuragi N, Dahiya R. Resveratrol suppresses growth of Ishikawa cells through down-regulation of EGF. *Int J Oncol* 2003; 23: 1167–72.
- 276 Lee JE, Safe S. Involvement of a post-transcriptional mechanism in the inhibition of CYP1A1 expression by resveratrol in breast cancer cells. *Biochem Pharmacol* 2001; 62: 1113–24.
- 277 El-Mowafy AM, Alkhalaf M. Resveratrol activates adenyllyl-cyclase in human breast cancer cells: a novel, estrogen receptor-

- independent cytostatic mechanism. *Carcinogenesis* 2003; 24: 869–73.
- 278 Bhat KP, Lantvit D, Christov K, Mehta RG, Moon RC, Pezzuto JM. Estrogenic and antiestrogenic properties of resveratrol in mammary tumor models. *Cancer Res* 2001; 61: 7456–63.
- 279 Basly JP, Marre-Fournier F, Le Bail JC, Habrioux G, Chulia AJ. Estrogenic/antiestrogenic and scavenging properties of (E)- and (Z)-resveratrol. *Life Sci* 2000; 66: 769–77.
- 280 Scarlatti F, Sala G, Somenzi G, Signorelli P, Sacchi N, Ghidoni R. Resveratrol induces growth inhibition and apoptosis in metastatic breast cancer cells via de novo ceramide signaling. *FASEB J* 2003; 17: 2339–41.
- 281 Subbaramaiah K, Chung WJ, Michaluart P, Telang N, Tanabe T, Inoue H, *et al*. Resveratrol inhibits cyclooxygenase-2 transcription and activity in phorbol ester-treated human mammary epithelial cells. *J Biol Chem* 1998; 273: 21875–82.
- 282 Shih A, Davis FB, Lin HY, Davis PJ. Resveratrol induces apoptosis in thyroid cancer cell lines via a MAPK- and p53-dependent mechanism. *J Clin Endocrinol Metab* 2002; 87: 1223–32.
- 283 She QB, Huang C, Zhang Y, Dong Z. Involvement of c-jun NH (2)-terminal kinases in resveratrol-induced activation of p53 and apoptosis. *Mol Carcinogenesis* 2002; 33: 244–50.
- 284 She QB, Ma WY, Wang M, Kaji A, Ho CT, Dong Z. Inhibition of cell transformation by resveratrol and its derivatives: differential effects and mechanisms involved. *Oncogene* 2003; 22: 2143–50.
- 285 Woo JH, Lim JH, Kim YH, Suh SI, Min DS, Chang JS, *et al*. Resveratrol inhibits phorbol myristate acetate-induced matrix metalloproteinase-9 expression by inhibiting JNK and PKC delta signal transduction. *Oncogene* 2004; 23: 1845–53.
- 286 Igura K, Ohta T, Kuroda Y, Kaji K. Resveratrol and quercetin inhibit angiogenesis *in vitro*. *Cancer Lett* 2001; 171: 11–6.
- 287 Ahmad N, Adhami VM, Afaq F, Feyes DK, Mukhtar H. Resveratrol causes WAF-1/p21-mediated G(1)-phase arrest of cell cycle and induction of apoptosis in human epidermoid carcinoma A431 cells. *Clin Cancer Res* 2001; 7: 1466–73.
- 288 Ahmad KA, Clement MV, Hanif IM, Pervaiz S. Resveratrol inhibits drug-induced apoptosis in human leukemia cells by creating an intracellular milieu nonpermissive for death execution. *Cancer Res* 2004; 64: 1452–59.
- 289 Huang C, Ma WY, Goranson A, Dong Z. Resveratrol suppresses cell transformation and induces apoptosis through a p53-dependent pathway. *Carcinogenesis* 1999; 20: 237–42.
- 290 Manna SK, Mukhopadhyay A, Aggarwal BB. Resveratrol suppresses TNF-induced activation of nuclear transcription factors NF-kappa B, activator protein-1, and apoptosis: potential role of reactive oxygen intermediates and lipid peroxidation. *J Immunol* 2000; 164: 6509–19.
- 291 Berge G, Ovrebo S, Botnen IV, Hewer A, Phillips DH, Haugen A, *et al*. Resveratrol inhibits benzo[a]pyrene-DNA adduct formation in human bronchial epithelial cells. *Brit J Cancer* 2004; 91: 333–8.
- 292 Hsieh TC, Wang Z, Hamby CV, Wu JM. Inhibition of melanoma cell proliferation by resveratrol is correlated with upregulation of quinone reductase 2 and p53. *Biochem Biophys Res Commun* 2005; 334: 223–30.
- 293 Zhang Q, Tang X, Lu QY, Zhang ZF, Brown J, Le AD. Resveratrol inhibits hypoxia-induced accumulation of hypoxia-inducible factor-1alpha and VEGF expression in human tongue squamous cell carcinoma and hepatoma cells. *Mol Cancer Ther* 2005; 4: 1465–74.